# METABOLIC AND NEUROHUMORAL ASPECTS OF ACUTE MYOCARDIAL ISCHEMIA IN MAN

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# METABOLIC AND NEUROHUMORAL ASPECTS OF ACUTE MYOCARDIAL ISCHEMIA IN MAN.

## METABOLE EN NEUROHUMORALE ASPECTEN VAN ACUUT MYOCARD-ISCHEMIE BIJ DE MENS.

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*To Pat, Patrick,*

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A. INTRODUCTION AND RATIONALE FOR THE STUDIES.



B. SPECIFIC STUDIES.

# Part 1

## Myocardial metabolic changes during acute ischemia in man. Relation to coronary flow and potential for evaluating pharmacological interventions.

### A. Introduction and rationale for the studies.

# INTRODUCTION.

For many decades, myocardial ischemia has been at the root of cardiovascular medicine. Prevention as well as treatment of this syndrome has been two of its major tasks and, consequently, certain improvements have been achieved in this area. Through a substantial reduction in primary risk factors, the incidence of coronary artery disease has declined. Also, preliminary reports indicate, that regression of coronary atherosclerosis, through pharmacological or dietary interventions, may actually become possible.
Furthermore, rapid technical developments in the area of mechanical, catheter-based interventions, aimed at improving coronary artery patency, have certainly added to the therapeutic achievements already afforded by cardiovascular surgery.
Both in this respect and on the prevention side, our recent, rapidly growing understanding of the important role of the vascular endothelium in the regulation of vasotone, in vascular growth and atherosclerosis, and in their interrelationship, will have a major impact on future management and prevention of ischemic heart disease.
At present, however, the incidence of ischemic heart disease still ranks high, despite a decline in coronary artery disease.
In the majority of patients, particularly those with so-called stable angina and ischemia, the therapeutic approach will be conservative for a number of reasons; the cost-effectiveness of non-pharmacological approaches being of major importance.
It is interesting to note, that despite its long standing history, the pharmacological "treatment" of ischemic heart disease is still very limited. Platelet-active agents aside, only three groups of drugs are officially recognized in this respect. Compounds, which are certainly not uniformly effective and often give rise to significant side effects. As such, there is a constant need for new cardiovascular drugs and, in particular, for alternative forms of pharmacological therapy. Consequently, sensitive and precise methods to analyse the mode of action and efficacy of these agents in man are greatly needed also. As the vast majority of anti-ischemic drugs act through their hemodynamic properties, invasive methods generally afford these to be better appreciated, in particular when fast sequential changes in and interaction of a multitude of cardiovascular parameters have to be studied.
Likewise, invasive screening allows for more precise as well as fast sequential monitoring of those markers of ischemia, which are otherwise inaccessible, e.g. specific myocardial metabolic indicators, such as lactate and the nucleosides. This thesis reflects our efforts in this field.
In sequential order, animal and human studies on the usefulness of nucleosides as markers of ischemia are discussed, followed by reports on the sensitivity of myocardial lactate metabolism in this respect in man and the reproducibility of lactate changes during short, repetitive periods of ischemia. Next, the relation of regional coronary bloodflow changes with these metabolic alterations will be considered. In subsequent chapters, the acute antiischemic properties of different vaso-active compounds are assessed through their effect on metabolism, both in patients with ischemic heart disease with and without left ventricular dysfunction.
Finally, a new area will be highlighted, that of the changes in systemic and transcardiac neuro-hormones during myocardial ischemia and the potential usefulness of specific therapy, e.g. converting enzyme inhibition, as anti-ischemic treatment, again demonstrated through their effect on cardiac metabolism.

To facilitate understanding the importance of these investigations, these specific chapters will be preceded by introductory notes on the normal regulation of cardiac metabolism, coronary flow and neurohormones, on the changes herein during ischemia and on those methodologies, which apply metabolic markers to detect myocardial ischemia.

# Chapter I
# CARDIAC METABOLISM.

# I.1.Regulation of normal cardiac metabolism.

## Oxydative phosphorylation.

Owing to the ever continuing sequence of contraction and relaxation, cardiac muscle, in contrast to other types of muscle, is not allowed an oxygen debt. The heart is in constant need of energy, locally generated and stored as adenine triphosphate (ATP) and, to a lesser extent, as creatinine phosphate (CrP). Under normal conditions, these high-energy phosphates are formed exclusively by oxydative phosphorylation, only 1% being generated through anaerobic pathways. In contrast, during extreme workloads, anaerobically-derived ATP-production may increase up to 7%[1].

Which substrates the heart prefers for its energy production depends on a number of variables, such as plasma substrate concentration, (neuro)humoral activity, e.g. insulin, glucagon and catecholamines, and the instantaneous metabolic rate of the heart. Besides extracellular substrates, e.g. free fatty acids (FFA), glucose, lactate and ketone bodies, there are endogenous cardiac stores of fuel, such as triglycerids and glucagon.

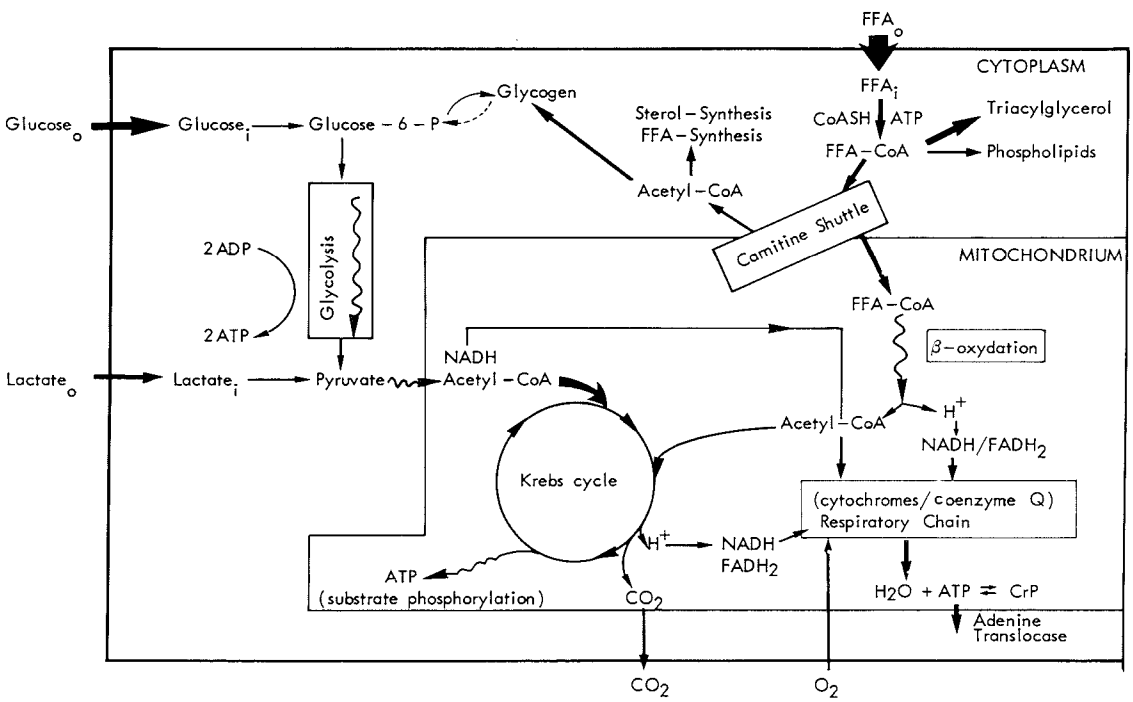


*Fig. 1: Myocardial substrate metabolism under normal, aerobic conditions. In declining order of prevalence FFA, glucose and lactate are taken up by the cell and metabolized, eventually to form acetyl-CoA, which enters the Krebs cycle. During the latter process 1 mol ATP is generated directly (substrate phosphorylation). However, more important is the formation of the reduced co-enzymes NADH and $FADH_2$, which subsequently are oxydized in the respiratory chain, eventually to form 33 mol of ATP. FFA enters the cell by diffusion or carrier-bound, where the majority is stored as glycerol. Only a small portion is metabolized in the ß-oxydation pathway after binding to acetyl-CoA and entering the mitochondria via the carnitine shuttle system. During ß-oxydation acetyl-CoA fragments are formed as well as the reduced co-enzymes NADH and $FADH_2$, which are then oxydized in the respiratory chain. (From reference 11 with permission).*

**FFA metabolism.**

In the resting, fasting state, FFA will be predominantly utilized for oxydative metabolism [2], providing up to 60-70% of substrate [2-6], although this may depend on concomitant plasma lactate levels [7,8]. FFA uptake by the cell depends on instantaneous blood levels, on the ratio of total FFA to high-binding sites on albumen and on chain length and degree of saturation. During first passage through the heart, approximately 40-50% of labelled FFA are extracted [4], membranal transport being both carrier-bound and through diffusion [9]. Once intracellular, FFA are mainly esterified to lipids and stored as glycerol. To a lesser extent, they partake in membrane function after transformation to phopholipids [10]. A small, but rapidly replenished soluble portion of the FFA is activated by acyl-CoA synthetase and, subsequently, translocated by carnitine-dependent transferases to the mitochondrial matrix space, where they are oxydized. During ß-oxydation, acyl-CoA is stepwise degraded to form acetyl-CoA fragments, which, subsequently, enter the citric acid or Krebs cycle (Fig. 1).

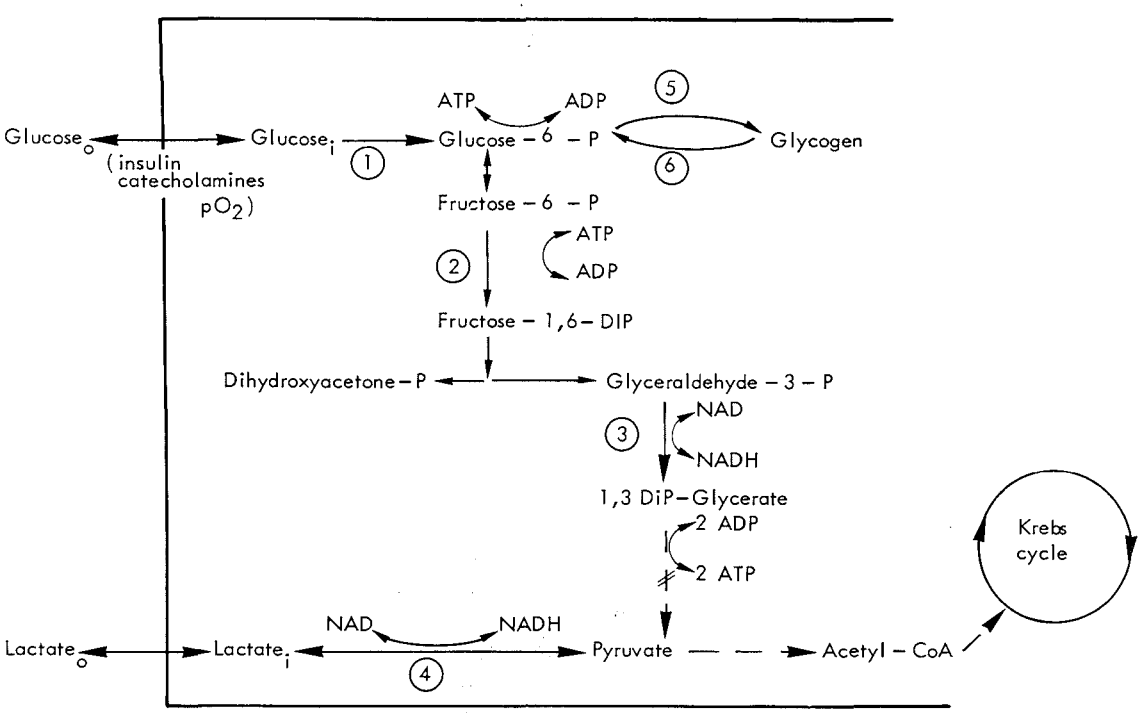


*Fig. 2: Glucose and lactate metabolism under normal aerobic conditions. Glucose uptake by the cell is carrier-bound and dependent on insulin and adrenalin stimulation. Once inside it is phosphorylated by hexokinase (1) to glucose-6-P. It then enters the glycolytic pathway or is transformed to glycogen by the enzyme glycogensynthetase (5). The glycolytic flux is mainly governed by the enzymes phosphofructokinase (2) and glyceraldehyde-3-P-dehydrogenase (4). When there is sufficient $O_2$ supply glycolysis is regulated mainly by the instantaneous ATP content at the phosphofructokinase level. Although ATP is utilized at 2 steps a net gain of 2 mol ATP per mol of glucose is achieved at the end of glycolysis. Pyruvate, formed both by the glycolytic pathway as well as from lactate is thereupon converted to acetyl-CoA and enters the Krebs cycle. (From reference 11 with permission).*

**Glucose metabolism.**

Under normal workloads and in combination with other substrates, the rate of glucose utilization is generally low (10-30%). In the presence of insulin the rate-limiting step for glycolysis in aerobic tissue is formed by the enzyme phosphofructokinase, the activity of which, under normal conditions, is limited by the tissue levels of high energy phosphates and citrate [12,13]. In the absence of insulin, glucose transport into the cell becomes rate-limiting [14]. When, however, glucose is the only fuel available, glycolytically-derived acetyl-CoA may increase with 70% during extreme workloads [1].
Sarcolemmal transport of glucose is carrier-bound, its activity stimulated by insulin, epinephrine and intracellular hypoxia. Next, it is transformed to glucose-6-phosphate by the enzyme hexokinase. From here, it may enter the glycolytic pathway to pyruvate, subsequently entering the citric acid cycle via acetyl-CoA, or it may be converted to glucagon and stored (Fig. 2).
Of importance, during its passage through the glycolytic pathway, a net yield of 2 mol ATP per mol glucose is gained anaerobically.

**Lactate metabolism.**

Under normal, non-ischemic conditions, lactate is always extracted by the heart [15] although, as a result of relatively low arterial levels (in man varying between 0.39 and 1.0 mmol/l in the supine, fasting state, personal communication), this does not provide sufficient energy for myocardial muscle function [8]. Hence, it only functions as an additional substrate for oxydative metabolism. The latter is achieved after transformation to pyruvate and to acetyl-CoA and subsequent processing in the Krebs cycle. The net extraction pattern of lactate varies between 0% and 46% (chapter V), depending on the arterial lactate level, substrate competition and catecholamine stimulation. It therefore follows, that when the heart produces lactate, there is bound to be some form of abnormality, the most common by far being myocardial oxygen deprivation [15].

**Substrate and oxydative phosphorylation and energy production.**

Although some ATP is produced during anaerobic glycolysis, this is largely insufficient for normal cardiac mechanical function. Of the total yield of 36 mol ATP per mol glucose, virtually all is generated aerobically by respiratory chain-linked oxydative phosphorylation.
Aerobic metabolism is dominated by the citric acid cycle, the primary function of which is to oxydize acetyl groups that enter the cycle in the form of acetyl-CoA molecules. After condensation of acetyl-CoA with oxaloacetate to citrate, the energy made available when the C-H and C-C bonds in citrate are oxydized, is captured in different ways during passage in the citric acid cycle. At the succinyl-CoA level, a high-energy phosphate link is created by a mechanism, which resembles that which operates during glycolysis, yielding 1 mol ATP (Fig. 3) [16].
The remaining energy from oxydation is channelled into the conversion of the co-enzymes $NAD^+$ and FAD to their reduced forms NADH and $FADH_2$. Under aerobic conditions, NADH and $FADH_2$ are subsequently oxydized during respiratory chain-phosphorylation, in which process the high-energy electrons, initially carried by these co-enzymes, are finally transferred under the influence of mitochondrial membrane-bound enzymes (cytochromes and co-enzyme Q) to oxygen. The energy, released during this process, is used to pump protons across the inner membrane of the mitochondrial matrix into the intermembranal space. This creates an electrochemical proton gradient across the mitochondrial inner membrane. The backflow of $H^+$ down this gradient in turn activates another membrane-bound enzyme, ATP synthetase, which catalyzes the conversion of ADP + inorganic phosphate to ATP, thus completing the process of oxydative phosphorylation.
The energy, thus produced, may be stored as ATP or CrP or used for a large variety of chemical processes including enzyme regulation, membrane function, contractility and relaxation. To this purpose, ATP has to be transported from the mitochondrion to the cytoplasm, a process which is carried out by the adenine nucleotide system.

**The role of L-carnitine in aerobic metabolism.**

Adenine nucleotide translocase is an exchange-diffusion transport protein, localized at the inner mitochondrial membrane, which catalyzes the exchange of ATP versus ADP on a mol per mol basis. Hence, it constitutes a key factor in linking mitochondrial energy production and cellular energy need. The activity of this transport system is regulated by the ratio long-chain acyl-CoA versus carnitine, indicative of the significance of myocardial cellular carnitine levels [17].

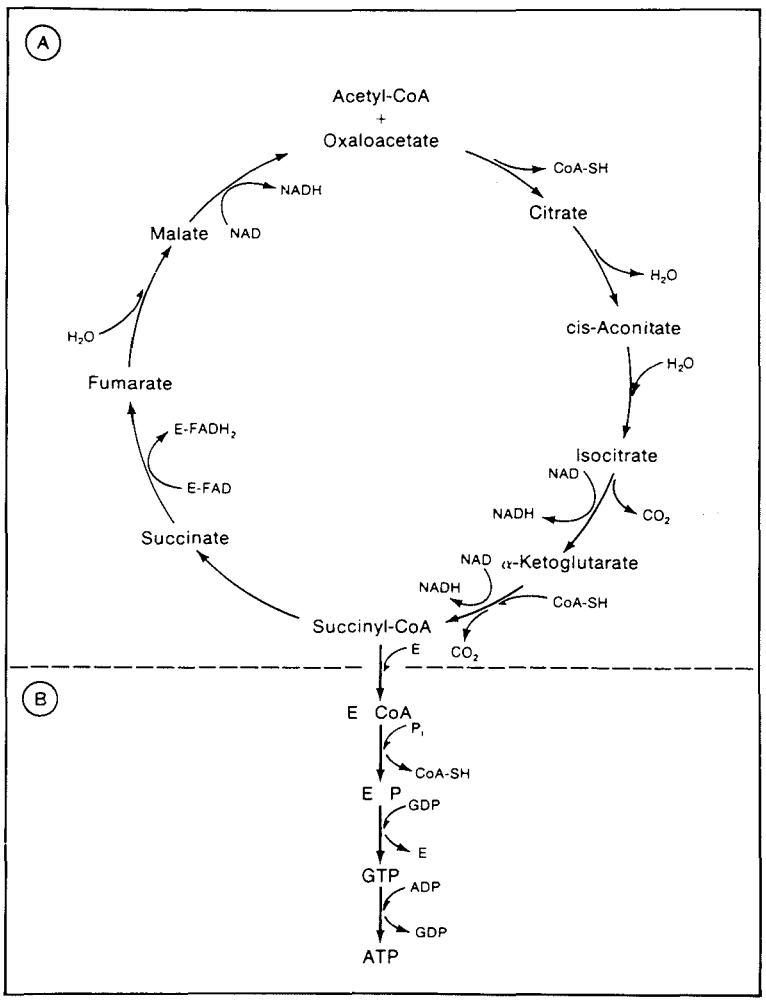


*Fig. 3: Pathways of acetyl-CoA oxydation.*
*a. Tricarboxylic acid cycle or Krebs cycle. Citrate is formed after condensation of acetyl-CoA with oxaloacetate and eventually transformed in the Krebs cycle to oxaloacetate, during a process of oxydation and decarboxylation, where a number of reduced co-enzymes are formed.*
*b. Substrate level phosphorylation. Each mol of enzyme-bound CoA released from succinyl-CoA provides for the generation of a single mol of ATP. (From reference 16, with permission).*

Moreover, L-carnitine is critically involved in the translocation of long-chain fatty acids from the extramitochondrial space to the mitochondrial matrix through the activity of carnitine acyltransferases. As these enzyme systems are both under hormonal and nutritional control, this carnitine-dependent process allows for a controlled access of long-chain fatty acids to their oxydation site, at the same time modulating the ratio of acyl-CoA and free CoA.

In view of the important regulatory role of L-carnitine in oxydative metabolism it is of interest that two of the major organs in need of L-carnitine, i.e. skeletal and cardiac muscle, are unable to synthesize this molecule, despite a high abundance of its primary precursor, ϵ-N-trimethyllysine, the reason being, that they lack the final enzyme in this process, deoxycarnitine hydroxylase. As a result, the direct precursor of carnitine, deoxycarnitine, has to be converted into carnitine in other organs, such as liver or kidney, and then transported back to the heart and skeletal muscles. Here, carnitine and deoxycarnitine are exchanged in a specific bidirectional exchange-diffusion pathway, which is only partially energy-related and depends on a membrane bound carrier system, which, like other transmembranal transport proteins, is sensitive to thiol reagents.

Besides raising several interesting questions which relate to genetic information and (changes in) gene expression of the various components of carnitine formation in the heart, this peculiarity of nature with respect to carnitine and the heart, also underlines the delicate position of this key control factor in aerobic metabolism and the critical dependency of normal carnitine supply to the heart.

**Amino acids, citrate and the malate-aspartate cycle.**

Another component of the metabolic complex in the heart, which deserves some consideration in view of its potential significance in myocardial ischemia, is the malate-aspartate cycle. Amino acids have been proposed as an additional source of fuel both during aerobic [18] and anaerobic conditions [19,20]. However, energy production derived from amino acid fermentation is minimal compared with that of glycolysis [21]. A more important function of several amino acids, such as aspartate and glutamate, is to enable the uphill transfer of reducing equivalents by the malate-aspartate cycle against an NADH-$NAD^+$ potential gradient [22] (Fig. 4).

In addition, the malate-aspartate cycle is required to coordinate mitochondrial and cytosolic metabolism in the heart [23]. Direct feedback and inhibition of glycolysis by citrate has, amongst others, generally been accepted as a primary mechanism for its control. However, as mitochondrial citrate is virtually unable to cross the mitochondrial membrane, the latter can only be accomplished through indirect means by way of the malate-aspartate cycle, which links the activity of the mitochondrial citric acid cycle with comparable isoenzymes in the cytosol. Thus, the glycolytic flux is made responsive to the redox state of pyridine nucleotides, to phosphorylation of adenine nucleotides and, in general, to the energy state of the heart.

The malate-aspartate cycle is dependent on the presence of glutamate, actively taken up by the heart. Changes in this uptake mechanism, together with alterations in alanine release, may reflect a malfunctioning of the cycle, such as occurs in ischemia, and may be used to indicate the severity of the latter.

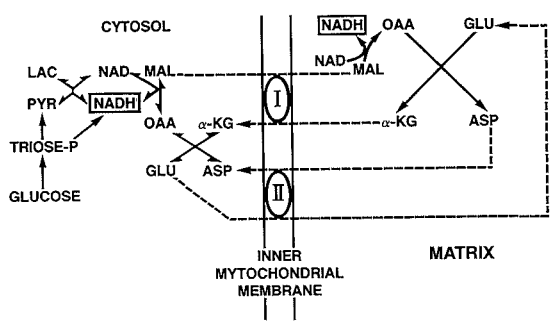


*Fig. 4: Schematic display of the malate-aspartate cycle and uphill transfer of reducing equivalents against and NADH/$NAD^+$ potential gradient over the mitochondrial membrane. After reduction of oxaloacetate (OAA) to malate (MAL), malate is transferred into the mitochondrion, where NADH and oxaloacetate are regenerated. Transfer of the latter to the cytosol is accomplished by transamination with glutamate (GLU), whereby α-ketoglutarate (α-KG) and aspartate (ASP) are formed, which are transported to the cytosol. Here oxaloacetate and glutamate are regenerated by reverse transamination.*
*LAC=lactate, PYR=pyruvate and TRIOSE-P=glyceraldehyde-3-P and α-glycerophosphate. (From reference 23, with permission).*

# I.2. Myocardial metabolism during ischemia.

## FFA metabolism.

With the occurrence of myocardial ischemia and the consequent unavailability of oxygen, aerobic metabolism and oxydative phosphorylation will quickly slow down and may even come to a complete stop, depending on the severity of ischemia. Secondary to diminished electron transport, as the ultimate acceptor oxygen is not available, and as a result of the subsequent rise in mitochondrial NADH/$NAD^+$ and $FADH_2$/FAD ratios, inhibition ß-oxydation, the most rate-limiting step of aerobic metabolism in ischemia, quickly follows [24]. This is accompanied by impaired utilization of fatty acids in the citric acid cycle. Also, during ischemia, membrane phospholipids are broken down with subsequent accumulation of non-esterified fatty acids and lysophospholipids [25-27]. Consequently, intracellular fatty acid amphiphiles of long-chain acyl-CoA and acylcarnitine rapidly increase. These fatty acid intermediates may affect the structural conformation and function of ionic channels, proteins and lipid bilayers in cellular membranes [28,29]. Moreover, they may inhibit important enzyme systems, such as adenylate cyclase [30], $Na^+/K^+$-ATP-ase [30,31], $Ca^{2+}$-ATP-ase [32] and adenine nucleotide translocase [33,34]. As such, FFA accumulation may add to the derangement in myocardial contractile function, observed in ischemia.
Incorporation of lipid intermediate amphiphiles in the membrane, either resulting in an insertion or exchange with the lipid bilayer or in a depletion through a detergent-like action, may alter the surface charge or permeability of the membrane with subsequent changes in ionic movements and cell swelling [35,36]. It has also been suggested that increased mitochondrial levels of long-chain fatty acids are directly involved in the uncoupling of electron transport [37]. Recent studies indicate the presence of a fatty acid binding protein (FABP) in the heart, that binds with high affinity to intracellular fatty acids and their thio-esters [38-40]. It has been suggested, that FABP's, which constitute 4-8% of the soluble proteins in the heart, may temporarily sequestrate FFA, thereby modulating their detrimental effects[41-42]. Alternatively, FABP may help transfer the poorly soluble fatty acids and fatty acyl-CoA from their site of entry into the cell to the site of oxydation or esterification [43]. Interestingly, these FABP's are lost from the heart during ischemia [44]. Also, a recent study has suggested that supplementation of these proteins may protect against myocardial ischemia, and may result in a significant preservation of high-energy phosphates and/or membrane phospholipids during ischemia and reperfusion [45].

## FFA and carnitine in ischemia.

The long-chain acyl-CoA/free carnitine ratio increases rapidly during ischemia [46]. Also, during ischemia, the ratio of long-chain acyl-CoA/CoA rises significantly with accumulation of long-chain CoA. This ratio modulates the activity of the enzyme pyruvate dehydrogenase, essential for the entry of pyruvate into the citric acid cycle. An increase in the ratio acyl-CoA/CoA inhibits enzyme activity. Through acyl removal from CoA as a consequence of the action of carnitine and carnitine-CoA acyltransferase, carnitine directly improves the flux of pyruvate in the Krebs cycle. Also, elevated levels of long-chain acyl-CoA inhibit adenine nucleotide translocase activity, whereas carnitine reverses this inhibition [17, 47]. This again suggests that the level of myocardial free carnitine is important during ischemia. Moreover, free carnitine is instrumental in the removal of long-chain acylcarnitines through an acylcarnitine-carnitine exchange process, equivalent to that between deoxycarnitine and carnitine [48].
Thus, the level of free carnitine in the heart is essential in reducing long-chain acylcarnitine and acyl-CoA levels during ischemia, removing their inhibitory effect on adenine translocase activity and, by diminishing the acyl-CoA/CoA ratio, modulating the inhibitory effect of the latter on pyruvate dehydrogenase during ischemia.
Our recent observation of significant free carnitine loss from the acutely ischemic myocardium in man [49] may be important therefore, and may indicate a

role for carnitine and its derivatives as an alternative way of limiting ischemia-induced myocardial functional changes.

## Myocardial ischemia and anaerobic glycolysis.

When oxydative phosphorylation is reduced during myocardial ischemia, glycolytic flux will be activated through increased activity of the enzymes phosphofructokinase and glyceraldehyde-3-P-dehydrogenase, secondary to accumulation of ATP metabolites and reduced feedback by citrate and ATP [23,50] (Fig. 5).
Also, cellular uptake of glucose increases as well as the formation of glucose-6-phosphate by hexokinase. However, this supply of substrate is critically dependent on the level of ischemia and the possibility of blood to reach the ischemic area.

An additional substrate for glycolysis is derived from glycogenolysis. However, the latter reserves are limited and will be exhausted within minutes during severe anoxia [51]. Also, it has been suggested that glycolytic flux, derived from glycogenolysis, is less beneficial than that derived from exogenous glucose during ischemia, in spite of similar rates of glycolytic ATP production [52]. Maintenance of cardiac contractile function and prevention of ischemic changes in diastolic tension may depend more on the source of ATP, e.g. glycolysis via glucose uptake, than on the concentration of ATP [53-55], at least during prolonged periods of ischemia. In contrast, in early ischemia, such as would be encountered in stress- or pacing-induced ischemia, the relative contribution of glucose flux in glycolytic ATP production and protection of function depends on available high-energy phosphates from other sources [52].

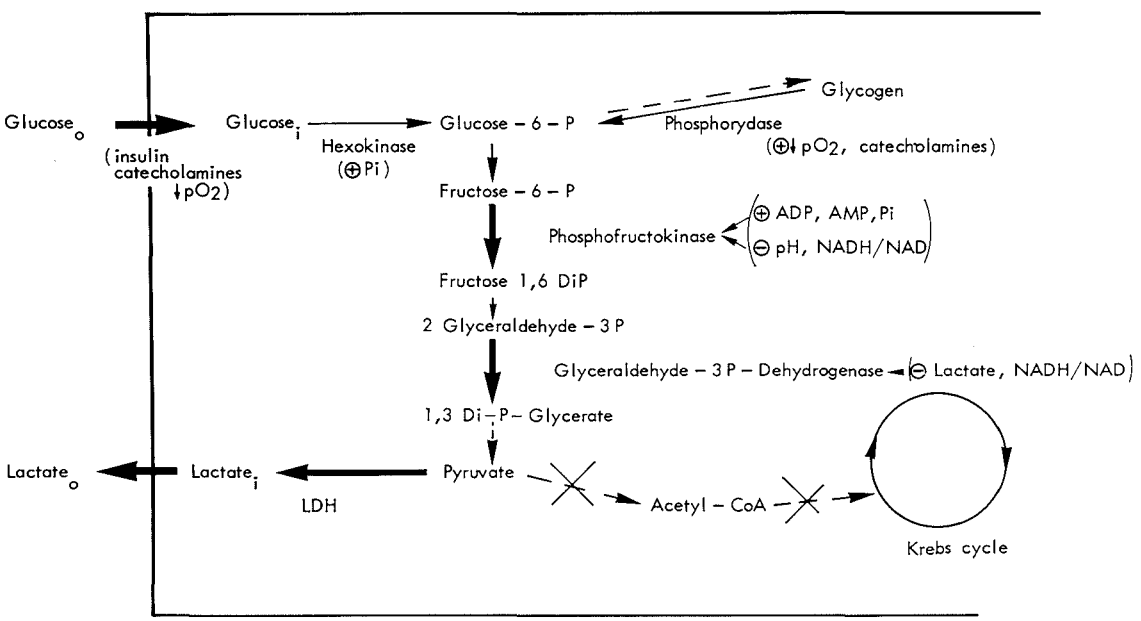


*Fig. 5: Stimulation of anaerobic glycolysis during myocardial ischemia and subsequent inhibition of oxydative phosphorylation due to enhanced activity of phosphofructokinase and glyceraldehyde-3-P-dehydrogenase in combination with increased cellular uptake of glucose. As a result, lactate is produced by the cardiocyte, instead of the usual extraction. (From reference 11 with permission).*

## Lactate.

As lack of oxygen will prevent normal functioning of the citric acid cycle, pyruvate is not further processed in the latter but, instead, will be transformed to lactate.
Thus, myocardial lactate production, rather than the normal extraction pattern, is found in an early stage during the ischemic process. The potential of this metabolic marker to indicate the presence of ischemia has been widely recognized [14,56,57], despite evidence, that lactate does not equilibrate freely over the cell membrane [58,59] and despite the fact that significant tissue-coronary venous lactate gradients are found in ischemia [57,60].
Although, theoretically, any kind of malfunctioning of aerobic metabolism will increase glycolysis, the specificity of myocardial lactate production to indicate ischemia in patients with coronary artery disease, is widely accepted.
Still, the concept may not be as simple as it looks at first sight. Cultured adult ventricular myocytes release lactate, despite optimal oxygenation [61]. Also, in non-ischemic canine and porcine models, lactate release from the heart has been clearly shown with isotope techniques in situations, where, using conventional methods, net chemical lactate extraction is found [62,63]. This observation is difficult to explain. Preferential usage of glycolytically derived ATP for sarcoplasmatic function and myocardial relaxation has been suggested. Also, some evidence indicates, that the subendocardial layers may be hypoperfused in the working heart during peak systolic pressures and, hence, may rely on anaerobic glycolysis to a greater extent than the subepicardial regions [64,65].
Besides some recent dispute on the specificity of myocardial lactate as a marker of ischemia, its sensitivity compared with other clinical markers, such as electrocardiographic or hemodynamic parameters, and with angina has also been debated (chapter V of this thesis). Part of this thesis relates to methods to improve the sensitivity of myocardial lactate production as indicator of ischemia in man (chapter V).

## Inhibition of glycolysis does affect its usefulness as marker of ischemia.

Whereas, as will be demonstrated in chapter V, myocardial lactate is a sensitive marker during short periods of ischemia, its effectiveness in this respect becomes progressively less during prolonged periods of ischemia. Due to inhibition of glycolysis, particularly at the glyceraldehyde-3-P-dehydrogenase level [66,67], myocardial lactate production eventually diminishes when the ischemic period is sustained. The latter is predominantly caused by proton and lactate accumulation [68] due to reduced venous efflux from the ischemic area. This results in pH changes and in a rise in the $NADH/NAD^+$ and $FADH_2/FAD$ ratios. Glycolysis is predominantly regulated by phosphofructokinase activity during early ischemia [69,70], whereas inhibition of glycolysis during prolonged periods of ischemia occurs at the level of glyceraldehyde-3-P-dehydrogenase [67]. Both enzymes are sensitive to pH changes and are inhibited by high-energy phosphates. Thus, glycolysis is dependent on the ratio of ATP versus ADP and AMP [68]. Consequently, the reduction in glycolytic flux leads to a decrease in anaerobic ATP production. Moreover, lactate production diminishes. Sustained inhibition of glycolysis after an ischemic event may explain why myocardial lactate production values are not reproducible when only short intervals are allowed between repeated periods of ischemia (chapter VI).

## Adenine nucleotide breakdown and myocardial ischemia.

In the absence of oxydative phosphorylation, synthesis of ATP from ADP cannot take place. As a result, ATP (and CrP) levels fall and ADP, AMP and inorganic phosphate ($P_i$) rapidly accumulate. The degree to which intracellular high-energy phosphate levels change, clearly depends on the severity of ischemia.
After complete coronary occlusion, CrP levels decrease by 75% during the first minute, accompanied by an equivalent rise in lactate levels [71]. In contrast, ATP content changes relatively little during the first 3 minutes following occlusion (Fig. 6).
During the subsequent 30 minutes, ATP falls progressively to approximately 50% of baseline values during severe (10% of control) coronary flow limitation and to 66% during moderate (20-70% of control) flow reduction [72]. When flow reduction is continued over 5 hours, a further decrease in ATP content to 6% of control in severe and to 52% in moderate ischemia follows. Also, repetitive short (3 minute) occlusions over 3 hours induce a 50% drop in ATP content of the ischemic tissue, but only during the first 20 episodes [73].

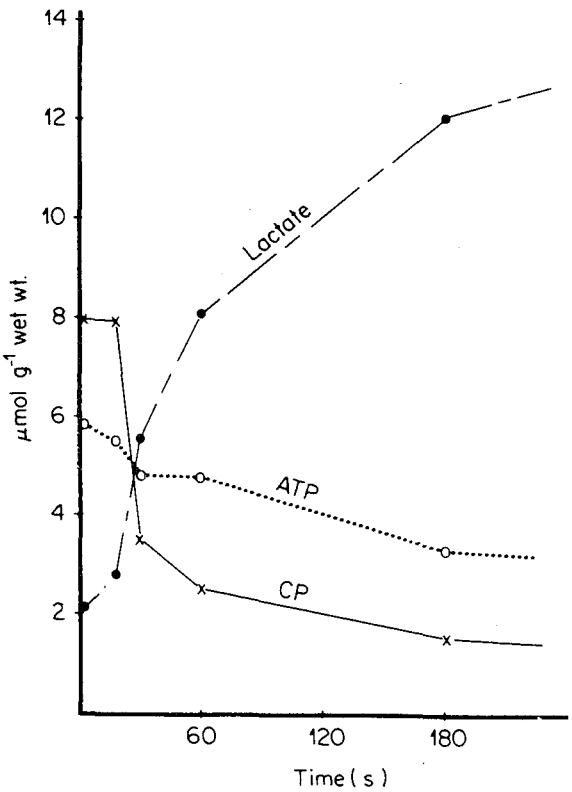


*Fig. 6: Immediate changes of intracellular high energy phosphate and lactate content after complete coronary occlusion in dogs. During the first 15 sec there is little change. However, a marked increase in lactate and decrease in CrP occurs during the following 15 sec, indicating both the appearance of anaerobic glycolysis and the immediate effect of depression of aerobic metabolism on high energy phosphate metabolism. Note that ATP content decreases relatively little during the first 3 min (from reference 71, with permission).*

Simultaneous with the decrease in ATP, an early rise in AMP levels is observed. As resynthesis to ADP is not possible in ischemia, AMP is either dephosphorylated to adenosine or deaminated to inosine monophosphate (IMP) (Fig. 7).
As the latter reaction will be inhibited by the concomitant rise in $P_i$ levels, this results in an increase in adenosine during ischemia. Changes in the myocardial purine nucleoside content have long been known to occur in anoxia and ischemia [74,75] with elevations in adenosine levels of 5-6 fold after only 15 seconds of coronary occlusion [76].

In well-oxygenated hearts, a substantial portion of adenosine is formed intracellularly by hydrolysis of S-adenosylhomocysteine [77] and reincorporated in the adenine nucleotide pool by adenosine kinase. Only a small portion is washed out by the coronary circulation.
During ischemia, adenosine is predominantly formed by dephosphorylation. In contrast, in well oxygenated hearts a substantial portion is derived from the transmethylation pathway [77]. Adenosine is the first ATP catabolite, able to pass the intact cell membrane into the interstitial space. Subsequent breakdown to inosine is under the influence of adenosine deaminase, an enzyme predominantly located in endothelial cells of blood vessels in the heart and adjacent pericytes, but not in myocytes or fibroblasts [78].
After cellular entry, adenosine, at low concentrations, is preferentially phosphorylated. At higher concentrations, above the phosphorylation potential of the endothelium, adenosine is broken down via inosine and hypoxanthine/xanthine to uric acid by the sequential actions of adenosine deaminase, nucleoside phosphorylase and xanthine oxydase (Fig. 7). Theoretically, hypoxanthine may be reincorporated into the myocytes in the form of inosine monophosphate through the action of hypoxanthine phosphoribosyltransferase. Thus, regeneration of adenine triphosphates is possible, but only with low efficacy [79,80].
Adenosine modulates a variety of physiologic effects in different tissues. Through coupling to adenosine-1 receptors it induces negative chronotropic, dromotropic and inotropic effects [81-84], whereas binding to adenosine-2 receptors results in its well-known vasodilator properties [85-87].
In addition, adenosine induces anti-adrenergic effects on the His-Purkinje system [88], reduces impulse formation in the sinus node [89] and inhibits high-affinity beta-adrenergic agonist

binding in myocardial membranes [83]. Moreover, it modulates platelet aggregation and inhibits superoxyde anion generation by neutrophils, thus attenuating neutrophil-mediated endothelial injury [90].

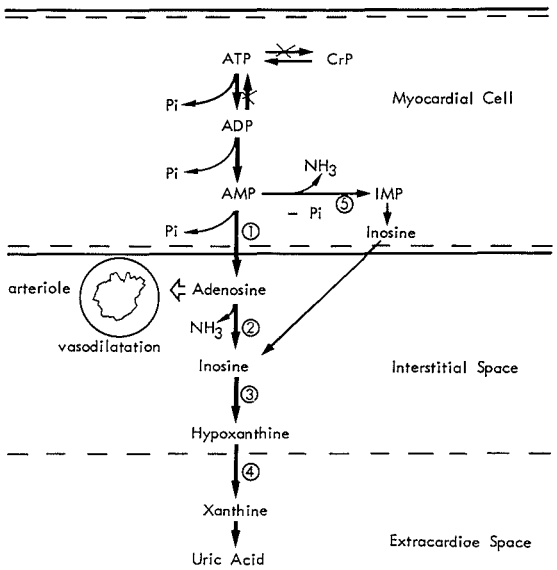


*Fig. 7: Adenine nucleotide breakdown during myocardial ischemia. In the absence of oxydative phosphorylation resynthesis of ATP from ADP is inhibited which results in the accumulation of AMP and $P_i$. AMP can either be dephosphorylated to adenosine or deaminated to IMP. The latter reaction, however, will be inhibited by the accumulating $P_i$, resulting in an increase of adenosine which is the first ATP catabolyte capable to pass the cell membrane into the interstitial space. It then may combine with specific adenosine receptors on the arterioles and induces vasodilatation. Adenosine is easily and quickly deaminated to inosine and is only found in very small amounts in the venous effluent. Its breakdown products inosine and especially hypoxanthine can be detected more easily and may be used as biochemical markers of myocardial ischemia.*
*1. 5'-nucleotidase*
*2. adenosine deaminase*
*3. nucleoside phosphorylase*
*4. xanthine oxidase*
*5. adenylic acid deaminase*
*(From reference 11 with permission).*

Finally, exogenous adenosine, administered intracoronary in humans, may provoke angina-like pain, which diminishes after aminophyllin, suggesting that purinergic receptor stimulation by adenosine may be responsible, at least in part, for ischemia-induced anginal pain [91].
Thus, adenosine may have a multiple role in cardiovascular functions, apart from its central role as metabolic regulator of (coronary) flow. For instance, one may speculate on the effect of adenosine during early ischemia on myocardial contractility. However, from a diagnostic point of view, its importance in ischemic heart disease relates predominantly to the fact, that, after sarcolemmal passage, it is easily and quickly deaminated to inosine.
Although the half-life of adenosine varies in different species from 3 minutes in the dog to 10 seconds in man [92], it is sufficiently short to prevent adequate detection of adenosine in the venous effluent during anoxia or ischemia [93,94], despite the accumulation of appreciable intracellular amounts [95]. In contrast, its immediate breakdown, inosine and hypoxanthine, are detectable.
Following preliminary data [96] in guinea pigs and in cats, which indicated that other nucleosides than adenosine could be useful as markers of ischemia, we embarked on a series of studies, investigating the sensitivity of these metabolites as indices of ischemia. A porcine model was used, as this better reflects the human situation than the commonly used canine, feline and rodent models of ischemia. In these studies, inosine and hypoxanthine were compared with other metabolic and with hemodynamic parameters. Next, these investigations were extended to patients with coronary artery disease. The results of these studies are presented in chapter VI.2. Moreover, the temporal relation of changes in myocardial nucleoside (hypoxanthine) release with alterations in regional myocardial perfusion, changes in different metabolites as well as electrocardiographic and hemodynamic markers of ischemia, are given in chapter VII.

**Effect of myocardial ischemia on other metabolites.**

*Inorganic phosphate.*

As a byproduct of adenine nucleotide breakdown during ischemia, inorganic phosphate accumulation and subsequent release from the ischemic area has received appropriate attention.
We and others have clearly shown that in the animal experiment, a significant rise in inorganic phosphate is found early after ischemia is induced [97-100], at least when determined from the direct effluent of the ischemic area. In fact, it has been suggested, that changes in inorganic phosphate are more pronounced than those of lactate and electrolytes, such as potassium [101]. However, in man, ischemia-induced changes are small when measured in the coronary sinus [102,103], which suggests that this parameter is of limited importance in the clinical assessment of ischemia. Nevertheless, we have attempted to relate ischemia-induced alterations in inorganic phosphate with simultaneous changes in nucleosides and lactate in patients with coronary artery disease, with and without angina, during pacing-induced stress, results of which are presented in chapter IV.2.

*Blood pH, $pCO_2$ and $pO_2$.*

Myocardial ischemia results in a significant reduction in pH and in an increase in $pCO_2$, presumably secondary to enhanced hydrogen ion formation during activated anaerobic glycolysis [57,104]. Similar to the observations with inorganic phosphate, changes are readily detected in the local venous effluent. However, detection in coronary sinus blood depends on the severity of ischemia.
In the studies by Opie et al., neither coronary sinus pH nor $pCO_2$ changed when ischemia was moderate, in contrast to significant alterations during severe ischemia [57].
For similar reasons we have never been able to detect any such changes with coronary sinus sampling during short periods of ischemia in man (see chapter IV and personal communications).

*Potassium.*

Electrolyte changes during ischemia are likely to occur as a result of functional and conformational membranal alterations as a result of ischemia. Clearly the most important are alterations in potassium fluxes. Myocardial potassium loss during ischemia has been known for a long time and attention has focussed primarily on its relation to ventricular arrhythmias in this setting [105-110]. In animal experiments with direct access to the venous effluent from the ischemic area, an immediate and sharp rise in potassium efflux is found [51,111], persisting throughout the ischemic period [111]. Again, in humans, the situation is far less clear.
Although early studies did claim excessive potassium loss during pacing-induced ischemia, an equally significant, albeit smaller egress of potassium from the heart has also been observed in patients, who did not become ischemic during the test [112]. As the latter observation suggested, that potassium loss during pacing was not specific for ischemia but rather dependent on the increase in heart rate per se, we compared changes in this electrolyte to more specific metabolic parameters of ischemia, applying an identical form of stress testing. In these studies (see chapter IV.2.) significant potassium loss from the heart in anginal patients was observed, however, at an early stage, before the development of angina.

*Ketone bodies.*

Ketone bodies, e.g. beta-hydroxybutyrate and acetoacetate, formed in the liver, have been proposed as an alternative substrate for oxydative metabolism. It is unlikely, however, that they are significant in this respect. Theoretically of more importance is the fact, that the ketone bodies may form a redox couple, which is in equilibrium with the mitochondrial $NADH/NAD^+$ ratio. Acute ischemia may manifest itself by a rise in the ratio beta-hydroxybutyrate/acetoacetate in the coronary venous effluent and a diminished cardiac uptake of beta-hydroxybutyrate. Unfortunately, ketone body concentrations in the heart are very low and, although small changes in the latter ratio can be found during ischemia [57,113], they are presumably of limited value as metabolic marker of ischemia.

*Glucose.*

Glucose uptake by the hypoxic heart increases as oxydative phosphorylation slows down. Thus, the degree of extraction may serve to indicate presence and degree of myocardial ischemia. However, different stimuli, such as stress, work, neurohormones and insulin, may similarly affect glucose uptake. Also, utilization of endogenous glucagon stores during the early phase of ischemia will affect glucose uptake. Finally, the rate of exogenous glucose is primarily determined by the

amount of arterial blood eventually reaching the ischemic area.

For these reasons, the identification of glucose metabolism in the heart has been of limited clinical value (see chapter IV), although the concept is still in use in non-invasive radionuclide techniques (chapter III).

*Amino acids.*

The main amino acids, transported in and out of the heart, are alanine, aspartate, glutamic acid and glutamine. Of these, alanine and glutamine are released whereas glutamate and aspartate are taken up. Alanine formation in the heart results from transamination of pyruvate [114]. As glutamate is the main amino acid for transamination in this process, a correlation between glutamate uptake and alanine release is present [115]. Interference with the glycolytic pathway may reverse this uptake-release pattern [114].

Alternatively, hypoxia and ischemia enhance alanine release from the heart and, concomitantly, glutamate uptake [114,116-118]. Hence, these amino acid fluxes may theoretically be useful as indices of ischemia.
The clinical significance of amino acid changes during ischemia are several. First, by shunting pyruvate away from lactate production to alanine formation, intracellular acidosis is reduced. Secondly, alanine and glutamate may be instrumental in binding and subsequent removal of excess ammonia, which accumulates during myocardial ischemia [7,119]. Finally, through the malate-aspartate cycle, amino acids allow reducing equivalents to be transported into the mitochondrion. These properties suggest, that the amino acids referred to above, may result in a reduction of the effect of ischemia. In vitro and in vivo studies have indeed suggested significant protective properties of glutamate against hypoxic damage [120-122].

*Citrate.*

Citrate, as already mentioned, is one of the key control elements of the enzyme phosphofructokinase and, hence, of glycolytic flux [123]. Exchange between the mitochondrial and cytosolic compartments is practically non-existent as a result of the low permeability of the mitochondrial membrane for citrate [124,125]. Instead, in different organs, such as the liver [126,127], citrate is formed in the cytosol from glutamate by the action of the cytosolic enzymes alanine aminotransferase, isocitrate dehydrogenase and aconitase. During ischemia, enhanced glutamate uptake and alanine release from the heart may be accompanied by citrate efflux. Thus, in combination with amino acid exchange, citrate may serve as an additional marker of myocardial ischemia [115,128,129].

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# Chapter II
# CORONARY FLOW.

# II.1. Regulation of coronary blood flow.

Regulation of vascular control is one of the areas in medicine, which has seen quite significant conceptual changes during the last decade.
Besides their conduit function, blood vessels are now known to be active synthetic and secretory organs, encorporating a number of intracrine, autocrine and paracrine systems, involved in the autoregulation of vessel size and function. In this respect, quite a variety of vascular growth promoting and inhibiting factors have been discovered, apart from novel vasoconstrictor and -dilator substances.
Of particular interest, several of these factors share properties relating to growth and vasotone. Thus, endothelin, a strong vasoconstrictor [1], has mitogenic activities [2], whereas the vasoconstrictor peptide angiotensin II not only promotes [3,4], but also inhibits vascular smooth cell growth [5]. Also, serotonin may stimulate smooth muscle cell mitogenesis [6]. In contrast, pure vasodilators, such as bradykinin and certain prostaglandins, have minimal mitogenic effects, although they stimulate protooncogene expression in fibroblasts to a similar degree as observed with typical growth factors, such as platelet-derived growth factors [7].

Besides vessel growth regulation, of which our knowledge is still in its infancy, regulation of vascular tone has seen considerable conceptual changes during the past decade.
Thus, the concept of endothelium-dependent vasodilatation, not appreciated until Furchgott and Zawadki's description of the obligatory role of intact endothelial cells in vasorelaxation induced by acetylcholine [8], has rapidly claimed its (important) place in the multitude of factors involved in the regulation of coronary flow.
Has it changed our thinking of how coronary flow is regulated? To a certain extent the answer is affirmative.
The concept of alterations in vasotone as a result of abnormal endothelial function in diseased coronary artery segments, has added to the picture of coronary vascular tone being regulated by metabolic, myogenic, autonomic and hemodynamic factors, such as we had before endothelial regulation of flow became known.
Now, a more complex but certainly more exiting pattern emerges, where not only the original hypotheses are better understood but also the interaction between endothelium, vascular smooth muscle cell and extravascular factors, implicated in vascular control, is gradually recognized.

**The coronary artery system.**

The coronary arterial system can be subdivided in the large epicardial or conduit vessels, from which smaller arteries branch off to penetrate the myocardial wall at an approximate 90% angle and eventually form the arterioles and capillaries. Resistance to coronary flow is determined mainly by the arterioles (the resistance vessels) which, under maximal pharmacological vasodilatation, have the capacity to increase coronary blood flow by a factor 4 to 5.
Normally, in the epicardial arteries, the conduit vessel resistance to flow is relatively low. In contrast, in the smaller intramyocardial vessels, an additional resistance to flow is created through the compressive forces of the tension developed in the ventricular wall.
This will be particularly noticeable in the subendocardial region, where wall tension is highest (Fig. 1). As a result, subendocardial resistance vessels are already dilated to some degree in the normal, non-ischemic situation. This merely ensures sufficient blood flow to the subendocardial cells, in which, due to the higher loading conditions, myocardial oxygen consumption is always greater than in the subepicardial cells. Consequently, during progressive coronary artery narrowing and the subsequent increase in conduit vessel resistance, the potential to vasodilate will be more readily exhausted in the subendocardial than in the subepicardial area.
Thus, when no other mechanisms interfere, myocardial ischemia, resulting from progressive epicardial coronary artery narrowing, will begin in the subendocardium, only to progress in an epicardial and lateral direction during more severe and more prolonged episodes of coronary flow reduction.

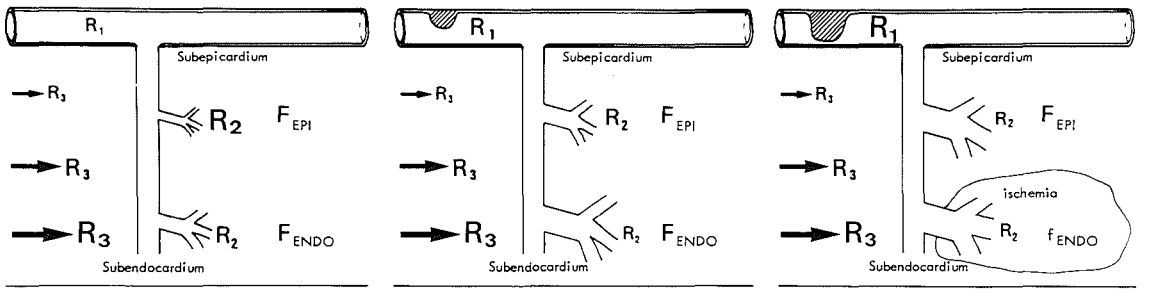


*Fig 1: Schematic representation of the various resistances in the coronary arterial bed and their effect on flow (F) in a normal coronary artery (fig. 1a) and in the situation of a moderate and severe stenosis (fig. 1b and fig. 1c, resp.). Due to higher wall tension and compressive forces (R3) in the subendocardial region, vasodilatation with decreased arteriolar resistance (R2) is already present in the normal situation (fig. 1a). During progressive coronary stenosis and hence increase in conductance vessel resistance (R1), a compensating vasodilatation and a decrease in arteriolar resistance is found, which in moderate lesions results in unchanged regional flow (fig. 1b). However, with a severe stenosis subendocardial arterioles eventually cannot further dilate and local coronary flow will diminish resulting in ischemia, even at rest (fig. 1c). (From reference 9 with permission).*

## Regulation of coronary flow.

With equal myocardial performance, coronary blood flow will remain constant in spite of varying perfusion pressures. The continuous adjustment of coronary resistance and flow to meet the instantaneous oxygen need of the myocardium, exists strictly on the basis of local mechanisms, which are mainly metabolic. This process is known as autoregulation [10,11] and occurs on a beat to beat basis at the microvascular level [12].
Besides metabolic control, myogenic regulation is also present. This old concept of vasoconstriction in response to increasing intraluminal pressures was recently investigated in subepicardial versus subendocardial arterioles of 80-100 micron [13]. This in vitro study clearly confirmed, that coronary arterial myogenic activity indeed exists and is capable to contribute to autoregulation. Furthermore, this study showed that myogenic control is more prominent in subepicardial than in subendocardial arterioles at all levels of perfusion pressure.
Under control conditions, 40-45% of coronary vascular resistance resides in small arterioles, which are 100 micron in diameter or less [14,15]. An additional 7-10% of vascular resistance is derived from the venous side. As a result, vessels over 100 micron in diameter are responsible for the remaining resistance under control conditions.
However, microvessels respond heterogeneously to changes in perfusion pressure with vasodilatation in small (<100 micron) vessels, as opposed to either no alterations or vasoconstriction in larger size microvessels during progressive reduction in perfusion pressure [16].
Of the metabolites involved in autoregulation, adenosine is one of the most important and instantaneous regulators of coronary flow. Through binding with $A_2$ receptors on the perivascular myocytes, it directly influences arterial tone [17]. This provides for an immediate metabolic link between energy production and oxygen delivery.

In order to explain work-induced vasodilatation, it has been hypothesized, that relative decreases in $pO_2$ are sensed by chemoreceptors on specific pericyte type cells, which then may increase local activity of the enzyme 5-nucleotidase and thus stimulate adenosine production [18]. Other metabolic factors, which influence coronary vascular tone without direct autoregulatory effects are pH, $pCO_2$ and osmolarity changes.
Although a direct autoregulatory effect of pH or $pCO_2$ is unlikely on quantitative grounds, e.g. non-physiological, large changes are needed to adapt coronary flow to instantaneous $O_2$ demand, changes in pH and $pCO_2$ presumably modulate the sensitivity of the autoregulatory (adenosine?) receptors. In general, metabolic acidosis will enhance coronary blood flow while, on the other hand, alkalosis induces vasoconstriction, accompanied by a (small) decrease in flow.

**Endothelium-dependent control of vasotone. A novel concept in autoregulation?**

*Endothelium-derived relaxing factor(s).*

Since the introduction of the concept of endothelium-dependent vasodilatation in 1980 [8], it has been demonstrated that the endothelium produces several potent vasodilator as well as vasoconstrictor substances. Of these, the endothelium-derived relaxing factor (EDRF), a labile humoral substance formed in the endothelium from L-arginine [19-21], is one of the more potent, albeit short-lived vasodilator agents in nature. EDRF is presumably identical to nitrous oxyde (NO) [22-25]. However, recent data on different responsiveness patterns of basal EDRF compared with EDRF, which is released by acetylcholine or compared with NO in proximal versus distal dog coronary arteries, have suggested that basal EDRF may not be analogous to NO and that more than one form of endothelium-dependent relaxing factors are present [26].
Various stimuli, mechanical, humoral or platelet-derived of nature, accelerate basal EDRF production or NO (Fig. 2). This then diffuses to the vascular smooth muscle cell to cause vasorelaxation through increased production of soluble guanylate cyclase [27,28], subsequently elevating cyclic guanosine 3,5' monophosphate (cGMP) and activating cGMP-dependent protein kinase [29].
It has been suggested, that EDRF, through stimulation of cGMP, inhibits (norepinephrine-induced) inositol triphosphate formation and hence modulates transsarcolemmal and intracellular calcium fluxes [30]. This effect is not specific, but is also observed with sodium nitroprusside and ANF in de-endothelialized vessels. In addition, and just as likely not a specific effect, EDRF, through cGMP-production, may inhibit norepinephrine release from adrenergic nerve endings [31]. As such, the endothelium can act as an endogenous inhibitor of sympathetic neurotransmitter release. The phenomenon of flow-mediated vasodilatation has been known for some time. It had already been observed in 1970, that this was indeed dependent upon flow and not upon pressure. Also, it was demonstrated, that neurogenic stimuli could not account for the observation [32].

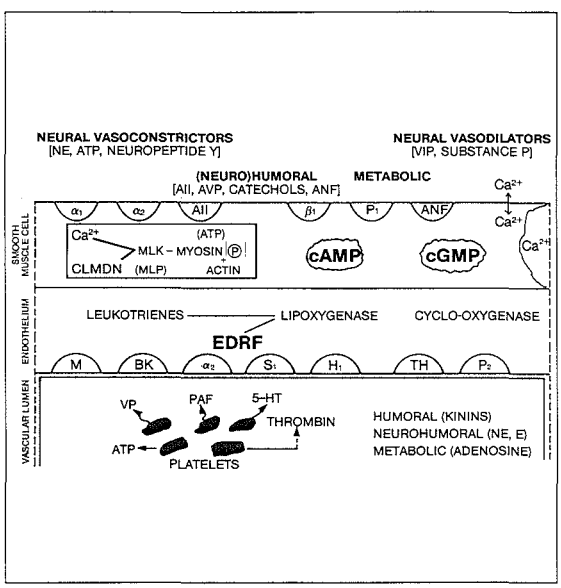


*Fig. 2: Schematic representation of different factors involved in control of vascular tone. A large number of circulating or platelet-derived factors may act on the endothelium through endothelial receptors. Several will be involved in formation of endothelial-derived relaxing factors (EDRF-1 and -2), which induce vasodilation through increased production of soluble guanylate cyclase, subsequent elevation of cGMP and interaction with transsarcolemmal and intracellular calcium fluxes. In addition, different vasodilator - and constrictor systems are indicated, acting on the endothelium or on the smooth muscle cell. By necessity, the figure is not meant to demonstrate all, vasodilating - or constricting presently known mechanisms (for this the reader is referred to the text in this chapter), but solely to indicate the multitude of factors, involved in vascular control. Even so, recent concepts, such as the endothelins, are not mentioned.*

Recently, flow-mediated vasodilatation was shown to be dependent on an intact endothelium [33-36], suggesting that increased blood flow and shear stress on endothelial cells result in enhanced basal release of EDRF.
Alternatively, transmission of a hyperpolarizing current from the endothelium to the vascular smooth muscle cell, through activation of specific ionic (potassium) channels following mechanical deformation of the endothelial membrane, has been proposed to explain flow-dependent vasodilatation [37,38]. Recent data from Cooke and coworkers clearly indicate, that flow activates a potassium channel (possibly the calcium-activated

potassium channel) on the endothelial cell membrane, which leads to the release of the endothelium-derived relaxing factor [39]. The latter observations did not exclude participation of the hyperpolarizing current or different endothelium-derived factors, e.g. the endothelium-derived hyperpolarizing factor [40,41]. However, as ouabain did not block the flow response, an additional effect of the hyperpolarizing factor, if present, must be limited. In contrast, specific EDRF blockers such as L-NMMA, abolished vasodilatation, unmasking an underlying vasoconstrictor tone.

Besides flow, a large number of exogenous substances also induce vasorelaxation by enhancing EDRF production, such as acetylcholine, adrenaline/noradrenaline, ADP, thrombin, vasopressin, substance P, leukotriene $D_4$, bradykinin and serotonin [42].
Recent observations suggest, that acetylcholine may trigger the release of two chemically different relaxing factors through stimulation of muscarinic-1 and -2 receptors, at least in canine arteries [43]. Stimulation of endothelial muscarinic-1 receptors induces EDRF (NO) release, whereas activation of the muscarinic-2 receptor results in the formation of the endothelium-derived hyperpolarizing factor (or EDRF-2). To complicate matters further, a recent report suggested, that superoxide-dependent relaxing factor(s) are released from the endothelium [44] and may induce vasodilatation by a different mechanism, other than obtained by EDRF stimulation.
Endothelium-dependent vasodilatation is not confined to the large conduit arteries [45]. It is also observed in resistance vessels following the administration of several (although not all) of the stimuli, which induce vasodilatation in conduit vessels [46]. However, the response to humoral agents in arterioles may not be similar to that observed in large arteries. For instance, serotonin appears to have differential effects at different levels of the coronary artery system, constricting larger coronary arteries (>90 micron), while smaller arterioles dilate when exposed to 5-hydroxytryptamine [46]. These contrasting actions of the same compound on the various levels of one arterial system may be explained by differential receptor populations. More likely, however, it is an expression of autoregulation with secondary endothelium-dependent dilation in down-stream microvessels, which compensates for upstream vasoconstriction following activation of 5-$HT_2$ receptors in the larger arteries. Also, endothelium-dependent relaxations may vary with the type of vessel and drug dosages used [47].
The observation, that basal EDRF production is under the influence of shear stress and is mediated by changes in ionic currents through potassium channels, may alter our concept of autoregulation to the extent, that different forms of autoregulation, not only metabolic or myogenic, but also hemodynamic of nature, may be operative side by side on the microvasculature.

**Prostanoids.**

In the endothelial cell, arachidonic acid, released from membrane phospholipids by the enzyme phospholipase $A_2$, is converted via the cyclooxygenase pathway and endoperoxyde formation into prostaglandin $H_2$ [48].
Subsequently, prostacyclin is formed, a potent vasodilator that enhances vascular cAMP production [49,50], or its counterpart, the potent vasoconstrictor thromboxane $A_2$, as well as other prostaglandins ($PGE_2$, $PGF_{2\text{-}\alpha}$, $PGD_2$). Whereas prostacyclin is the major product of the cyclooxygenase pathway of the vascular wall, thromboxane is predominantly formed in platelets. Other prostanoids, which are also formed in the endothelium, although in small amounts, such as prostaglandin $F_2$-$\alpha$ and prostaglandin $E_2$ , may result in vasoconstrictory or dilatory responses depending on the experimental conditions used [51].

Phospholipase $A_2$ activation is observed after mechanical or chemical actions on the endothelial membrane. This may explain, to a certain extent, why prostacyclin release is flow-dependent and why pulsatile flow results in a significantly greater prostacyclin production than constant flow. Prostacyclin release is also triggered by hypoxia-induced calcium influx in the endothelial cell [52]. Besides, several of the substances that induce EDRF release, such as acetylcholine, histamine or thrombin, may also release prostacyclin [53]. Interestingly, angiotensin II stimulates prostacyclin production in the canine renal, but not in the femoral artery [54].
Despite the high level of prostacyclin synthetase in the intima and the potential to synthesize the prostanoid after various stimuli, the role of prostacyclin as an endogenous vasodilator is not quite clear. Inhibition of this autocoid does not result in substantial effects on blood flow in many organs [48], in contrast to marked effects after inhibition of nitrous oxyde synthesis [55,56].

**Endothelium-dependent vasoconstriction.**

Until recently, considerably less attention has been paid to endothelial-related vasoconstriction than to vasodilatation. Although several stimuli, including arachidonic acid, have been demonstrated to induce endothelium-dependent contractions, presumably mediated by the release of eicosanoids, these could not account for all conditions under which vasoconstriction took place[57,58].

During recent years, attention has focussed on a family of peptides, endothelins, 25 amino acids long, with strong vasoconstrictor properties and produced in normal functioning vascular endothelium. Endothelin induces long lasting vasoconstrictor effects of relatively slow onset in most vascular beds[59]. Its mechanism of action may involve acceleration of intracellular calcium fluxes, a conclusion predominantly based on the inhibitory actions of several calcium antagonists[60-62]. However, high-affinity binding sites for endothelin-1, the endothelin derived from human endothelial cells, are not affected by calcium antagonists. Thus, although the level of extracellular calcium may modulate contractions by endothelin, a distinct functional pathway, which involves phospholipase C-mediated phosphoinositide breakdown has been proposed. This results in intracellular mobilization of $Ca^{2+}$ and activation of protein kinase C, with subsequent inositide triphosphate and diacylglycerol formation[63-65]. These intracellular actions are directly related to other biological effects of endothelin, such as an impairment of myocardial relaxation[66] or the induction of vascular (and myocardial?) growth[67-69].

Whether endothelin is involved in acute autoregulation is unknown. Its long lasting effect and relatively slow onset of action would argue against this. On the other hand, endothelin production, like nitrous oxyde, is regulated by shear stress[70].

Most likely, the peptide institutes a continuous baseline vasoconstrictor tone, easily overridden by dilator mechanisms, such as prostacyclin and nitrous oxyde.
Under pathological conditions, where endothelial function is compromised, the vasoconstrictor actions of endothelin may, however, take the overhand.

**Non-endothelium-mediated regulator mechanisms of coronary flow.**

*Vascular smooth muscle cell receptor modulation of vascular tone.*

The vascular smooth muscle cell (VSMC) ultimately controls its own contractile state and, hence, vasotone. Vascular tone is regulated by the cytosolic $Ca^{2+}$ level, by the interaction of calcium with other second and third messenger systems (calmodulin) and, subsequently, by myosin light-chain phosphorylation, which enables the interaction of the contractile elements and, hence, contraction.
Important regulators, besides calcium, are the G-proteins, the inositol-phosphatidyl pathway and Na/H antiporter systems. The signals for vasoregulation either induce direct intracellular actions in the VSMC or act through one of the numerous receptors on the cell membrane. Examples of direct stimuli are NO and prostacyclin from the endothelium. Also, various agents may act directly on intracellular guanylate cyclase (nitrates and sodium nitroprusside) or on adenylate cyclase (forskolin and phosphodiesterase inhibitors) to induce vasodilatation (Fig. 3).

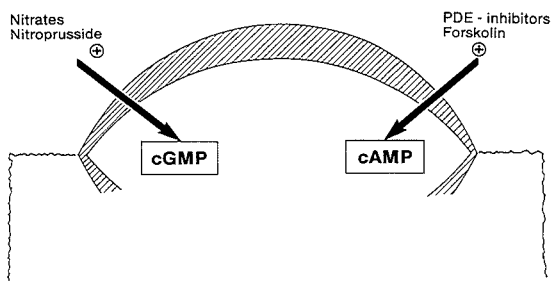


*Fig. 3: Endothelium-independent vasodilator mechanisms. Several agents may act directly on cAMP and cGMP, thereby inducing vasodilatation (see the text of this chapter). (From reference 71 with permission).*

However, the majority of stimuli for vasodilatation or vasoconstriction act through receptor coupling.
For instance, binding of endothelin-1 to high-affinity receptors, as a first step to vasoconstriction, has already been mentioned. Several compounds, which induce endothelium-dependent vasodilatation, may actually behave as vasoconstrictors through direct interference with specific receptors on the VSMC, when the endothelium is absent or is functionally impaired to such an

extent, that protection against these actions is not possible anymore[72] (Fig. 4).
An example here is acetylcholine, which will directly induce vasoconstriction through its $M_2$ receptor on the vascular smooth muscle cell membrane, either by reducing adenylate cyclase activity or by enhanced turnover of the phosphoinositide cycle.

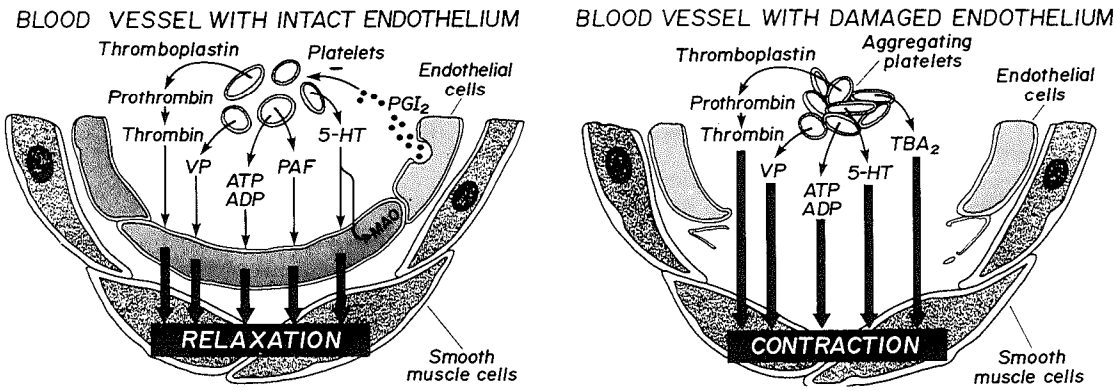


*Fig. 4: Schematic representation of the role of the endothelium to protect against vasoconstriction by several products of aggregating platelets, such as vasopressin (VP), serotonin (5-HT), thromboxane ($TBA_2$), platelet-activity factor (PAF), adenine nucleotides (ATP, ADP) and thrombin. The intact endothelium (left figure) provides the vasodilator prostacyclin ($PGI_2$), degrades serotonin and, through activation of specific endothelial receptors by most other products, forms vasodilating substances. Also, it prevents platelet aggregation ($PGI_2$, EDRF). In contrast, when the endothelium is damaged (right figure), platelets aggregate and several derived substances may induce vasoconstriction, depending on the blood vessel. (From reference 72 with permission).*

In contrast, when the endothelium is intact, acetylcholine releases EDRF with subsequent vasodilatation. Receptor-linked vasodilatation may result from inhibition of the postsynaptic alpha-1 receptor with prazosin, whereas calcium antagonists attenuate postsynaptic alpha-2 receptor-mediated vasoconstriction[73] (Fig. 5).

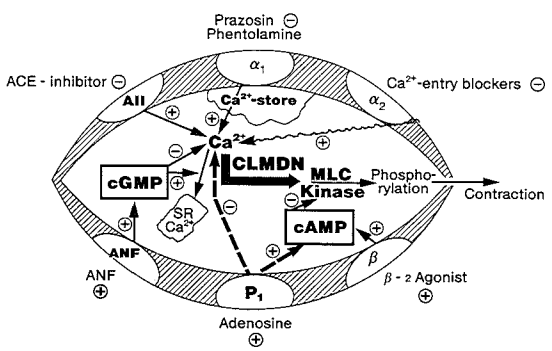


*Fig. 5: Vascular smooth muscle cell receptor-linked vasoregulation. Activation of specific receptors by several stimuli interfere with the contractile apparatus through modulation of adenylate- and guanylate-cyclase, G-proteins, the inositol-phosphatidyl pathway, $Ca^{2+}$ and Na/H antiporter systems (see text on page 38).*
*AII = angiotensin receptor; ANF = atrial natriuretic peptide; CLMDN = calmodulin; MLC = myosin light-chain; $P_1$ = purinergic receptor; SR = sarcoplasmatic reticulum. (From reference 71 with permission).*

Vascular angiotensin II receptor[74] blockade may have similar effects on intracellular calcium fluxes, reducing vascular tone.
Alternatively, receptor-dependent vasodilatation may be achieved by beta-2 stimulation and subsequent cAMP production, which inhibits the activation of myosin light-chain kinase by calcium and calmodulin.
Likewise, purinergic (adenosine $A_1$) stimulation promotes cAMP production and, besides, impedes $Ca^{2+}$ entry into the cell.
Atrial natriuretic peptides, after activation of their specific receptor(s)[75], result in an increase in, particularly, (membrane-bound) cGMP[76], which presumably has a dual effect on $Ca^{2+}$ kinetics, restricting $Ca^{2+}$ entry into the cell and promoting its sequestration from the cytosol into the sarcoplasmatic reticulum; effects, which are also achieved through cAMP activation[77].
Both in the heart and the arterial system, calcitonin gene-related peptide binding sites have been identified. CGRP binding may stimulate adenylate cyclase[78,79]. Both peptides I and II, present in the peripheral nervous system in heart and blood vessels, induce vasodilatation, apart from positive inotropic and chronotropic effects[80].
Moreover, activation of specific dopamine-1 receptors will result in vasodilatation in various arterial systems, including coronary vessels[81].

**Presynaptic modulation of vasotone.**

An additional, important but indirect way to induce receptor-dependent vasodilatation is through presynaptic modulation of neurotransmitter release (Fig. 6).
Besides its direct effect on postsynaptic adrenergic receptors, norepinephrine, when released into the synaptic cleft, also stimulates the presynaptic alpha-2 receptor, thereby inhibiting further norepinephrine release.
In addition, stimulation of other presynaptic receptors, such as the serotonergic, histaminic ($H_2$), purinergic ($A_1$), dopaminergic ($DA_2$) and muscarinic receptors, also results in diminished norepinephrine release and vasodilatation.
Conversely, presynaptic beta-adrenergic and angiotensin II receptor activation leads to enhanced neurotransmitter release and vasoconstriction.
Thus far, alpha-adrenergic vasoconstrictor mechanisms have been mentioned in particular. However, it should be realized, that from the same nerve endings, neuropeptide Y and ATP are coreleased with norepinephrine[82,83] and may in turn affect vasotone.
Besides, ATP is also released from sensory motor nerves and from non-sympathetic purinergic nerves. Depending on which subtype of the purinergic-2 receptor is activated it may induce opposing effects on vessel tone[80]. Moreover, adenine nucleotides are instrumental in eliciting EDRF formation via endothelial $P_2$ receptor stimulation, which suggests a complex mode of action of the purine nucleotides on overall flow regulation. However, the relative importance of these various effects of ATP on coronary flow is as yet speculative.
Neuropeptide Y, although predominantly a neurotransmitter, which modulates noradrenaline release, may by itself induce coronary vasoconstriction[85], either directly or by facilitating alpha-adrenergic vasocontriction[86].

**Adrenergic control of coronary flow.**

Whether an alpha-adrenergic vasoconstrictor tone is present under absolute resting conditions, is still a matter of debate[87-90].
Studies in cardiac transplant patients suggest, that resting coronary resistance is less than in normally innervated individuals[91], at least when overall

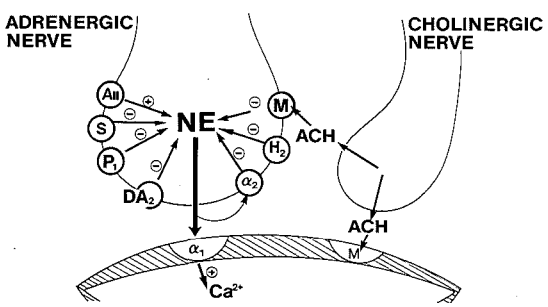


*Fig. 6: Presynaptic modulation of neurotransmitter (norepinephrine: NE) release and indirect receptor-mediated vasodilatation. AII = angiotensin; ACH = acetylcholine; $DA_2$ = dopaminergic receptor; $H_2$ = histaminic receptor; M = muscarinic receptor; $P_1$ = purinergic receptor; S = serotinergic receptor. (From reference 71 with permission).*

coronary sinus flow (measured by thermodilution) and resistance is compared between transplant patients and normal individuals. It has been suggested, that a constant degree of neurally-induced vasoconstriction exists under normal conditions, continuously reflex- modulated and limiting metabolic coronary dilatation by 30%[92,93].

Vasoconstriction follows alpha-adrenergic stimulation and is opposed, to a certain extent, by beta-adrenergically-induced relaxation. The latter is the primary adrenergic response to endogenously released norepinephrine[94]. Although early studies have suggested, that adrenergically-induced changes in resistance are low (30-40%) compared with metabolically-induced alterations, which can be 5-6 fold from baseline, later investigations have indicated, that norepinephrine administration increases coronary resistance and is able to compete with metabolic vasodilatation[95-97]. Also, in conscious dogs on beta-blockade, alpha-adrenergic coronary vasoconstriction limits exercise-induced metabolic coronary dilatation and the subsequent increase in coronary flow[98].

This alpha-adrenergic effect on vascular tone during exercise is exerted predominantly by circulating catecholamines and not by endogenous amine release from cardiac sympathetic nerves.

Both alpha-1 and -2 adrenoceptors are present in the coronary system, including that of humans[99-103]. Although the relative distribution of the 2 types may vary in conduit and resistance vessels, both types are present in the large epicardial as well as in the resistance vessels[104,105]. However, as with autoregulation, the response to alpha-adrenergic stimulation varies with vessel size, i.e. constriction of microvessels greater than 150 micron but dilatation of vessels less than 100 micron[104]. Under control conditions, resistance vessels respond equally with vasoconstriction to specific stimuli[103]. In contrast, alpha-adrenergic vasoconstriction opposing exercise-induced vasodilatation is predominantly achieved by alpha-1 stimulation[106]. Whereas during exercise alone, adrenergic vasoconstriction appears transmurally homogeneous[107], during ischemia following coronary flow restriction, alpha-adrenergic vasoconstriction has been claimed to redistribute coronary blood from the epicardium to the endocardium and, hence, may be beneficial in alleviating transmural steal during coronary hypoperfusion[108].

The question arises whether this implies that alpha-adrenergic stimulation is actually beneficial in myocardial ischemia or whether it increases ischemia through enhance vasoconstriction in the post-stenotic coronary vessels.

Also, how do the numerous regulator mechanisms of coronary vasomotor tone behave during ischemia?

# II.2.

# Coronary blood flow regulation and myocardial ischemia.

## The classic concept of coronary insufficiency.

The classic concept of coronary insufficiency is that of a pathophysiological disturbance in coronary perfusion and therefore of oxygen and substrate supply to the myocardium in relation to its instantaneous demand. In most instances, coronary insufficiency results from significant luminal narrowing of a large coronary conduit artery and subsequent inability to increase coronary flow sufficiently to meet instantaneous oxygen demand during exercise or stress. This "stenosis" may be more or less fixed, following atherosclerotic thickening of part of the arterial wall or dynamic, as a result of abnormal, regional vaosconstriction.
Alone, these mechanisms may not appreciably reduce coronary flow. When they are operative in concert, however, as often happens, coronary insufficiency may become sufficient to induce a relative or absolute shortage of oxygen and substrates in the post-stenotic area as well as reduced drainage from it, culminating in the clinical picture of myocardial ischemia.
The vasodilator reserve of the coronary vasculature prevents flow reduction in the presence of moderate coronary artery diameter narrowings of 40-50% or less.
In contrast, a progressive decrease of coronary flow with only minimal or no vasodilator reserve, is observed when the coronary artery diameter is acutely reduced by 85% or more[109]. In the intermediate range, blood supply improves but only to a certain extent, depending on the remaining coronary vasodilatory reserve (Fig. 7).

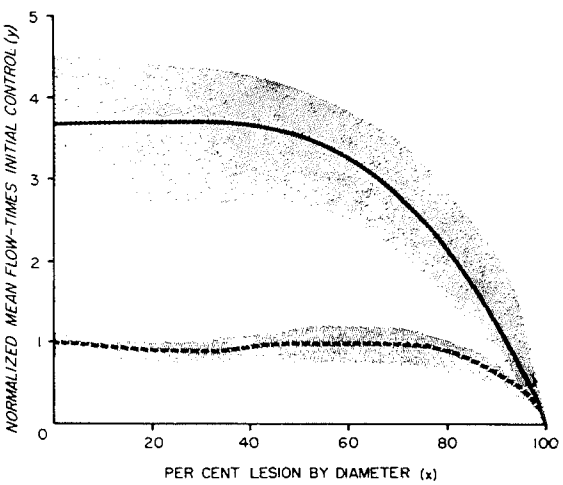


*Fig. 7: Coronary artery flow and vasodilatory reserve. The relation between percentual coronary artery diameter constriction to resting mean flow (- - - - - - - -) and the hyperemic response (————) to intracoronary contrast injections in dogs is shown. Flows are expressed as ratios to control resting mean values at the beginning of each experiment. The shaded area indicates the limits of the relation plotted for individual dogs. (From reference 109 with permission).*

Whether, in the latter situation, coronary flow will be sufficient to prevent ischemia, depends not only on the remaining vasodilator reserve, but also on direct myocardial oxygen demand. Hence, the occurrence of myocardial ischemia becomes critically dependent on the instantaneous supply/demand ratio.
Myocardial oxygen demand is determined to a small extent only by the basic cellular functions needed for cell viability. Whilst the beating (canine) heart consumes 8-15 ml $O_2$/min/100 gr myocardial tissue, only 2 ml is needed in the quiescent, non-beating heart[110].
Wall tension, heart rate and, to a lesser extent, contractility are the major determinants of myocardial oxygen consumption. The occurrence of myocardial ischemia in the event of a critical stenosis will depend on these hemodynamic variables. However, this is often not the only cause and it may not even be the determining

factor, as to the possible occurrence of ischemia.

**Coronary vasoconstriction and spasm.**

The concept of the myocardial supply/demand ratio for the development of ischemia is of less importance when spasm occurs in normal or near normal epicardial arteries. Although the original concept considered vasospasm as a form of increased smooth muscle tone surrounding a critical stenosis[111,112], it has subsequently been shown, that coronary vasospasm may occur on top of any kind of lesion, but also in normal or subnormal coronary arteries[113,114].

Significant coronary vasospasm in patients with normal or near-normal coronary arteries, is presumably of less clinical importance than moderate reductions in artery diameter as a result of coronary vasoconstriction, when superimposed on a fixed stenosis, at least not in terms of incidence.

In recent years we have seen an explosion of evidence for abnormal coronary artery narrowing in lesion vessels, following a multitude of stimuli, including acetylcholine, sympathetic stimulation, exercise and fast atrial pacing[115-119]. Even relatively small luminal reductions, due to increased vasotone, may alter a moderate lesion into a critical stenosis without any coronary flow reserve. Such examples of a 20% reduction in coronary diameter as a result of vasoconstriction, as presented in Fig. 8, are clinically relevant.

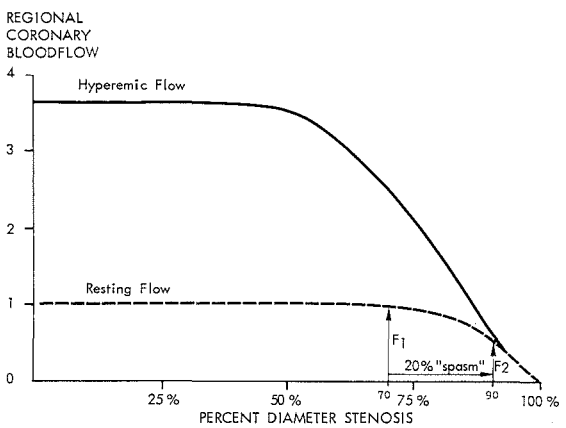


*Fig. 8: Theoretical example of the effect of a relatively small reduction in diameter by increased vasomotor tone on the severity of an underlying coronary artery lesion. A 20% narrowing due to vasoconstriction can change a moderate lesion with sufficient coronary reserve into a critical stenosis without any reserve left or with even a decrease in resting coronary flow (From reference 9 with permission).*

In humans, both excercise[119] and intracoronary acetylcholine[115] induce similar reductions in diameter in stenotic coronary vessels. When these constrictions occur superimposed on lesions of 70% or greater, the resultant reduction in overall coronary flow could be sufficient to induce ischemia without the need to alter myocardial oxygen demand as well. Thus, our concept of the supply/demand ratio should take into account the dynamic behaviour of the large conduit vessels over and beyond the lesion area. Assuming that the stenosis is sufficiently eccentric to allow for vasomotion, this certainly means an addition to the old concept.

Due to the variability of dynamic changes in vasotone superimposed on an existing fixed lesion, different degrees of ischemia may be found in the same patient despite similar changes in myocardial oxygen demand.

Alternatively, similar changes in coronary perfusion may occur in an individual but resulting from entirely different stimuli. An example of the latter is given in Fig. 9, where, in the same patient, a comparable reduction in regional coronary flow is observed during spontaneous angina at rest and during pacing-induced ischemia, at a heart rate of 120 beats/minute.

**Mechanisms of abnormal vasomotor regulation**

Evidence is accumulating, that abnormal vascular behaviour, e.g. a diminished or absent dilator response following stimuli, which normally would induce vasodilatation, is not restricted to severely stenotic arteries, but is also observed in vascular regions with only minimal atherosclerosis[116-118] (Fig. 10). Moreover, recent data suggest, that in hypercholesterolemia per se, without atherosclerosis, endothelium-dependent relaxation is already impaired, even after a short exposure to cholesterol[120]. Also, several studies indicate, that the vasoconstrictor response to calcium and norepinephrine is increased in hypercholesterolemic animals before the development of atherosclerosis[121-123].

A disturbance in endothelial function in atherosclerosis and hypercholesterolemia with subsequent inappropriate EDRF or NO production, has been implicated to explain the abnormal vasodilator response after certain stimuli. Likewise, a persistent dysfunction of regenerated endothelium after angioplasty and reperfusion injury has been reported[124,125]. In the first model, a sustained, abnormal vasodilator response to acetylcholine and aggregating platelets

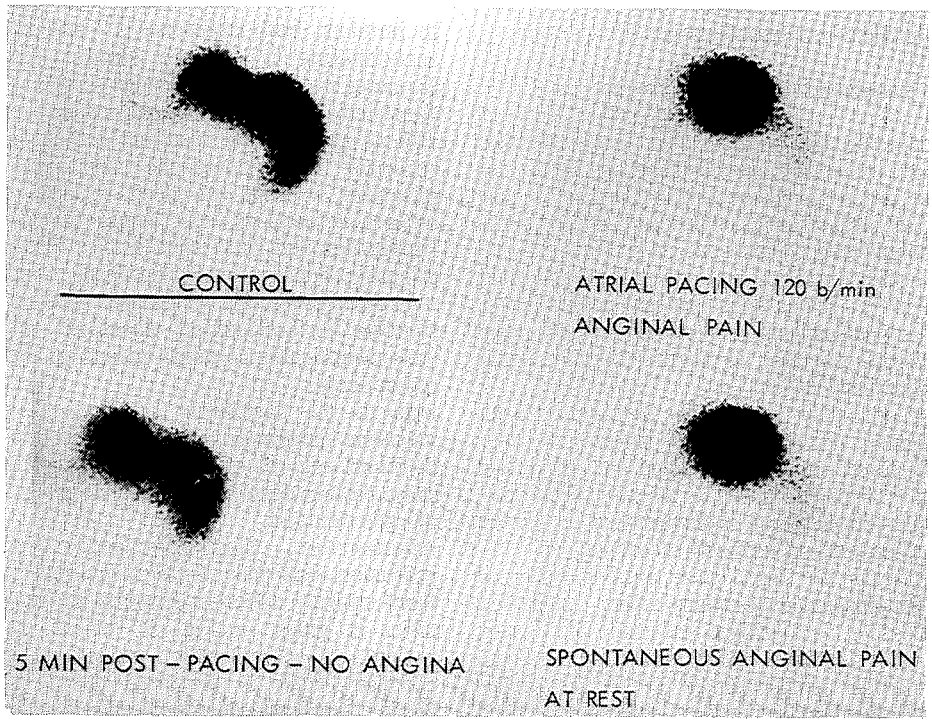


*Fig. 9: Reduction of regional coronary flow during spontaneous angina pectoris at rest in the same area as during atrial pacing-induced ischemia. In this patient Krypton-81m is continuously infused into the left circumflex artery which has a 70-90% stenosis (arrow). During pacing-induced anginal pain $^{81m}Kr$ distribution is decreased over the post-stenotic area with an increase over the normal area. 5 min after pacing the krypton changes have nearly returned to the control situation after angina has subsided for several minutes. However, thereafter, during spontaneous anginal pain Krypton-81m again diasappears in the same area with an increase over the normal region suggesting a reduction in coronary flow due to spasm of the artery at the site of the stenosis. (From reference 9 with permission).*

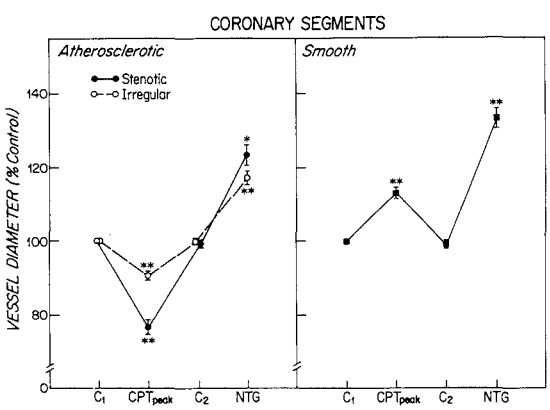


*Fig. 10: Abnormal vasomotor response of atherosclerotic coronary artery segments following the cold pressure test (CPT). Whereas smooth segments dilate, severely stenotic artery diameter decreases significantly. However, also minimal atherosclerotic segments (irregular) behave abnormally with vasoconstriction. (From reference 116 with permission).*

was found[124] . In contrast, in the second model with reperfusion injury, provided that such a pathophysiogical condition does indeed exist[126], this was only due to aggregating platelets[125].

Enhanced platelet aggregation, not counteracted by EDRF, could favour platelet adhesion, aggregation and the release of platelet-derived contractile (and growth-promoting) agents, favouring coronary

vasoconstriction, spasm and ischemia.
It is interesting to note, that besides a persistent abnormal endothelial response to certain stimuli, the effect of others, such as bradykinin, becomes impaired only after a long time following denudation and regeneration of the endothelium[127,128]. This observation and the relatively high frequency of restenosis following angioplasty strongly indicates the need for extensive research in the area of restenosis and endothelial changes at the molecular and cellular level.
The endothelium, although popular, is not the only culprit when it comes to abnormal vasodilator behaviour during ischemia or clinical situations related to ischemia. Despite arguments to the contrary from most in vitro workers, preliminary data on the vasodilator response of non-endothelium-dependent vasodilator stimuli suggest, that in hypercholesterolemic humans abnormalities in both endothelial and smooth muscle function are present (M.Creager: personal communication).
Moreover, several studies suggest an important role for alpha-adrenergic vasoconstriction in ischemia, either by initiating or by amplifying the ischemic process. During progressive reduction of coronary flow, intracoronary norepinephrine or cardiac sympathetic nerve stimulation reverses metabolic coronary vasodilatation to alpha-adrenergic vasoconstriction[129-131]. Whether specific adrenoceptor subtypes are preferentially involved, is not yet clear.
The bulk of the evidence, coming from one research group, suggests, that vasoconstriction is predominantly caused by alpha-2-linked mechanisms, whereas alpha-1 adrenergic vasoconstriction actually diminishes during ischemia[132,133]. Besides specific alpha-2-blocking agents, calcium-antagonists are shown to antagonize adrenergically-induced coronary vasoconstriction during ischemia[134,135]. In contrast, in anesthetized open-chest dogs, where baseline sympathetic tone is likely to be elevated, alpha-adrenergic vasoconstriction during ischemia is alleviated by alpha-1 but not by alpha-2 blocking agents[136].
This, together with the observation, that alpha-1 adrenergic vasoconstriction specifically limits the increase in coronary flow during exercise[106], again a situation where overall sympathetic tone is equally enhanced, suggests a differential alpha-subtype interference with vascular resistance, which depends on the instantaneous level of overall sympathetic tone.

**Relative contribution of abnormalities in vasodilator control to the clinical picture of myocardial ischemia in man.**

The relative contribution of the multitude of factors, which govern the instantaneous myocardial oxygen demand/supply ratio and regional coronary vasotone to occurrence and severity of myocardial ischemia in man, will vary considerably from one clinical setting to the other. The origin of myocardial ischemia in conditions, such as Prinzmetal-type variant angina, or occurring as a result of abnormal microvascular behaviour in syndrome X, hypertrophic or dilated cardiomyopathy, will be clearly different from that in the patient with coronary artery disease, the subject of this thesis. Even in the latter it is impossible, however, to create a uniform picture.
One factor, which may be rather consistent although difficult to determine sequentially in vivo, is the "coronary steal phenomenon", e.g. the propensity of coronary arterial blood to be shunted away from high to low resistance areas.
Thus, oxygen and substrates are preferentially transported to normal regions with unaffected vasodilator reserve capacity, away from the coronary lesion area with its increased stenosis resistance. Furthermore, if coronary artery lesions are severe and eccentric, further downstream vasodilatation, as a result of stress or vasodilator mechanisms, may actually increase stenosis resistance and reduce coronary flow[137-139]. Proposed mechanisms here include a collapse of the normal compliant part of the arterial wall as a result of pressure loss in the stenosis area or enhanced outflow turbulence and subsequent increased resistance. In addition, turbulent flow and, as a consequence, abnormal flow patterns along the endothelial wall, might affect basal EDRF release.
To what extent an abnormal regulation of vasomotor tone by the various mechanisms outlined above, really contributes to the onset and propagation of ischemia over and above the factors given before, is as yet unknown. Largely, because sufficient human data are not yet available.
Sympathetic activation by isometric exercise may induce significant coronary artery narrowing, followed by objective and subjective clinical signs of ischemia[140,141].
Also, the cold pressure test may result in increased coronary vascular resistance, mediated by alpha-adrenergic vasoconstriction and, subsequently, in ischemia[142,143]. However, the latter patient studies only examined overall coronary flow and not stenosis resistance.

In contrast, several recent angiographic investigations do indeed indicate, that abnormal vasomotor tone and vasoconstriction are present in lesion areas during exercise or fast atrial pacing. However, the underlying mechanisms are not clear from these studies. Neither do they provide insight as to whether these alterations in coronary diameter and in flow do result in ischemia and, if so, what the temporal relation is to myocardial ischemia.
We have tried to establish this relation in a model of pacing-induced myocardial ischemia, where regional coronary blood flow was continuously monitored with a short-lived isotope, Krypton-81m, administered intracoronary in patients with coronary artery disease. Changes in Krypton-81m distribution during ischemia were assessed in areas with moderate and severe coronary lesions as well as in regions without coronary disease. Subsequently, the temporal relation of these changes with metabolic, electrocardiographic and hemodynamic variables are described. The results of these investigations, presented in chapter VII, are preceded by a more detailed description of the Krypton-81m method.

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# Chapter III

# METABOLITES AS MARKERS OF ISCHEMIA.

# III.1.

## Usefulness and applicability of myocardial metabolic markers as indicators of myocardial ischemia in man.

Myocardial ischemia results in early and profound metabolic alterations in the heart, predominantly in the ischemic area. Consequently, the identification of myocardial ischemia in man by way of these metabolic alterations has been one of the diagnostic goals in cardiovascular medicine since the introduction of coronary sinus catheterization in man by Bing et al in 1947[1].
Although, in theory, a sensible approach when defining the presence and magnitude of ischemia, several questions arise, e.g. how useful are changes in metabolites for the assessment of myocardial ischemia; which metabolites are particularly useful in humans and how applicable are the methods to determine these metabolic changes in man? Moreover, are they sufficiently reproducible to allow for (pharmacological) interventions to be tested? And finally, are there specific metabolic alterations, which allow us to define specific interventions?

Currently existing methods to identify ischemia-induced metabolic alterations consist of invasive and non-invasive techniques (Table I).

Invasive techniques are best categorized as:

- methods, which identify ischemia through chemical evaluation of metabolites in arterial and coronary venous blood.
- tracer methods, which apply radiolabelled substrates in the setting of arterial and coronary venous catheterization
- techniques, which use specific catheter tip electrodes positioned in the coronary venous effluent.

Non-invasive techniques consist of radionuclide methods, in particular positron or single photon emission tomography with labelled fatty acids, glucose or amino acids as substrates. In addition, nuclear magnetic resonance spectroscopy, although still in its infancy where the human heart is concerned but very promising in smaller species as well as in peripheral human tissue, should also be mentioned.

**Table I: Methods to identify ischemia-induced metabolic alterations in humans.**

| Method | Technique | Substrates |
|---|---|---|
| | **INVASIVE** | |
| Arterial/coronary sinus catheterization | Biochemical evaluation (arterial and coronary venous blood) | lactate, pyruvate, inorganic phosphate, potassium, citrate, alanine, glutamate, FFA, carnitine, nucleosides, prostaglandins, neurohormones |
| | Radio-labelled substrates | $^{14}$C-glucose, $^{14}$C-lactate, $^{3}$H-norepinephrine, $^{13}$C-lactate |
| | Catheter tip electrodes | pH, $O_2$, potassium |
| | **NON-INVASIVE** | |
| Radionuclide methods | Planar/SPECT | $^{123}$I-phenylpentadecanoic acid |
| | PET | $^{123}$I-hexadecanoic acid |
| | PET | $^{11}$C-palmitate |
| | PET | $^{18}$F-deoxyglucose |
| | PET/SPECT | $^{13}$N-glutamate |
| | PET | $^{11}$C-acetate |
| Magnetic resonance spectroscopy | - Rotating frame technique<br>- Depth-resolved surface coil spectroscopy<br>- Image-selected in-vivo spectroscopy<br>- Spectroscopic imaging | P-31, H-1, N-14, C-13, Na-23, F-19 |

*Abbreviations: PET = positron emission tomography; SPECT = single photon emission computed tomography*

## III.2. Non-invasive techniques.

As emphasis in this thesis is on the evaluation of myocardial metabolism through invasive methods in humans, non-invasive techniques, important as they are, will be reviewed only briefly.

### NMR-spectroscopy.

Nuclear magnetic resonance spectroscopy is a new, non-invasive technique to study myocardial metabolism. Using the spin properties of certain nuclei, such as P-31, H-1, N-14, C-13, Na-23 and F-19, specific metabolic steps in the heart have been evaluated in vivo. The technique now allows reproducible experiments with a temporal resolution of several seconds to minutes. Thus, in animal experiments, relatively fast changes in high-energy phosphates, inorganic phosphate and pH are noted during ischemia and reperfusion[2,3]. The technique enables the determination of intracellular free calcium concentrations using F-19[4]. Moreover, C-13 NMR spectroscopy has been used to identify certain aspects of glycogen metabolism and citric acid fluxes[5,6]. Also, direct detection of free radicals with NMR spectroscopy has been described[7]. Although promising, the applicability of nuclear magnetic resonance spectroscopy in the human heart has been limited thus far by issues such as sensitivity and localization[8].

### Radionuclide techniques.

The radionuclide techniques, employed in the detection of myocardial metabolism, consist primarily of positron (PET) or single photon (SPECT) emission tomography with labelled fatty acids, acetate, deoxyglucose and glutamate as substrates. Earlier studies predominantly employed planar scintigraphy. With this technique the half-life of disappearance from the heart of phenylpenta-, hexa- and heptadecanoic acids, labelled with gamma-emitting iodine isotopes can be determined, which supposedly reflects their metabolic turnover in the ß-oxydation pathway. Moreover, a recent study applied planar scintigraphy with $^{13}$N-glutamate during symptom-limited bicycle exercise testing in patients with single-vessel coronary artery disease. In this investigation, glutamate accumulation as a marker of ischemia in reversible ischemic areas was compared with with thallium-201 kinetics, which reflect myocardial perfusion[9].
Labelling of medium-chain FFA with iodine-123 in the omega position does not significantly alter their normal biological behaviour[10,11]. Apart from visualization of areas with diminished uptake, e.g. infarct areas, abnormal turnover rates of FFA labelled with iodine-123 in ischemic or infarcted regions may be determined[12,13].
However, some doubt has arisen, as to whether the measured changes in radioactivity really reflect the metabolic turnover of FFA or merely the kinetics of free iodine[14,15]. Moreover, beside other limitations inherent in the use of single photon-emitting radionuclides[16,17], planar scintigraphy also precludes precise delineation of regional and transmural metabolic changes. The latter, however, does improve considerably with tomography.
Whereas with SPECT similar fatty acid isotopes are used as with planar scintigraphy[18], cyclotron-produced positron emitters, such as oxygen-15, nitrogen-13, carbon-11 and fluorine-18, are employed with PET for metabolic studies[19-24].
Moreover, with the latter technique, myocardial perfusion can be monitored simultaneously with metabolic imaging, using oxygen-15[25], nitrogen-13[26], or the generator-produced rubidium-82[27-28].
Thus, regional glucose uptake (labelled with fluorine-18) can be compared with $^{11}$C-palmitate turnover and myocardial perfusion ($^{13}$N-ammonia)[23] during pacing- or exercise-induced ischemia[29,30] (Fig. 1). As such, the methodology allows for a useful approach towards the detection of regional alterations in metabolism and flow and is one which is applicable in man.

In general, non-invasive methods are preferable to invasive techniques, at least to a certain extent.
Besides obvious advantages for the patient, as well as being less vigorous for the investigator, these methods do allow for long term, sequential investigations to be carried out in the same patient. Moreover, the technical advancements of some of the techniques, e.g. positron and single photon emission tomography, enable us currently to identify regional as well as transmural changes in myocardial metabolism, coupled to changes in regional coronary flow. In no way is this possible

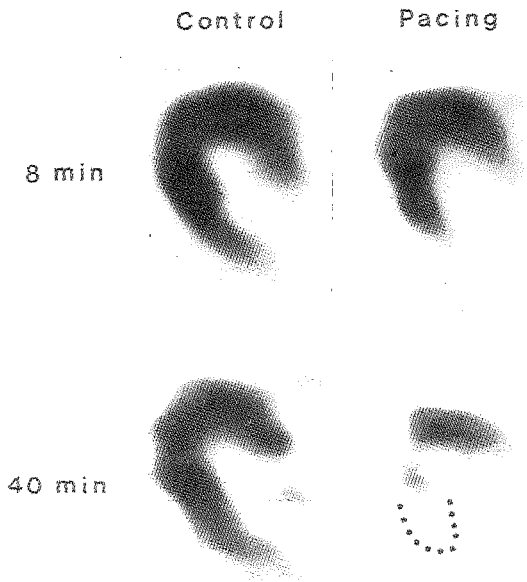


*Fig. 1: Myocardial $^{11}$C-palmitate activity at control and during atrial pacing in a patient with a 90% proximal stenosis in the left anterior descending coronary artery. Midventricular images are shown with the anterior wall at the upper left, septum at the upper right and lateral wall at the lower left. Images at the top (8 min) reflect $^{11}$C-palmitate uptake and bottom images (40 min) clearance. Uptake is normal and homogenous both during control and pacing. At rest, clearance is also homogenous. However, during pacing, clearance became heterogenous with retarded clearance in septum and anterior wall, indicative of impaired fatty acid oxydation during pacing in the area at risk (from reference 29, with permission).*

with any of the invasive methods available.
Obviously, these are very significant advantages of the non-invasive techniques.
On the other hand, a number of important limitations are present as well, which make these methods less attractive than would appear at first sight (Table II).
First, tracer uptake in the heart depends on several mechanisms, which are difficult to control, such as local perfusion, the integrity of active transport mechanisms and the overall metabolic state of the heart. For instance, extraction and turnover of fatty acids, such as palmitate, are clearly dependent on substrate availability[31]. In contrast, a recent study[32] indicates, that myocardial fuel supply and selection have only limited effects on acetate kinetics, supporting previous suggestions that the early rapid clearance phase of $^{11}$C-acetate truly reflects tricarboxylic acid cycle flux without much influence by changes in substrate supply[33,34]. Secondly, the quantitative delineation of tracer distribution may be complicated by wall motion, partial volume effects and spillover of the isotope.

In addition, in the case of planar scintigraphy, tracer activity uptake by non-myocardial structures or the overlapping of ischemic and normal areas, may be a significant complicating factor.

However, most importantly, the relatively long half-life of most tracers, used for metabolic imaging, as well as the long duration of tomographic imaging time, do preclude the fast sequential determination of metabolic changes, which is a prerequisite for monitoring of myocardial metabolism during (the early phases of) myocardial ischemia.
In addition, the various substrates used as tracer, may induce specific problems in the interpretation of ischemic metabolic changes. Labelled fatty acids, extracted by the ischemic myocardium, may back-diffuse in a considerable amount in unaltered chemical form[35]. Also, that part of the tracer time-activity curve that primarily reflects the oxydation process may be overshadowed by retarded wash-out curves, particularly during ischemia, thus obscuring the real metabolic rate.
Glucose, labelled with fluorine-18 as fluoro-2-deoxyglucose[36] is trapped after being converted to $^{18}$F-2-deoxyglucose-6-P and does not enter the glycolytic pathway. It therefore only indicates the rate of cellular uptake of glucose and subsequent phosphorylation.
Although the latter is related to glycolysis, it also depends on glycogenolysis. Moreover, the half-life of this tracer (109.7 min, $\beta^+$ 97%) makes its value rather dubious, particularly in the event of a progressive reduction in coronary flow.

**Table II: Metabolic imaging techniques in myocardial ischemia - limitations.**

| | |
|---|---|
| Tracer uptake | perfusion, integrity active transport systems, overall metabolic state |
| Tracer clearance | back-diffusion |
| Imaging | tracer uptake myocardial tissue - overlapping normal areas |
| Tracer distribution quantification | wall-motion, partial volume effect, tracer spill-over |
| Imaging duration/tracer half-life | fast sequential determination metabolic changes not possible |

# III.3. Invasive methods.

## Catheter tip electrode techniques.

In contrast to the long lasting procedures generally encountered with non-invasive radionuclide metabolic imaging, catheter tip electrodes enable both very fast as well as accurate determinations of specific metabolic changes in blood passing by the catheter tip.
Thus, in patients with coronary artery disease, significant, albeit small changes in coronary venous pH or potassium are readily detected during ischemic episodes with the catheter postioned in the coronary sinus. During angioplasty procedures, the coronary venous pH is shown to fall transiently, 4 to 6 seconds after deflation of the balloon, returning to control values within 65 seconds[37]. As to be expected, the peak pH change is related to the duration of coronary artery occlusion. However, at least 15 seconds of balloon inflation is needed for coronary venous pH to change.
Similar changes in coronary venous potassium are observed during angioplasty, again not during, but following balloon deflation, with increments up to 0.6 mmol/l[38]. In a different set-up, but by the same investigators the effect of fast atrial pacing on coronary venous pH was studied in patients with coronary artery disease. In all but one patient, who developed ischemia during the test as shown by characteristic electrocardiographic changes, a significant increase in coronary venous pH (>0.02 units) was observed[39]. Interestingly, maximum changes were not found during, but at 20 seconds post-pacing (Fig. 2). This coincides with maximum changes in coronary venous lactate levels under identical circumstances, e.g. 15 seconds post-pacing, in patients with ischemic heart disease, as reported by us in the same year[40].
The catheter tip electrode technique allows for a continuous and sensitive assessment of the metabolic parameter under study.
With chemical analysis of coronary venous blood, significant pH changes have never been established in man, neither during nor directly after pacing (unpublished observations). Although, early studies using biochemical methods did indicate, that coronary venous pH

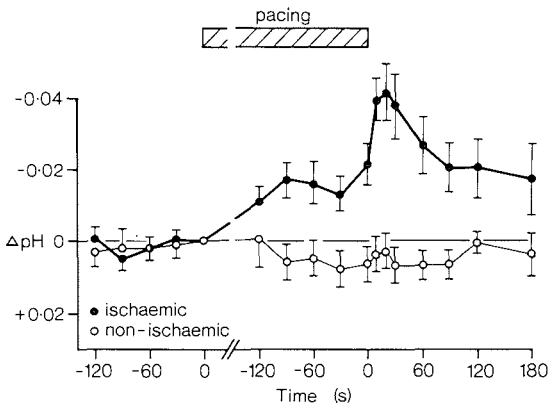


*Fig. 2: Sequential changes in coronary venous pH during and after atrial pacing, measured by catheter tip electrode technique. In ischemic patients a maximal decrease in pH is observed at approximately 20 seconds after pacing.*

increased during (pacing-induced) ischemia, arterial pH rose also and to a greater extent. Consequently, the difference between arterial and coronary venous pH actually increased significantly[41].
Also, significant potassium release as a result of ischemia has never been consistently established using biochemical methodologies[42,43], indicative of the sensitivity of the catheter tip electrode technique.

Moreover, the method should allow for reproducible recordings to test interventions, providing that the usual precautions, necessary with coronary sinus catheterization studies, are taken care of and that sufficient time is allowed between tests for restoration of metabolism.
The main disadvantage of the technique, apart from being invasive, is that it does not allow for concomitant determinations of coronary flow and metabolites, and like all other invasive methods, is not wel suited for the determination of regional changes in metabolism.

**Biochemical evaluation of metabolites in the coronary venous effluent.**

The inability to determine regional alterations in metabolism is a major disadvantage of all invasive catheter-based techniques, particularly those, which rely on coronary venous blood being sampled for subsequent chemical analysis. To determine coronary flow by the latter method, to collect blood at short intervals and, at the same time to ensure reproducible measurements after lengthy intervals, the coronary venous catheter is optimally positioned in the mid-portion of the coronary sinus. The collecting orifice, usually at or near the tip of the catheter, should be at least 2-3 cm away from the ostium of the coronary sinus, to prevent reflux of atrial blood interfering with the coronary venous sample[44]. Even then, the presence of atrial reflux should be excluded by measuring the effect of bolus infusions of saline in the right atrium on the coronary sinus thermodilution curve.

Atrial reflux is a major concern in invasive studies of the coronary sinus, particularly when right atrial pressure becomes elevated following ventricular pacing or atrial cannon waves, such as may occur during fast atrial pacing.

On the other hand, when coronary sinus flow is measured and blood is sampled for metabolic determinations, the catheter should not be positioned too high up in the coronary sinus, as then the combined procedure of sampling blood and measuring flow over relatively long periods will become virtually impossible. Repetitive fast sampling of blood from a restricted area such as that drained by the great cardiac vein through thermodilution flow-type catheters, is usually very difficult and unpredictable. If, in the latter situation, atrial pacing is carried out to produce ischemia, failure to capture or the occurrence of ventricular instead of atrial pacing may be an additional problem.

Thus, the preferred and commonly used position of the catheter will be somewhere in between these two extremes, in the mid-portion of the coronary sinus.

In this position, the venous admixture from the anterior, high-mid septal and lateral part of the left ventricle will usually contain blood from areas with and without significant coronary lesions. Hence, during ischemia, blood from non-ischemic regions will invariably dilute the effluent from the ischemic area(s). As during a procedure, such as exercise or atrial pacing, post-stenotic blood inflow falls and, subsequently, venous outflow diminishes, coronary sinus samples may mainly reflect blood from normal, non-ischemic regions, thus obscuring the main event to be studied.

**Sensitivity and usefulness of metabolic markers of ischemia.**

A host of metabolites, eicosanoids and neurohormones have been assessed, using coronary sinus catheterization, in patients with coronary artery disease in an attempt to better identify the ischemic event. Of these, lactate, pyruvate, inorganic phosphate, blood gases, potassium, citrate, alanine, glutamate, FFA, carnitine, nucleosides, prostanoids and neurohormones have been studied using various forms of stress testing. With respect to the first, the parameters to be studied, lactate, nucleosides and the neurohormones will be addressed in particular, as they are the key targets of this thesis. With respect to the second, the preferable stress tests in a setting, where emphasis is on myocardial metabolic changes, are fast atrial pacing and coronary balloon occlusion.

Coronary angioplasty has provided for a unique model of myocardial ischemia in man. The potential of the technique to induce ischemia has already been referred to on page 59. With respect to reproducibility, both coronary hemodynamics and myocardial metabolites have been reported to be comparable following the first 2 occlusions but not thereafter[45,46]. In contrast, more recent studies have expressed concern about the unpredictable effect of partial obstruction by the deflated balloon before the first inflation, which may render the subsequent occlusion period less reliable[47].

Besides questions concerning reproducibility, ethical considerations may interfere with the usefulness of the model, as they may prohibit a strict adherence to the study protocol in terms of occlusion times.

Ethical considerations are generally less of a problem where the atrial pacing stress test is concerned. Although the majority of patients will experience anginal pain during pacing-induced stress, as with all other procedures aimed at inducing myocardial ischemia, this rapidly diminishes immediately after pacing in nearly all patients. Often, angina diappears during the first 30-second period following pacing, which is in sharp contrast with the relatively slow regression of pain after exercise. Although the latter may be an argument against exercise, an even stronger argument is the fact that reproducible measurements of coronary flow and metabolites, using a coronary sinus catheter, are in fact impossible.

Although reproducible flow measurements during supine exercise have been claimed[48], the inherent movement of the catheter with exercise and hence the probability of sampling different venous outflow beds in the course of the study, precludes usage of the method other than in very selected cases. Moreover, arterial levels of metabolites and of neurohormones change significantly during exercise, irrespective of ischemia, interfering with myocardial extraction patterns and invalidating a proper evaluation of small ischemia-induced changes herein. In contrast, alterations in arterial levels generally do not occur during pacing.
Other forms of stress, such as the hand grip test, do not result in a similar degree of myocardial ischemia and ischemia-induced metabolic changes as fast atrial pacing[49]. The cold pressure test is also relatively insensitive in the diagnosis of ischemia, both compared with exercise testing[50] and atrial pacing (unpublished observations).
Hence, atrial pacing has been the preferred mode of stress in studies of coronary hemodynamics and cardiac metabolism, since its introduction in 1967 by Sowton et al[51].
Likewise, we have used the test intensively over the past 17 years. As a result, the patient studies, mentioned in this thesis, have all been carried out with incremental atrial pacing, using a standard pacing protocol, as described in chapter V.

**Myocardial lactate metabolism as indicator of ischemia.**

During fast atrial pacing and ischemia, there is a relative prevalence of venous admixture from non-ischemic regions, as a result of reduced arterial inflow and diminished venous outflow from ischemic areas. This, presumably, is the major reason for the relatively low sensitivity of metabolites, such as lactate, to indicate myocardial ischemia in humans, at least when measured directly, through chemical analysis of coronary sinus blood. Moreover, where lactate is concerned, several other factors interfere with its normal extraction pattern. During exercise, arterial lactate levels increase, accompanied by markedly enhanced cardiac extraction. This already suggests, that this parameter is not well suited as indicator of ischemia in this setting, besides the near impossibility to conduct meaningful investigations by way of coronary sinus catheterization during exercise. In contrast, conditions such as hyperventilation, alternative substrate availability (FFA) and catecholamines, all reduce cardiac lactate extraction by affecting either glucose uptake or several key enzymes in the glycolytic process[52-55]. As a result, a reduction of lactate extraction alone under a certain level, e.g. 10%, as has been used in human studies to indicate ischemia[45,56-58], cannot be taken as an objective sign of ischemia (chapter V)[59]. Neill and Kremkau[60] compared the second largest group of patients with and without a significant (>70%) coronary diameter narrowing reported in the literature (see chapter V for comparison) and observed that during fast atrial pacing average values +/- 2 SD for lactate extraction in the normal group ranged between 46% and 0%. A similar range during pacing was reported by Gertz et al. in normal subjects[59]. Hence, the stringent criterion of lactate production should be adhered to, to obviate false positive results. If so done, the sensitivity of lactate as marker of ischemia in patients with coronary artery disease indeed appears low[61] as suggested by the majority of studies in humans[41,60-66]. However, this information is derived from studies where changes in myocardial lactate metabolism were only assessed during the stress test. In Table III (next page) the percentage of patients with significant coronary artery disease, who developed myocardial lactate production during pacing are compared to those with significant electrocardiographic or hemodynamic criteria or ischemia. In contrast to a variable but rather high incidence of anginal pain and to relatively low but consistent frequencies for ischemic ST-changes, the occurrence of lactate production is also more variable and, at the same time, relatively low, varying between 35% and 69%. Likewise, abnormal elevations in left ventricular end diastolic pressure are also inconsistent, ranging between 39% and 76%. Does this mean, that we are restricted to a relatively non-specific and subjective marker as anginal pain as the primary indicator of ischemia in this model[64] or are there ways to increase the sensitivity of objective indices? More specifically, can we improve the potential of abnormal myocardial lactate metabolism to indicate ischemia in man? This particular question will be addressed in this thesis.

**How to improve identification of myocardial ischemia through lactate determination.**

All data considered, in patients with coronary artery disease myocardial lactate production averages 50% during specific stress tests, such as fast atrial pacing, which is not encouraging[41,60-66].
However, it should be realized, that available human data are derived from relatively small patient populations (Table III).

**Table III: Percentage of patients with metabolic, electrocardiographic, hemodynamic and symptomatic signs of ischemia during pacing.**

| Author | N | Percentage of patients with | | | |
|---|---|---|---|---|---|
| | | Lactate-production | ST-depression | LVEDP increase | Angina |
| Parker 1969 [41] | 21 | 62 | 90 | 76 | 81 |
| Linhart 1972 [63] | 31 | - | 55 | 39 | 55 |
| Neill/Kremkau 1974 [60] | | | | | |
| submax pacing | 89 | 28 | - | - | 51 |
| max pacing | 24 | 35 | - | - | 71 |
| Chiong 1974 [61] | 23 | 69 | 52 | - | 78 |
| Thadani 1979 [62] | 12 | 58 | 58 | 58 | 100 |
| Ihlen 1983 [65] | 40 | 54 | - | - | 100 |
| Markham 1983 [64] | 18 | 50 | 56 | 44 | 67 |
| Thomassen 1988 [66] | 64 | 62 | 73 | - | 100 |

*Abbreviations: LVEDP = left ventricular end diastolic pressure; N = number of patients*

Also, the relative presence of proximal versus distal left coronary artery disease or right coronary artery disease has generally been given little attention. More importantly, the assessment of lactate metabolism was always carried out during pacing. As far back as in 1981 we reported, that the sensitivity of measurements of myocardial lactate metabolism as a definitive marker of ischemia, significantly improves when the cardiac extraction values of this metabolite are not only determined during maximal pacing rates, as is customary, but in particular when they are assessed during the immediate post-pacing period, e.g. at 15 seconds after pacing[40]. Several years later, this observation was confirmed by Ihlen et al[65]. These authors observed that, whereas average lactate extraction values were still positive during maximal pacing heart rates, they became negative and significantly different from control at 15 seconds after pacing. However, in their study also, only 13 out of 20 patients had signs of abnormal lactate metabolism at some point during the test, which is little different from the values from other studies, given in Table III.

This predominant increase in lactate release from the heart at this particular point of time after pacing has subsequently been observed in all our published material dealing with ischemic metabolic changes in man. The potential of improved assessment of myocardial lactate metabolism as indicator of ischemia has not been given further official attention. Instead, working on the hypothesis, that relatively large numbers of patients would be needed to underscore our initial observations and to better understand the relative impact of lesion location and size on ischemia-induced metabolic changes,a prospective study was carried out in approximately 450 patients, all undergoing the same form of stress testing under identical circumstances. Of these, patients with and without coronary artery disease, with and without pre-existing signs of myocardial ischemia, and with single left or right versus multivessel disease were studied, all undergoing myocardial lactate determinations at prefixed time points during and after pacing. Moreover, coronary flow was also measured in a subpopulation.

As the data analysis has only recently been completed, two of the most important questions have been addressed and are presented in this thesis.

One compares the sensitivity and specificity of lactate versus other markers of ischemia in patients with and without significant left coronary artery disease (see chapter V), the other investigates the reproducibility of lactate changes following variable intervals between the pacing stress test (chapter VI.2). Preliminary data indicate, that the optimal time point to assess myocardial lactate production is between 15 and 30 seconds after pacing and not at any other point of time during or following the test. Moreover, by concentrating on this particular period after pacing, the sensitivity of the method significantly improves and becomes superior to commonly used markers as angina, ST-segment changes or alterations in left ventricular end diastolic pressure.

**Evaluation of myocardial lactate metabolism: radionuclide tracer technique versus chemical evaluation.**

Can we further improve the sensitivity of myocardial lactate metabolism as an index of ischemia in man?

It has been suggested, that when only chemical changes in lactate are measured, regional alterations in lactate release and their contribution to the instantaneous, overall lactate extraction pattern, may be underestimated or may remain unnoticed. Gertz and coworkers assessed myocardial lactate metabolism in patients with coronary artery disease, measuring arterial and coronary venous levels through simultaneous biochemical and radionuclide techniques, using $^{14}C$-1-lactate as tracer for the latter method[67]. At rest, they clearly showed labelled lactate release from the heart during net chemical extraction, thereby underscoring the suggestion, that, in fact, chemical analysis may significantly underestimate regional ischemia. Moreover, in a later study, they observed, that during atrial pacing, the radioisotope release, already present at rest, increased markedly and more significantly than chemical lactate release[68].

However, they also demonstrated, that in a greater percentage of patients myocardial lactate release, as indicated by the tracer technique but not by the chemical method,apparently occurred in the complete absence of other objective or subjective symptoms of ischemia. More disturbingly, significant radioisotope lactate release was also present in patients without coronary artery lesions, under resting conditions[67] (Fig. 3). Without the latter observation, it could be argued, that silent ischemia is apparently present (continuously or transiently?) in patients with significant coronary artery disease. Transient perfusion effects have been documented with thallium scintigraphy in resting patients without further obvious signs of ischemia. Likewise, we have observed resting

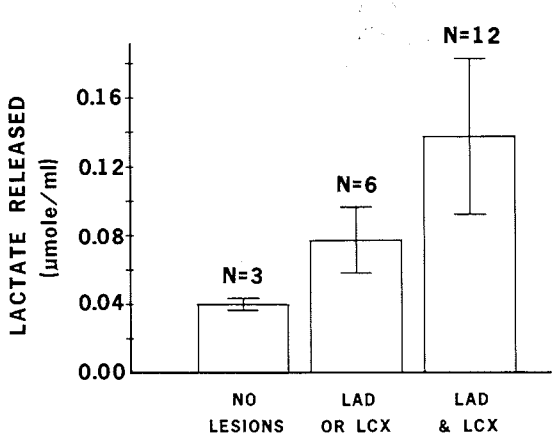


*Fig. 3: Myocardial lactate release, as determined by a tracer technique, using $^{14}C$-1-lactate. Already at rest, myocardial lactate is released as a function of the degree of coronary artery disease. However, lactate release, not appreciated by conventional biochemical techniques, is also present in patients without coronary lesions (from reference 67, with permission).*

*abnormalities in myocardial perfusion with Krypton-81m, distal to significant coronary lesions in patients without chemical lactate production or other objective signs of ischemia*[69] (chapter IV). However, the abnormal tracer release of lactate in patients without coronary lesions, without any sign of ischemia, is far more difficult to explain. Both in vitro studies and animal studies have indicated, that lactate release from the heart may occur despite normal oxygenation[70,71], which suggests that under certain conditions, some background efflux of lactate from specific areas of the heart may be "physiologic" (see chapter I, page 23 for further discussion) or, at least, not directly related to hypoxia of cardiac tissues, as already suggested by Huckabee[72].

To further confound the issue, Hanet and coworkers recently reported the opposite in silent ischemia[73]. These authors observed a significant increase of labelled lactate extraction and of myocardial lactate uptake in non-anginal patients, in whom, in contrast, chemical lactate production and cardiac release was present, taken to indicate the presence of asymptomatic myocardial ischemia (Fig. 4).

To date, more data are obviously needed to settle this issue. However, it certainly underscores the necessity to accept only net chemical production values for lactate as a definite sign of ischemia.

**Can we improve the sensitivity of lactate through the determination of additional metabolites?**

*Pyruvate.*

The ratio of lactate to pyruvate has been proposed to improve the sensitivity of the first in the assessment of hypoxia. A number of early workers in the field reported that this ratio increased in the coronary venous effluent during myocardial hypoxia[74-76].However, Henderson et al. observed that lactate and pyruvate do not equilibrate freely across the cell membrane and that the pyruvate efflux from the isolated rat heart exceeds that of lactate by the factor 10[77]. This suggests, that although potentially more sensitive, the ratio of lactate to pyruvate might also be less reliable. Besides, on theoretical and mathematical grounds, the original concept of "excess lactate" presumably does not hold. In addition, later animal and human studies during ischemia have indicated that pyruvate measurements do not improve the sensitivity of lactate as a metabolic marker of ischemia[78].

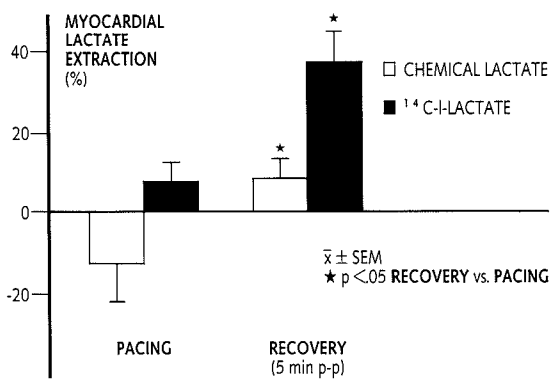


*Fig. 4: Net chemical lactate release during fast atrial pacing in asymptomatic patients with coronary artery disease, indicative of silent ischemia. In contrast, a simultaneous tracer method with L-(+)[1-$^{14}C$]lactate suggests the opposite, i.e. net cardiac lactate uptake (adapted from reference 73).*

*Alanine and glutamate.*

Besides lactate, the concomitant determination of glutamate and alanine fluxes may provide a more sensitive means to assess ischemia in man. The hypothesis being, that to alleviate lactate accumulation during ischemia and the subsequent inhibition of various enzymes of the glycolytic chain, transamination of glutamate with pyruvate occurs. This, subsequently, results in the formation of $\alpha$-ketoglutarate, as a potential fuel for the Krebs cycle, and of alanine, which is then released from the heart and can be measured. In order to maintain the redox state of the $NADH/NAD^+$ system, aspartate needs to be converted to succinate through the malate-aspartate cycle (see chapter I, page 20, for more detailed discussion).
Hence, alanine release or enhanced glutamate uptake, besides lactate production, should theoretically improve the assessment of anaerobic glycolysis.
Thomassen and coworkers recently compared 21 normal individuals and 64 patients with coronary artery disease[79]. Of the latter, 40 did produce lactate during and after atrial pacing, Their data do not support the theory that, in humans, glutamate-alanine changes have a significant impact on the overall assessment of metabolism during ischemia. Although, as shown in Fig. 5, some extra alanine production was present in the group with lactate production and some enhanced uptake of glutamate in both lactate and non-lactate producers in the recovery phase, these effects were relatively moderate when compared with the significant changes in lactate metabolism.

*Citrate.*

In the same study, Thomassen and coworkers stressed, that cytosolic citrate release may be more relevant as an additional marker of ischemia than the amino acids glutamate or alanine. Previous studies by the same group already demonstrated citrate release in both normal individuals and patients with coronary artery lesions during pacing-induced stress[80]. As such, this parameter discriminated between groups during the period after pacing. Of importance, a significant augmentation of citrate release from the control situation was also observed in patients who did not produce lactate during pacing [79]. However, as no data were provided to show that this group did really develop ischemia during pacing, the real value

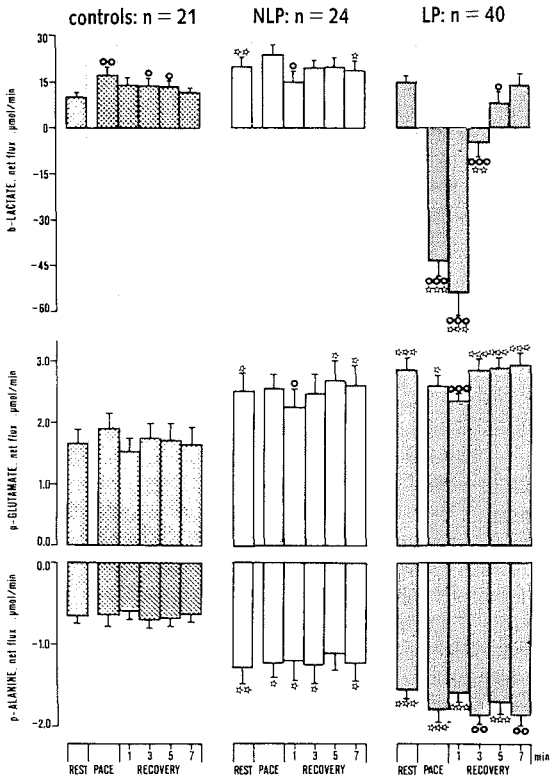


*Fig. 5: Myocardial fluxes of lactate, glutamate and alanine during and after pacing in patients without ischemia and ischemic patients with (LP) and without lactate production (NLP) during pacing. Despite evidence for some extra alanine production and enhanced glutamate uptake in LP, compared with other groups, changes are moderate and variable, compared with the significant effects in lactate metabolism (from reference 79, with permission).*

of citrate release as marker of ischemia over and above lactate production remains obscure. Also, in the study by Thomassen, absolute changes in citrate kinetics were minimal. Moreover, they occurred in all groups with changes all pointing in the same direction. At present, the real significance of citrate as a marker of ischemia awaits further clarification, preferably from different centres as well.

*Nucleosides.*

The determination of cardiac nucleoside release provides for an entirely different approach to assess myocardial ischemia and one, that is not as dependent on substrate delivery as lactate.

Contrary to observations in various animal species, such as the dog, hypoxanthine efflux, rather than adenosine or inosine release, is found in humans during ischemia, due to specific differences in nucleoside degradating enzyme activity (see chapter I).
We have been the first to report on inosine and hypoxanthine release during ischemia in the porcine heart and of hypoxanthine, in particular in patients with coronary artery disease[43,81-85]. The results strongly suggest the usefulness of these nucleosides as indicators of myocardial ischemia. Some are presented in chapter IV.
In man, our observations were recently confirmed by Edlund et al[86]. Like us, these authors were also unable to detect inosine in appreciable quantities or find significant changes in adenosine release during ischemia. As such, our results contrast with the original publication by Fox et al., who reported a significant increase in coronary venous adenosine in patients who, during atrial pacing, became lactate producers[87]. The latter study should be considered with some reservations, though. First, only coronary venous blood levels and not arterial values for adenosine were reported. Secondly, the separation in lactate versus non-lactate producers was not exact. Thirdly, the amount of blood needed for one adenosine determination (90 cc) precludes fast and easy sampling with the inherent danger of erythrocyte damage during the procedure. Our results are in even sharper contrast to the observations by Kugler[88], who did not find significant changes in coronary venous or arterial hypoxanthine during pacing-induced ischemia in man, although myocardial extraction changed to production. Moreover, in his study, ischemia significantly affected coronary venous inosine levels and changed cardiac extraction of this nucleoside to production.
The main difference between Kugler's experimental design and ours is the immediate inhibition of blood element metabolism with 8% perchloric acid after blood sample collection in our studies. Also, the amount of blood needed in our studies was significantly less than in Kugler's experiment, which must have had an important impact on the results. Clearly, Kugler's results may have suffered from the additional measurement of nucleosides from non-cardiac origin.
In our earlier studies on myocardial nucleoside efflux during ischemia in man, cardiac hypoxanthine release was compared in anginal versus non-anginal patients, as was customary in those days. In fact, by so doing, a significant difference was observed between these groups. Hypoxanthine release occurred predominantly in anginal patients and was apparently more pronounced than lactate production in these patients (Fig. 6).

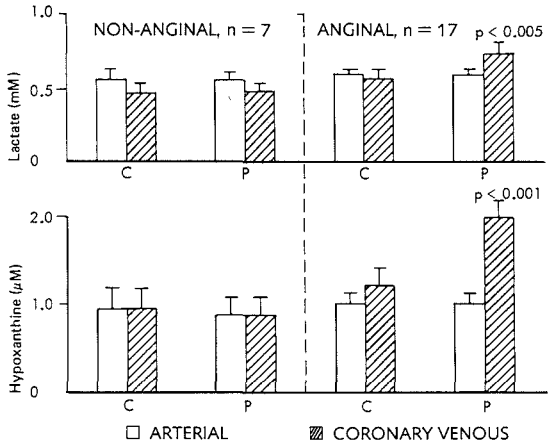


*Fig. 6: Pacing-induced changes in arterial and coronary venous lactate and hypoxanthine levels in patients who developed angina versus non-anginal patients. Coronary venous hypoxanthine levels rise markedly in anginal patients, more so than coronary venous lactate (from reference 55, with permission).*

In later studies, patients were never grouped as anginal or non-anginal. Instead, patients with significant coronary artery lesions were studied as one group. Even so, significant hypoxanthine efflux again could be demonstrated during pacing-induced stress in patients who became ischemic, also in individuals without angina.
Two important procedural differences exist between our early studies and later experiments, the results of both periods given in chapters IV and VII.
First, the biochemical analytical technique improved markedly by introducing high-pressure liquid chromatography[89,90]. Subsequently, much higher levels of coronary venous hypoxanthine were observed during ischemia[91]. Also, the new technique allowed separation of hypoxanthine and xanthine, which before then was not possible.
Secondly, later studies focussed on the post-pacing period. Thus, we found that hypoxanthine efflux was most marked after pacing, in analogy with the results obtained with lactate. However, the maximum for hypoxanthine was at 1 minute post-pacing and not at 15 seconds, as with lactate. Moreover, hypoxanthine efflux was more prolonged than lactate production, still being

present at 5 minutes after pacing. Data are presented in chapter VII.3.
Also, the temporal relation of these metabolic changes with myocardial perfusion, electrocardiographic changes and hemodynamic alterations are reported.

**Other metabolic markers of ischemia.**

The usefulness of several other metabolites as indicators of ischemia have been studied in man, using invasive techniques and, generally, by applying fast atrial pacing as stress test.
In contrast to the metabolic changes mentioned before in this chapter, most other metabolites have not really added in improving the diagnostic armamentarium of the invasive cardiologist in this area.
Substances, which are potentially important have already been described in detail in chapter I and will not be rediscussed here in view of their limited or absent applicability and/or sensitivity in man. Moreover, a number have been investigated in concert with lactate and hypoxanthine in our patient studies and will be duly commented upon in the respective manuscripts (chapter IV).
The effect of ischemia on cardiac prostaglandins we believe falls outside the scope of this thesis, although we have reported on them in patients with silent ischemia at rest as well as during pacing-induced ischemia[92,93].
Furthermore, neurohormones and myocardial ischemia will be considered separately in part II of this thesis.
As an exception, however, the effect of ischemia on cardiac carnitine fluxes deserves some attention.

*Carnitine.*

The role of carnitine in normal cardiac metabolism and during ischemia has been commented upon in chapter I. It has been suggested, that the level of free carnitine in the cardiac myocyte is essential for a number of transport functions, linked with free fatty acid flux into the ß-oxydation pathway and transport of high-energy phosphates over the mitochondrial membrane.
Ischemia-induced loss of free carnitine from the heart may therefore have important implications. Reduction of cardiac free carnitine levels has been demonstrated in animal studies of acute as well as relatively long lasting and severe ischemia proportional to the reduction in blood flow[94-97].
We have recently shown, that relatively short and moderate episodes of myocardial ischemia in man also result in a significant efflux of free carnitine from the heart[98] (Fig. 7).

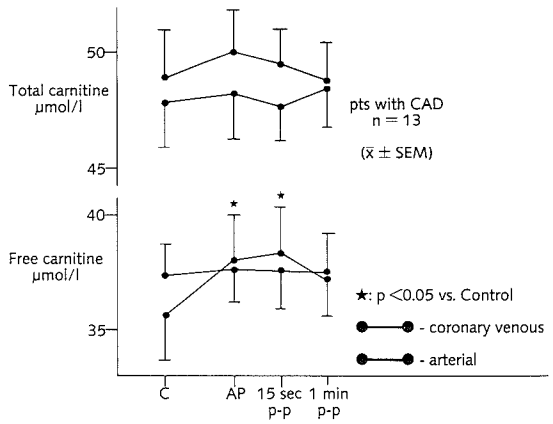


*Fig. 7: Significant increase of coronary venous free carnitine levels during pacing-induced ischemia without changes in arterial free carnitine or in total carnitine levels. Concomitantly, cardiac free carnitine uptake changes into release.*

Moreover, our data suggest, that this release is from the ischemic area. In contrast, total carnitine levels do not change, which implies an increased uptake of acyl-carnitines. Whether specifically long-chain acyl-carnitines accumulate is unknown at present, but is the subject of further studies.
Animal studies have indicated, that L-carnitine suppletion may protect against myocardial ischemia[99]. Whether, in man, free carnitine release during ischemia is affected by carnitine suppletion is unknown. However, Ferrari et al., in 1984, have suggested that the administration of intravenous L-carnitine may have beneficial effects on metabolites during pacing-induced ischemia, reversing lactate production to extraction with a simultaneous decrease in glucose uptake and enhanced FFA extraction[100].
Moreover, Thomsen and coworkers reported improved pacing tolerance after administration of DL-carnitine[101]. However, as the interval between pacing with and without the drug was only 15 minutes, the general lack of reproducibility of objective variables of ischemia (see chapter VI.2.) does not allow for such a conclusion. In contrast, several non-invasive exercise studies in patients with angina do suggest an antiischemic effect of L-carnitine (102-104).
On theoretical grounds, not L-carnitine but instead propionyl-L-carnitine may be the preferred substance to reverse changes in myocardial

carnitine content during ischemia, as myocardial extraction of this derivative is better than that of the mother compound.
Moreover, propionyl-L-carnitine may have additional beneficial effects in ischemia through an anaploretic effect of propionyl itself. A recent placebo-controlled trial did indeed indicate a significant improvement with long term propionyl-L-carnitine[105]. Unfortunately, L-carnitine was not investigated in this study.

# III.4.

# Usefulness of metabolic markers in delineating the effectiveness of pharmacological interventions.

## Reproducibility.

The first requirement of any method to test interventions is reproducibility. In view of the invasive nature of the metabolic techniques, discussed in this thesis, any study, designed to delineate the effectiveness of pharmacological interventions during ischemia in humans, will usually be performed only once. Likewise, such studies have to be completed within a reasonable time frame.
The question therefore will be whether metabolic changes, induced during repetitive periods of ischemia, are reproducible within a time span, which is acceptable both in logistical and ethical terms.
Unfortunately, this aspect has received very little attention thus far. As a result, the bulk of invasive metabolic studies has been performed in an uncontrolled way, using various metabolites, in particular lactate, to identify the effect of interventions on myocardial ischemia.
Often, the interval between tests has been short, presumably too short, to allow cardiac metabolism to recover from ischemia before embarking on the second episode of ischemia.

## Reproducibility of myocardial metabolic markers of ischemia using atrial pacing as stress test.

With respect to the pacing stress test model, which is central in this thesis, only a few reports can be found in the literature, dealing with the objective of reproducibility of metabolic alterations during repetitive periods of pacing-induced stress. Of these, all but a few have only considered changes in cardiac lactate extraction.
Jackson and coworkers, in 1978, performed a few placebo tests in the context of studies on the effect of propanolol on cardiac ischemia[106]. Based on observations in 5 patients, of whom only 2 produced lactate, they concluded, that an interval of 45 minutes was needed for reproducible lactate production values. In contrast, 3 patients, in whom shorter, albeit unspecified intervals were applied, had variable lactate production values. In a preliminary report, a few years later, we could confirm their observations, albeit in a definitely larger patient population. Our studies suggested, that an interval of 15 minutes resulted in significantly less average lactate production values. In contrast, when the interval was lengthened to 30 minutes, mean values were comparable but individual values did vary considerably. This was less after 45 minutes.
Although the results of subsequent studies by different groups generally tended to agree with our preliminary observations, there is as yet no consensus as to the optimal interval between atrial pacing stress tests and the reproducibility of metabolic values. Thus, whereas with an interval of 45 minutes excellent reproducibility of individual lactate values were found by Ferrari et al.[107], Bagger et al. reported a large variability after exactly the same period[108]. Also, whereas Drobinsky et al.[109] and Pouleur and coworkers[110] reported significantly less lactate production after an interval of 20 minutes, which agrees with our observations, Ihlen and coworkers found good reproducibility in lactate extraction values following a similar period[111]. Although the latter discrepancy may relate to different pacing protocols it still reflects the absence of a consensus as to the resting period needed between stress tests. Moreover, dissimilar results have been reported with respect to FFA levels after intervals varying between 20 and 45 minutes[107,108,110]. In addition, the determination of FFA in conditions where heparin has to be administered does not make much sense[112]. In contrast, cardiac exchange of amino-acids and citrate may be more reproducible[108-110].
In general, these results do not depend on the model used, e.g. whether pacing is incremental or at a continuous high rate. However, the duration of pacing may have an effect. In this respect, the study by Ihlen[111] is interesting, which implicates that very short periods of pacing may allow for lactate changes to be reproducible, whereas longer periods result in a suppression of lactate production. This suggests, that in the latter

instance, glycolysis becomes depressed.
Besides the fact, that very little information exists concerning the model, i.e. the reproducibility of pacing-induced ischemia, available data stem from small patient groups. Also, the number of intervals studied between tests is limited and generally not performed by the same investigators.
Hence, we have carried out a prospective, comparative study in a larger patient population. All patients were investigated at the same time of day, fasting and without premedication, submitted to a similar type of pacing protocol, the only difference being the interval between tests, which ranged from 15 minutes to 1 hour. Results on the reproducibility of lactate values are given in chapter VI.2.
At present, insufficient data ara available on the reproducibility of other metabolites, in particular of the nucleosides. However, an early study in the ischemic pig model suggests, that during a second 30-minute period of 60% flow reduction, following 35 minutes of reperfusion, cardiac nucleoside release is similarly reduced as lactate production when compared with the first ischemic period (see chapter VI.1).
These data indicate, that this model of relatively severe and protracted ischemia is not suited for the study of interventions. Moreover, they suggest that the reproducibility of metabolites, such as the nucleosides, should be carefully checked before embarking on intervention trials using these metabolites to indicate antiischemic effects.
In general, whenever new parameters, whether metabolic, prostanoid or neurohumoral of origin, are being used to delineate the effectiveness of a therapeutic intervention, their reproducibility ought to be investigated in a double-blind, placebo-controlled manner. Moreover, when changes in metabolites, such as lactate, are used routinely, each laboratory should establish the reproducibility of this parameter in their particular setting. We have carried out a number of investigations on the effect of different vasoactive compounds, primarily based on lactate as marker. These investigations were performed after we had established the minimum interval for its reproducibility (see chapter VI).
Our studies were carried out under very specific conditions, with the patient supine and resting and the stress applied in the form of an increase in heart rate. This has a number of advantages from the model point of view. With pacing, only the heart is stressed under very controlled and reproducible conditions without general systemic effects, which may interfere with ischemia-induced cardiac alterations. On the other hand, it may be argued, that the model is not very physiologic, ischemic attacks usually being evoked by different stimuli, e.g. exercise or cold.
The arguments against the latter forms of stress, where metabolic monitoring is concerned, have already been mentioned.
It may also be suggested, that interventions, which act mainly by their effect on heart rate, cannot be studied in this set-up. This, however, is not entirely true, as the agent, which strictly acts by a negative chronotropic effect, has yet to be discovered. Thus, several studies, using pacing-induced stress, have been carried out with different beta blocking agents, nearly all showing a reduction in ischemia following this intervention, despite artificially preventing the agent's negative chronotropic properties[106,113]. Likewise, in a small, early study we have observed a significant reduction in myocardial lactate production following 5 mg intravenous atenolol, a selective beta-1 blocking drug, in patients with coronary artery disease, being studied during repetitive atrial pacing stess tests, 1 hour apart (unpublished observations). In the latter instance, these effects could be explained by an acute, drug-induced reduction in arterial pressures as well as in contractility and, hence, in myocardial oxygen demand. Unfortunately, no data were available at that time on coronary flow.
It has been suggested that beta blocking agents, even the selective types, may diminish coronary flow[114], either by an autoregulatory effect secondary to a reduction in myocardial oxygen demand or by an unopposing effect on alpha-adrenergic tone.
Indeed, by using the model of pacing-induced stress we have observed that a direct vasoconstrictory effect of beta blockade may be important in limiting its antiischemic activity, at least in the resting patient. These studies were carried out with low- and high-dose intravenous administrations of labetalol, thereby altering the relative effects of alpha versus beta blockade on systemic and coronary hemodynamics[115].
Normotensive patients with coronary artery disease either received 0.5 mg/kg, or 2 mg/kg labetalol intravenously. Studies were carried out at rest, measuring coronary and systemic hemodynamic effects of labetalol for 15 minutes after drug administration. Next, its effect on myocardial ischemia was investigated during two successive, identical atrial pacing stress tests, one hour apart.
At rest, only the high dose reduced coronary vascular resistance, despite a proportionally greater decrease in coronary perfusion pressure, reflecting more pronounced alpha-adrenergic

blocking properties of the high dose compared with the low dose. In contrast, during pacing, coronary vascular resistance increased significantly less following the high dose, compared with the pretreatment pacing test (Fig. 8).

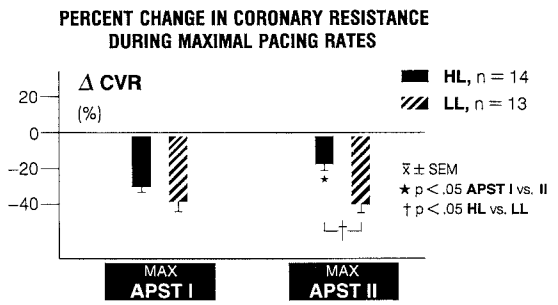


*Fig. 8: Effect of high dose (2 mg/kg, HL) or low dose (0.5 mg/kg, LL) intravenous labetalol on coronary vascular resistance (CVR) during 2 successive, identical atrial pacing stress tests, 1 hour apart (APST I and II, resp.). Whereas the decrease during maximal heart rates (MAX) is similar in low dose patients, the reduction in coronary vascular resistance is significantly less following the high dose.*

With the low dose, comparable increases were seen during, before and after pacing. This difference in effect can be explained in two ways. One, the more pronounced, unspecific beta blocking properties of the higher dose labetalol may limit coronary vasodilatation following the increase in myocardial oxygen demand, enabling alpha-adrenergic coronary vasoconstriction to become the limiting factor[116-118]. In this respect, our recent observation of enhanced norepinephrine uptake in the ischemic myocardium[119], may be relevant. Apparently, the more pronounced alpha-adrenergic blocking effects are not able to counteract the effect of the beta blocking part of labetalol at this stage. The second explanation for the reduction in coronary vascular resistance relates to the equally more pronounced reduction in myocardial oxygen demand, also observed in the high dose group. Consequently, there might be less need for coronary flow. However, if this were true, one would also expect myocardial oxygen consumption to be less, which did not occur. Moreover, pacing-induced myocardial ischemia should be less in the high dose group. This again did not occur. Objective signs of myocardial ischemia were reproducible in the high dose group (Fig. 9). In contrast, the low dose patients demonstrated a significant improvement in myocardial lactate metabolism, whereas ST-segment depression was less, indicating anti-ischemic effects with the low but not with the high dose.

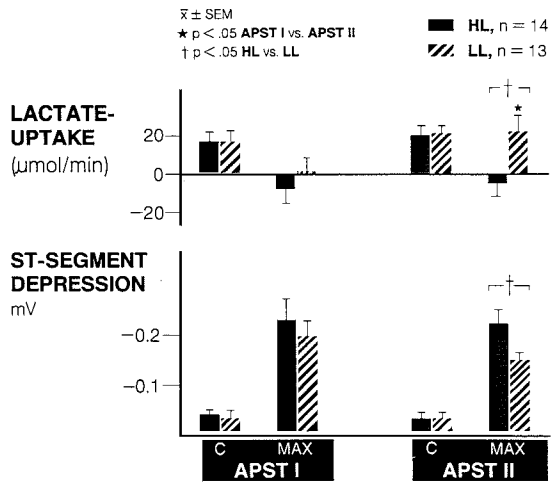


*Fig. 9: Antiischemic effects of low dose labetalol (LL) during 2 successive pacing tests (APST I and II, resp.), indicated by significant improvement of myocardial lactate metabolism. In contrast, no such effects are observed with the high dose patients (HL).*

This observation favours the concept that beta blocking agents may limit the necessary decrease in coronary vascular resistance during pacing-(and possibly exercise-)induced stress in the post stenotic area.

This example identifies one of the major advantages of the atrial pacing stress test protocol, its potential to examine the underlying pathophysiologic mechanisms of ischemia in a well defined and controlled manner.

By using this technique we have been able to identify mechanisms, through which different types of vasoactive agents and specific neuro-humoral modulators may result in acute anti-ischemic effects in the resting patient.

In this respect amiodarone, bepridil and diltiazem have been studied. Results are presented in chapter VIII. Moreover, we have differentiated the effects of some of these compounds in patients with a normal versus depressed cardiac pump function (see chapter IX).

The latter may be clinically important as it helps identifying the responsiveness to and tolerability of these drugs in patients with severe ischemia at rest, in whom ventricular function usually is depressed.

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# Part 1

## Myocardial metabolic changes during acute ischemia in man. Relation to coronary flow and potential for evaluating pharmacological interventions.

## B. Specific studies.

# Chapter IV:
# ADENINE NUCLEOTIDE METABOLISM AND ISCHEMIA.

# IV.1.

# Myocardial Nucleoside and Carbohydrate Metabolism and Hemodynamics During Partial Occlusion and Reperfusion of Pig Coronary Artery.

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# Myocardial Nucleoside and Carbohydrate Metabolism and Hemodynamics During Partial Occlusion and Reperfusion of Pig Coronary Artery

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J. W. DE JONG, P. D. VERDOUW AND W. J. REMME. Myocardial Nucleoside and Carbohydrate Metabolism and Hemodynamics During Partial Occlusion and Reperfusion of Pig Coronary Artery. *Journal of Molecular and Cellular Cardiology* (1977) **9**, 297–312. The effect of local ischemia on myocardial metabolism and hemodynamics was studied in open-chested pigs. Coronary artery flow was reduced by 74% with a screw clamp for one hour, after which period the clamp was released. Arterial and local coronary venous samples were taken repeatedly during control, occlusion and reperfusion. During control, the myocardium extracted lactate (20%) and inosine (24%). No or minor arteriovenous differences were measured for glucose, potassium, inorganic phosphate and hypoxanthine. During ischemia, the heart consumed glucose (about 25%); lactate and inosine extraction decreased to minimal values of —126% and —642%, respectively. Hypoxanthine, potassium and inorganic phosphate showed relatively small changes in arteriovenous difference. Extraction patterns approached control values during release. Myocardial oxygen uptake decreased by 68% after occlusion. Immediately following release, uptake returned to control value, but subsequently it fell again by 20 to 30%. Extensive and long-lasting reactive hyperemia was observed during reperfusion. However, flow debt was only partially repaid. Throughout the experiment heart rate increased, and cardiac output and mean aortic pressure decreased. During occlusion, the peak derivative of left ventricular pressure decreased about 24%; peripheral resistance and left ventricular end-diastolic pressure increased 20 to 30%. These changes were only partially reversed during reperfusion. It is concluded that, in addition to changes in lactate metabolism, myocardial inosine production is a good marker for myocardial ischemia in the pig, because it correlates well with carbohydrate extraction and ventricular function, and the occlusion-induced changes are relatively large.

KEY WORDS: Myocardial ischemia; Inosine; Hypoxanthine; Lactate; Glucose; Inorganic phosphate; Potassium; Left ventricular pressure; Cardiac output; Peripheral resistance; Coronary constriction; Flow and oxygen debt; Contractility.

## 1. Introduction

Electrocardiographic and hemodynamic measurements have not been able to quantitatively delineate functional changes due to ischemic heart disease. Abnormalities of myocardial lactate metabolism can indicate the severity of this disease [*20*, *27*]. However, the usefulness of the latter has been questioned [*17*, *19*].

Brachfeld [*2*] and Cohen [*5*] summarize a number of conditions in which lactate changes were not associated with hypoxia. At a symposium of the World Health Organization [*24*] a search for other metabolic indicators of myocardial ischemia was suggested.

Catabolites of adenine nucleotides accumulate in the ischemic heart [*1, 7, 14, 25*]. Also the release of these compounds during ischemia has been demonstrated. Coronary venous and coronary sinus levels of inorganic phosphate increase after coronary artery occlusion in dogs [*11, 23, 28–30*]. Phosphate [*4*], adenosine [*12*] and hypoxanthine [*32*] release could be found from human hearts during angina induced by rapid atrial pacing. Pig hearts released some inosine and hypoxanthine after partial occlusion of a coronary artery [*8, 15*]. The present study was undertaken in order to compare the myocardial release of several ATP-catabolites and other metabolites, and relate these to changes in hemodynamic parameters during ischemia and reperfusion. Myocardial inosine release seems to be useful as a marker for ischemia of the pig heart.*

## 2. Materials and Methods

### *Animal preparation*

Male or female Yorkshire pigs (20 to 25 kg) were fasted for about 24 h. The animals were sedated with 120 mg azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium) i.m. Subsequently 150 mg metomidate (Hypnodil, Janssen Pharmaceutica) was administered *via* a vein on the dorsal surface of the ear [*21*]. The animals were intubated and connected to a Pulmonat respirator for artificial respiration with a mixture of 33% oxygen and 67% nitrous oxide, at a frequency of 12/min (tidal volume about 20 ml/kg). Ventilation was controlled by continuous monitoring of expired carbon dioxide (kept at about 4%) with a Capnograph (Jaeger, Würzburg, GFR) and intermittent measurement of arterial blood gases. Oxygen saturation was measured with an *in vitro* hemoreflectometer (American Optical Company, Framingham, Mass.). Arterial oxygen saturation was maintained above 95%. The temperature of the animals was kept between 35° and 37°C with a heating pad and infrared lamps. ECG leads I, II and III were recorded throughout the experiment. A single 8F Cournand catheter was positioned in the thoracic aorta *via* the left femoral aretery. The lumen was used for central aortic pressure measurements by connecting it to a P23Db Statham pressure transducer, and for the withdrawal of blood samples for the determination of blood gases and biochemical parameters. Through the right femoral vein a 7F triple lumen balloon-tipped thermodilution catheter (Edwards, Santa Anna, Ca.) was positioned in the pul-

* Part of this work has been presented at the 25th Annual Scientific Session of the American College of Cardiology, New Orleans, La., 1976 [*9*].

monary artery for the determination of cardiac output [*13*]. Through the left carotid artery an 8 MMC Dallons-Telco catheter (Thomson, Paris, France) with a frequency response flat to 1000 Hz was placed in the left ventricle for the recording of left ventricular pressure. From the right jugular vein an 8F Cournand catheter was placed in the right atrium for the administration of anesthetics consisting of a mixture of 2 mg $kg^{-1}$ $h^{-1}$ azaperone and 8 mg $kg^{-1}$ $h^{-1}$ metomidate [*21*]. The catheter was connected intermittently to a P23Db Statham transducer for the determination of right atrial pressure.

### *Surgical procedure*

The left ventricle was exposed by means of a midsternal incision and sufficient parts of the cleaved fourth and fifth ribs were removed to get easy access to the left anterior descending coronary artery (LAD) and accompanying vein. The LAD was then dissected free from its origin to its first branch. This allowed for about 1 cm of free artery around which an electromagnetic flow probe (Skalar, Delft, The Netherlands; 20 to 25 mm in diameter) was applied. Distal to this flow probe, but before the first branch, a J-shaped screw clamp was placed. Zero flow was obtained by occluding the LAD with an atraumatic forceps. In 12 pigs the accompanying cardiac vein was cannulated with a polyethelene catheter (internal diameter 1.0 mm) with its tip in the zone expected to become ischemic. Care was taken for a free runoff of cardiac venous blood. The blood was collected and returned to the animal. After dissection of the vessels a total of 3 ml lidocaine was applied to prevent spasm. The system was flushed with about 3000 units heparin.

Three sham-operated animals served as control. Blood sampling and hemodynamic measurements were carried out as in the other animals.

In a third group of six animals also the ramus circumflex was dissected and a flow probe was placed around the vessel. Mechanical interference prevented cannulation of the vein accompanying the circumflex, when flow probes were placed around the left anterior descending and the circumflex. No biochemical data were obtained from these experiments.

### *Hemodynamic measurements*

All tracings were recorded on a Siemens Oscillomink B recorder. Cardiac output recordings were made in duplicate. Between the cardiac output determinations, left ventricular pressure, its first derivative (LV$dP/dt$) and aortic pressure were recorded at high speed. During this recording the ventilator was switched off to avoid baseline drift due to respiratory assist. From the tracings the following parameters were obtained: left ventricular end-diastolic pressure (LVEDP), maximum rate of rise of left ventricular pressure (peak LV$dP/dt$) and mean aortic pressure.

For an accurate assessment of LVEDP, the gain of the left ventricular pressure signal was magnified five times during part of the recording. Heart rate was counted from the ECG, while peripheral resistance was calculated from:

$$\text{peripheral resistance} = \frac{\text{mean aortic pressure—right atrial pressure}}{\text{cardiac output}}.$$

*Biochemical measurements*

Blood samples were taken and handled as described earlier [*8*]. Plasma glucose [*36*], potassium [*37*] and inorganic phosphate [*38*] were assayed on an AutoAnalyzer II (Technicon, Tarrytown, NY) with Technicon SMA reference serum/CPK as the standard. The following determinations were performed in duplicate. Lactate was assayed enzymically in deproteinized acid samples on the AutoAnalyzer [*8*]. Standard curves were made with lithium lactate in 4% $HClO_4$. Inosine and hypoxanthine were determined at room temperature (22 °C) in neutralized samples as described before [*8*]. Samples containing less than 5 μM inosine were assayed according to Olsson [*25*].

*Experimental protocol*

After a stabilization period of 30 min control hemodynamic measurements were performed and simultaneously blood samples drawn from the descending aorta and the local coronary vein. Subsequently the mean LAD blood flow was reduced to about 25% of control by tightening of the screw clamp. The clamp was adjusted, when needed, to control the flow at a stable level during the entire ischemic period of 60 min. During ischemia, the same parameters were recorded and samples collected as during the control run. Subsequently the clamp was released. All hemodynamic measurements were carried out again and blood samples were collected for 50 min.

*Statistical analysis*

Student's paired *t*-test (two-tailed) was used. $P > 0.05$ was considered to be not significant (N.S.). *P*-values are given for comparison with the mean control value, unless otherwise indicated.

## 3. Results

*Ventricular arrhythmias*

Ventricular arrhythmias were observed frequently in the first half hour after the flow was reduced by about 75%. After 30 min ventricular arrhythmias became

rare. Ventricular fibrillation occurred in 25% of the pigs, all within the first half hour. No attempts were made to defibrillate these animals and they were excluded from the study. Complete occlusion of the left anterior descending coronary artery always causes ventricular fibrillation of pig heart within 30 min [see also ref. *33*], which excludes complete ligation in the open-chested anesthetized pig as a model for the study of longer periods of ischemia.

### *Coronary blood flow*

In all six animals in which the flows through the left anterior descending coronary artery and the ramus circumflex were measured simultaneously, the LAD flow was dominant: it was 2 to 2.5 times the circumflex flow. During the LAD occlusion period (up to one hour) there was no increase in circumflex flow. Also no change in LAD flow and in the oxygen saturation of its accompanying vein was found, when the circumflex was occluded for as long as one hour.

Figure 1 shows the time course of LAD flow during the experiment. Partial occlusion of this artery resulted in a decrease in coronary blood flow from 26.5 ±

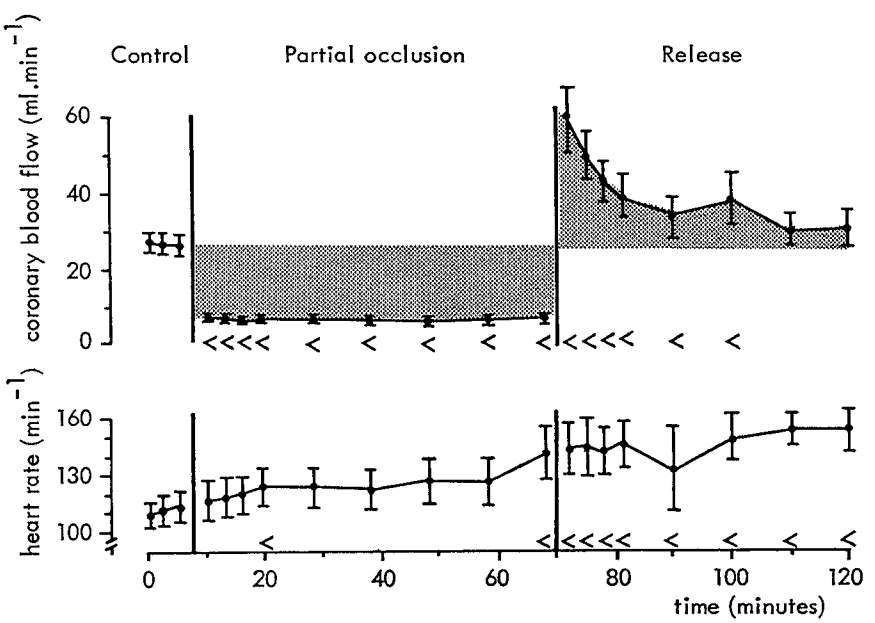


FIGURE 1. Time course of coronary blood flow and heart rate during control, partial occlusion of the LAD, and release. Mean values ± S.E. are presented ($n$ = 6–9). < indicates $P < 0.05$. The shaded areas during occlusion and release represent the flow debt and reactive hyperemic flow ("repayment"), respectively.

2.3 (mean ± S.E.) to 7.0 ± 0.4 ml/min ($P < 0.001$). After release of the clamp, extensive and long-lasting reactive hyperemia was observed. Peak hyperemic flow was reached after about 2 min (58.8 ± 8.7 ml/min, $P < 0.01$). Flow debt [*26*] during the occlusion period was 1170 ml, while the reactive hyperemic flow turned out to be 375 ml. Consequently there was no total repayment of the flow debt as (reactive hyperemic flow)/(flow debt) was only 0.32.

At the end of the experiment the flow values approached the control level.

### *Heart rate*

Heart rate (Figure 1) increased gradually from 113 ± 7 to 142 ± 13 beats/min ($P < 0.05$) during ischemia and continued to rise during the release period to 155 ± 11 beats/min ($P < 0.01$).

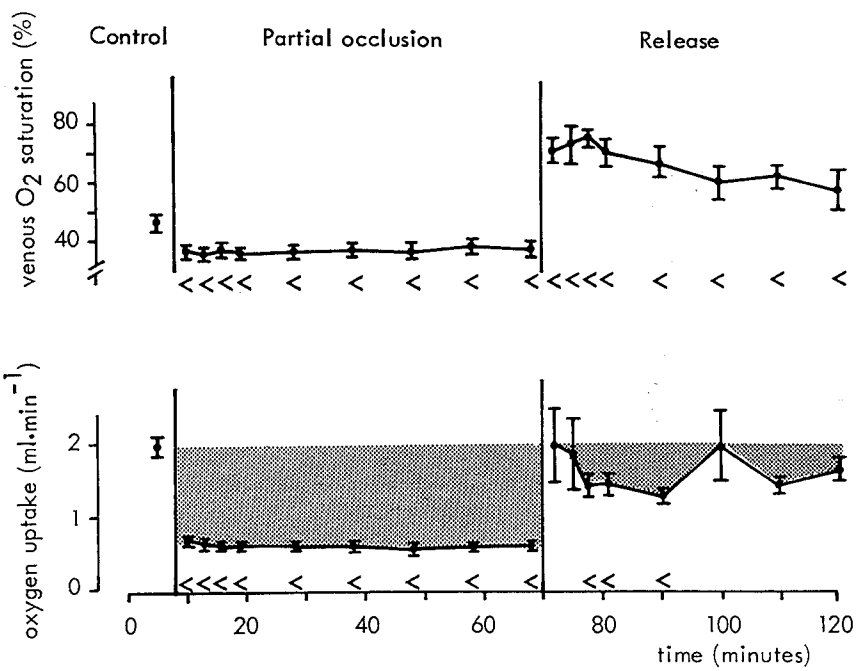


FIGURE 2. Coronary venous oxygen saturation ($O_2$ sat) and myocardial oxygen uptake during control, occlusion and reperfusion ($\bar{X}$ ± S.E., $n$ = 4–9). Oxygen uptake was calculated as (A–V) $O_2$ sat × coronary flow × [hemoglobin] × hemoglobin-bound $O_2$. No correction was made for physically dissolved oxygen. Mean arterial $O_2$ sat was 97% throughout the experiment, and mean hemoglobin concentration was 120 g/l. Hemoglobin binding capacity was taken to be 1.34 ml $O_2$/g hemoglobin. < indicates $P < 0.05$. The shaded area during occlusion represents the oxygen debt, which is not repaid during release.

*Myocardial oxygen uptake*

Local venous oxygen saturation during control was 47.1 ± 3.2%. This was reduced to 35.9 ± 2.0% ($P < 0.005$) after 2 min of occlusion and remained decreased during ischemia (Figure 2). After release, oxygen saturation increased to 71.3 ± 4.1% ($P < 0.001$) and dropped gradually to 57.5 ± 5.9% ($P < 0.05$) at the end of the experiment.

Oxygen uptake decreased from 1.91 ± 0.09 ml/min during control to 0.62 ± 0.03 ml/min ($P < 0.001$) during the ischemic period (Figure 2). During reperfusion, there was a return to control value in the first minutes, but subsequently oxygen uptake dropped somewhat. Thus no repayment of oxygen debt was observed.

*Pressures, cardiac output and peripheral resistance*

Peak LVd$P$/d$t$ decreased about 24% during ischemia. The decrease was only statistically significant during the first half hour of occlusion (Figure 3). During reperfusion, 90% of control value was found (N.S.) A few minutes after occlusion LVEDP increased from 6.8 ± 0.9 to 8.7 ± 1.0 mmHg (N.S.). LVEDP remained at that level during the ischemic period. After release, LVEDP dropped to 5.8 ± 1.3 mmHg (N.S. *versus* control, $P < 0.05$ *versus* ischemic values). Mean aortic pressure decreased gradually during ischemia by 11% (Figure 3). Mean aortic pressure

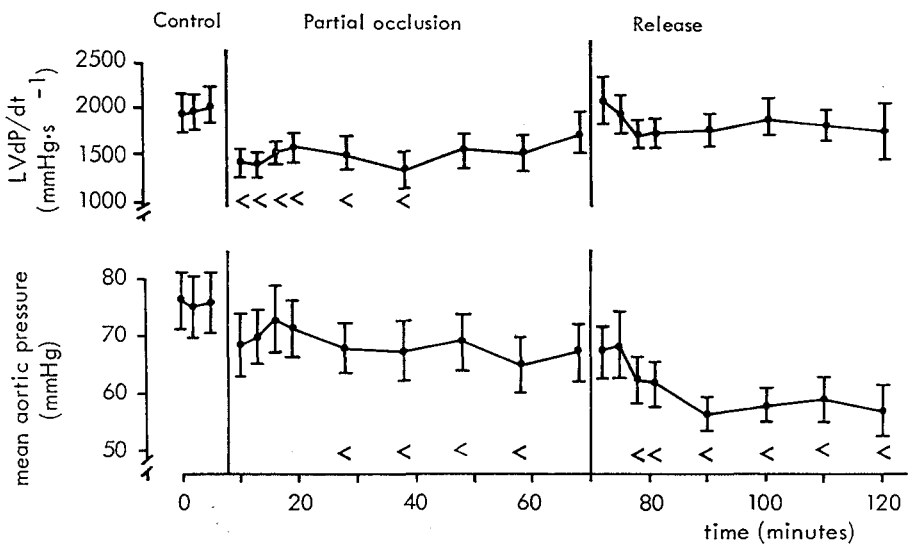


FIGURE 3. Changes in peak LVd$P$/d$t$ and mean aortic pressure during the experiment ($\bar{X}$ ± S.E., $n = 6$–9). < indicates $P < 0.05$.

continued to decrease during release: At the end of the experiment it was 76% of control.

A gradual decrease in cardiac output was observed during occlusion (Figure 4). The values remained decreased during reperfusion (64% of control). Peripheral resistance (Figure 4) increased gradually during occlusion. The resistance exceeded at the end of occlusion control values by 28% ($P < 0.005$); during release, it levelled off, but remained elevated.

*Biochemical changes*

The control value of arterial lactate (3.84 $\pm$ 0.52 mM) rose gradually to 6.29 $\pm$ 1.03 mM ($P < 0.001$) at the end of the experiment (Figure 5). Occlusion-induced changes in myocardial carbohydrate extraction were observed. Lactate extraction during control (20.4 $\pm$ 2.9%) decreased immediately, with minimal values ($-126 \pm 16\%$, $P < 0.001$) during the first 10 min of occlusion (Figure 5). During control, myocardial glucose consumption was of minor importance. After occlusion, however, the heart extracted about 25% ($P < 0.001$) of glucose (Figure 5). Both lactate and glucose extraction approached their control values at the end of release.

During control, inosine is extracted by the heart (Figure 6). The average venous control value (14.3 $\pm$ 2.5 $\mu$M) is different from the arterial one (17.9 $\pm$ 2.5 $\mu$M, $P < 0.001$). This inosine extraction (24.4 $\pm$ 6.1%) decreases, with a minimal value of $-642 \pm 116\%$ ($P < 0.001$) at 10 min of ischemia.

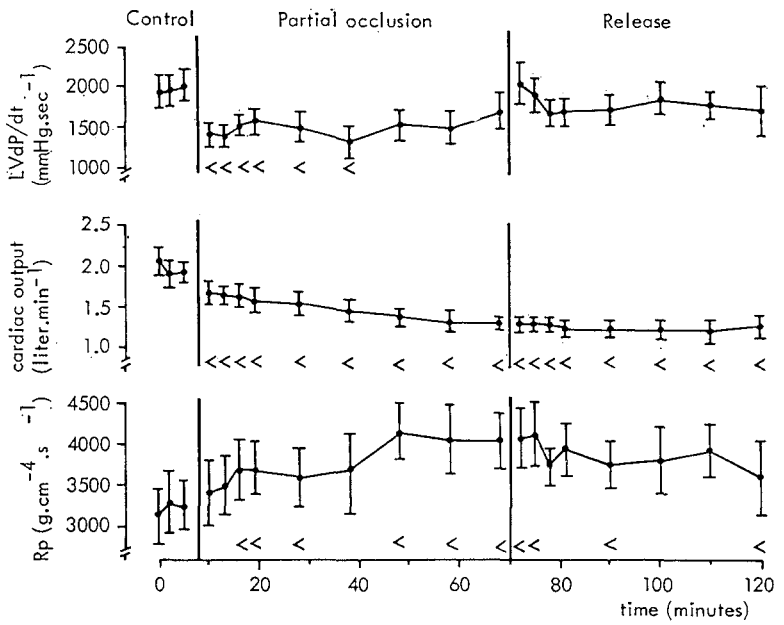


FIGURE 4. Cardiac output and peripheral resistance (*R*p) during the experiment ($\bar{X} \pm$ S.E. $n = 6$–9). < indicates $P < 0.05$.

Figure 6 also shows release of hypoxanthine from the heart during ischemia. At the end of release the coronary venous levels of both ATP-catabolites approach the arterial levels (which increase gradually during the experiment).

Figure 7 shows the changes in potassium and inorganic phosphate. The venous levels of both electrolytes exceed the arterial values somewhat during control. During occlusion, venous potassium increases rapidly by 50% ($P < 0.001$) followed by a return towards the arterial level. The elevation in venous inorganic phosphate is most pronounced during the first 15 min of occlusion (up to 35%, $P < 0.001$); a gradual decrease is observed thereafter. Venous electrolyte levels approach arterial values during release.

### *Correlations*

In Table 1 correlations between several parameters are given. High correlations ($r = |0.94—0.97|$) were observed for the arteriovenous difference of inosine and

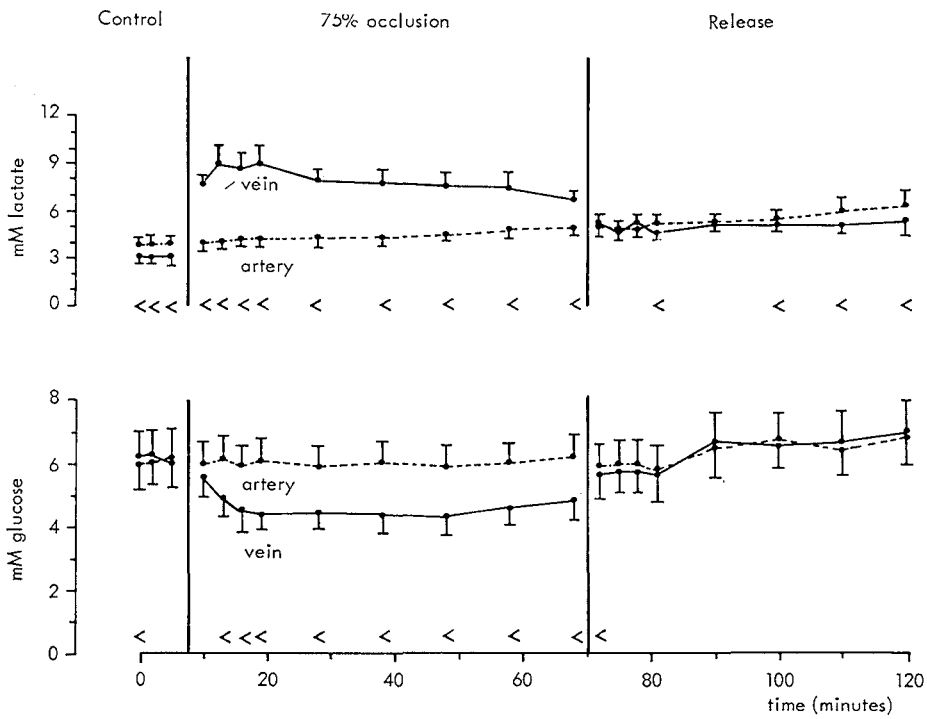


FIGURE 5. Lactate production and glucose consumption by the ischemic heart. The arterial (● – – ●) and coronary venous (●——●) concentrations of whole blood lactate and plasma glucose are indicated (mean with one S.E., $n = 6$–9). < indicates $P < 0.05$ for comparison of coronary venous with arterial levels.

hypoxanthine with lactate and glucose extraction. Figure 8 shows the correlation between coronary venous inosine and myocardial lactate extraction ($r = -0.93$). Lactate and glucose extraction correlated significantly less with the arterio-venous difference of potassium ($r = 0.74$ and $-0.52$, respectively, Table 1). The same table shows the correlation between several biochemical parameters and LVd$P$/d$t$. Relatively high degrees of correlation were found for expressions of carbohydrate, inosine or inorganic phosphate utilization *versus* LVd$P$/d$t$. Such a correlation was absent *versus* peripheral resistance.

## 4. Discussion

Left anterior descending flow exceeds circumflex flow 2 to 2.5 times. This is in agreement with the findings of Schaper [*33*], who injected pig coronaries with an epoxy-polymer; the LAD cast weight was double the circumflex cast weight. Thus, the LAD provides more blood to the pig left ventricle than the circumflex does. The

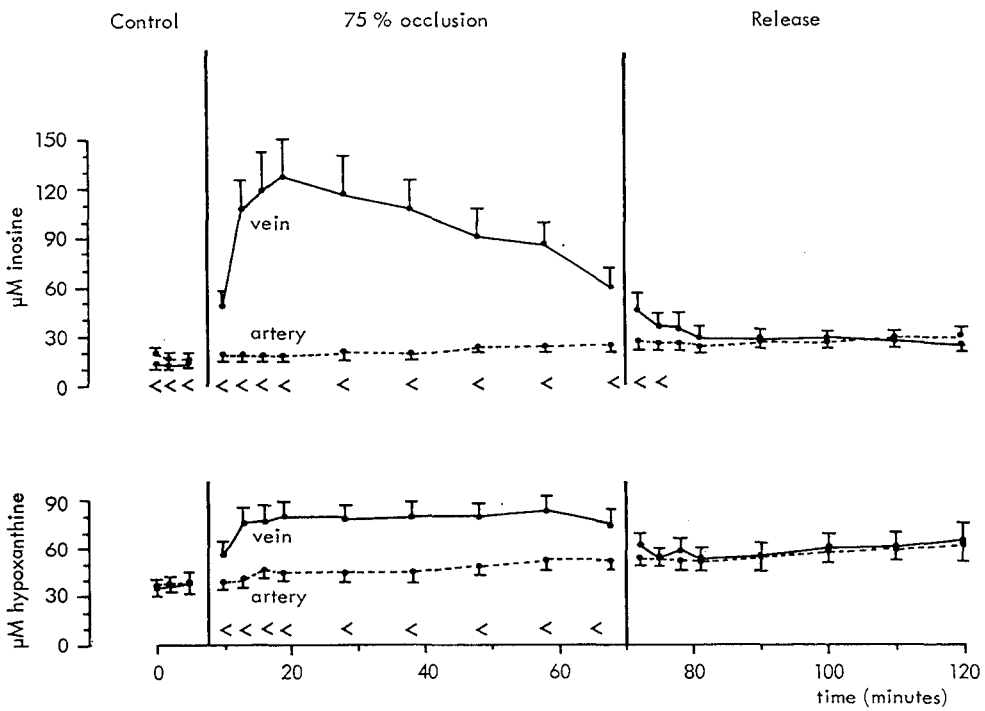


FIGURE 6. Inosine and hypoxanthine release from the ischemic heart. Whole blood arterial (● – – ●) and coronary venous (●——●) levels of both ATP-catabolites are shown (mean with one S.E., $n = 7$–9). < indicates $P < 0.05$ for comparison of venous with arterial levels.

TABLE 1. Correlation between changes in myocardial metabolite levels with lactate and glucose extraction and the first derivative of left ventricular pressure, LVd*P*/d*t*. Correlation coefficients (*r*) were calculated for the mean values ($n = 20$, obtained during control, occlusion and release) in 9 pigs, of extraction or arterio-venous difference (A–V) of several biochemical parameters with lactate and glucose extraction and LVd*P*/d*t*

| | *Versus* lactate extraction | | *Versus* glucose extraction | | *Versus* LVd*P*/d*t* | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Lactate extraction | — | — | —0.87 | <0.001 | 0.86 | <0.001 |
| Glucose extraction | —0.87 | <0.001 | — | — | —0.75 | <0.001 |
| A–V inosine | 0.94 | <0.001 | —0.94 | <0.001 | 0.77 | <0.001 |
| A–V hypoxanthine | 0.94 | <0.001 | —0.97 | <0.001 | 0.58 | <0.005 |
| A–V potassium | 0.74 | <0.001 | —0.52 | =0.001 | 0.65 | <0.001 |
| A–V inorganic phosphate | 0.94 | <0.001 | —0.77 | <0.001 | 0.74 | <0.001 |

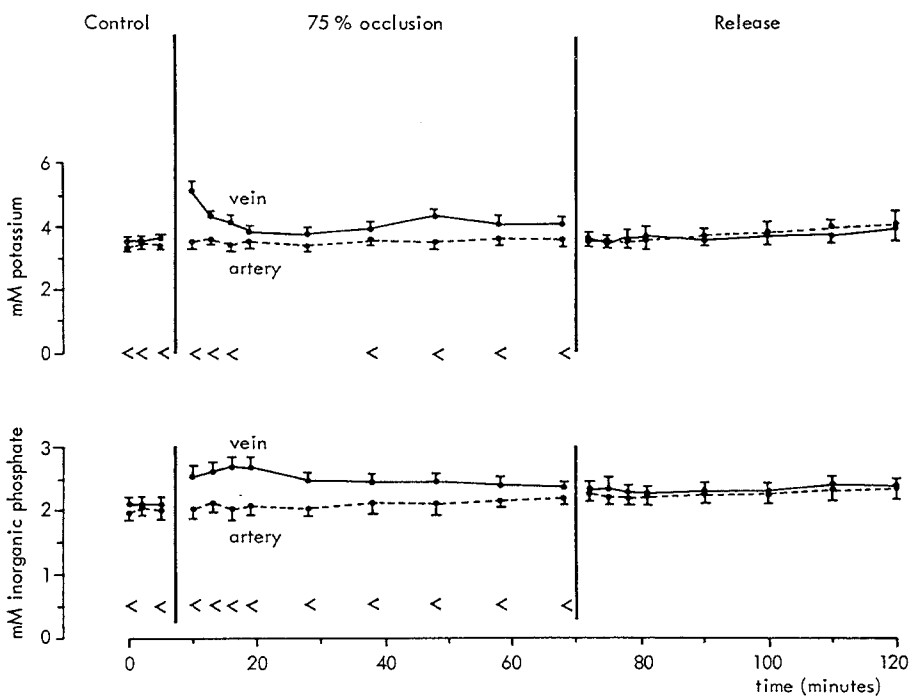


FIGURE 7. Release of potassium and inorganic phosphate from the ischemic myocardium. Plasma arterial (● – – ●) and coronary venous (●——●) concentrations are shown (mean with one S.E., $n = 7$–9). < indicates $P < 0.05$ for comparison of venous with arterial levels.

right coronary artery is of the same size as the left anterior descending coronary artery. These distributions resemble more closely the situation in man than the dog heart does [*3*, *33*].

A number of investigators emphasized that the metabolic consequences of coronary artery occlusion are best studied by assay of samples draining the zone of ischemia [see, for instance, refs. *6*, *11*, *30*]. In an earlier study [*8*] we reduced the *peak* flow in the LAD by 50%, which caused an average initial increase in coronary venous inosine of 70%. However, in 7 out of these 14 pigs increases in coronary venous inosine were below 35%. In the present study *mean* flow in the LAD was reduced by 74% resulting in a remarkable increase in the release of inosine (Figure 6). Within 5 min of partial occlusion coronary venous inosine exceeded the arterial level by more than 230% in all pigs. This release of inosine is accompanied by excessive production of lactate (Figure 5). Coronary venous inosine levels and myocardial lactate extraction correlate very well, except for the values found within 2 min of occlusion: the initial change of lactate extraction to lactate production is more pronounced than that of inosine (Figures 5, 6 and 8). This difference during the first few minutes of ischemia could be due to the fact that lactate is an end-product (of anaerobic glycolysis), whereas inosine can be catabolized. Inosine and lactate production reach a maximum at about 10 min of ischemia and gradually level off thereafter. It is doubtful that the decrease of inosine and lactate production

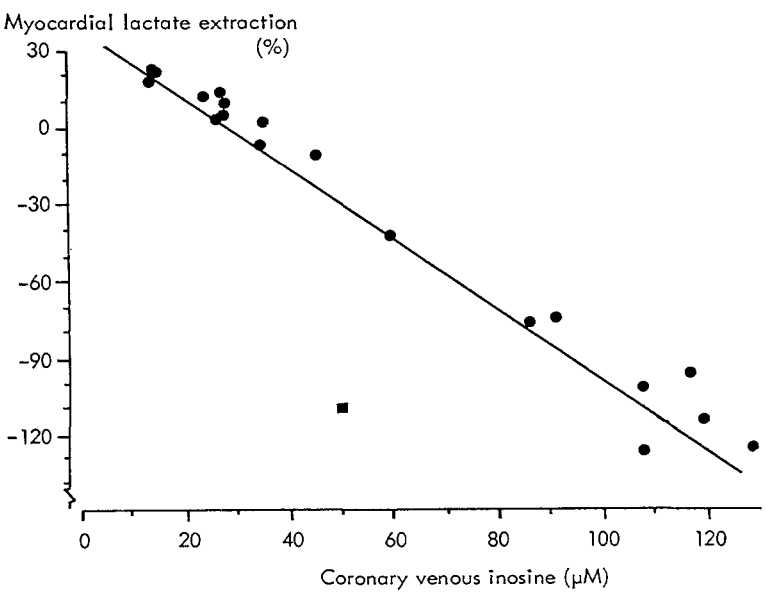


FIGURE 8. Correlation between the mean values of lactate extraction and venous inosine concentration during control, partial occlusion and reperfusion. (■) Value found 2 min after occlusion. $r = 0.93$, $P < 0.001$, $n = 20$ (9 pigs).

is due to the mobilization of collateral flow in the myocardium. The pig heart has a very poorly functioning collateral system; it has the ability to develop a sizable functional collateral circulation [*18*], but this is not likely to occur within one hour [*34*]. Measurements of microspheres or by casts are necessary to obtain more information on the collateral system under our experimental conditions.

It can be speculated that the extraction of inosine observed during control (24%) reflects an active salvage pathway of purine nucleosides in pig heart, *via* hypoxanthine, IMP and adenylosuccinate [*22*].

During control, lactate is the main substrate for the pig heart under these experimental conditions. If one assumes that the lactate extracted by the pig myocardium is converted to carbon dioxide and water, more than 70% of the myocardial oxygen consumption is accounted for. The extraction of glucose is only important during ischemia; during control, insignificant amounts of glucose (and free fatty acids, unpublished observations) are extracted. Myocardial utilization of carbohydrates depends on the arterial concentration of a variety of substrates of the heart (fatty acids, ketone bodies, lactate, glucose). The concentration of substrates may vary considerably: for instance, under azaperone-metomidate anesthesia arterial lactate levels are 3 to 4 times higher than those observed under pentobarbital [*8*], fentanyl [*10*] or halothane [*10*] anesthesia. Brachfeld [*2*] pointed out that despite great care, one may still fail to obtain evidence of ischemia by reliance on evidence of anaerobic glycolysis alone. Throughout the study coronary venous inosine or its arteriovenous difference correlates very well with lactate extraction and glucose extraction (Table 1, Figure 8). Change in arteriovenous inosine difference appears to be useful as a (additional) parameter for myocardial ischemia in the species used in this study. Changes in myocardial inosine metabolism also were found to correlate with changes in LV$dP/dt$ (Table 1). In earlier studies [*8*, *16*] a correlation between local venous inosine and the degree of myocardial wall thickening was observed during ischemia. In this study the myocardial ATP content was not measured. It is speculated that the nucleosides released during occlusion reflect depletion of ATP from a compartment involved in contraction.

Opie *et al.* [*28*, *29*] and Mathur and Case [*23*] occluded the LAD in the dog, which results in a reduction in flow to 12% of control [*34*]. Potassium and inorganic phosphate releases were much less evident than changes in lactate metabolism after LAD occlusion [*23*, *28*, *29*]. Also in the present study in which the LAD was partially occluded, release of these ions was small compared to changes in the myocardial metabolism of carbohydrates and nucleosides.

Arterial lactate, inosine and hypoxanthine rose gradually by 60 to 70%. At the same time heart rate increased throughout the experiment, whereas cardiac output and mean aortic pressure decreased. These changes indicate that the condition of the pigs deteriorated in the course of the experiment. This is further evidenced by our control studies with sham-operated animals in which these biochemical and hemodynamic parameters remained at their initial value. Peripheral resistance,

LV$dP/dt$ and LVEDP did not return to control values after the occlusion period, which also indicates that irreversible changes took place. As was shown recently [*31*, *35*], after (total) occlusion of longer duration, reperfusion failed to restore contractility to any significant extent.

During 50 min of reperfusion flow debt was only partially repaid and oxygen debt was not repaid (Figures 1 and 2). This contrasts findings with occlusions lasting up to minutes that oxygen and flow debt are invariably overpaid [see review, ref. *26*]. Sharma *et al.* [*35*] reported that the severe myocardial damage induced by total occlusion of canine LAD for 60 to 90 min was not reversed by reperfusion for one hour. They stated that longer periods of reperfusion may be beneficial. In our experiments no biochemical evidence was found for anaerobic myocardial metabolism after 50 min of reperfusion. Lactate was again utilized by the heart, and glucose extraction was nil. At the end of the experiment oxygen and lactate uptake were not significantly different from control values.

It is concluded that, in addition to changes in lactate metabolism, myocardial nucleoside production is a good marker of myocardial ischemia, because it correlates well with carbohydrate extraction and the occlusion-induced changes are relatively large. Changes in contractile properties of the heart correlate with changes in myocardial lactate and inosine metabolism.

*Acknowledgements*

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## IV.2.

# Effects of Pacing-Induced Myocardial Ischemia on Hypoxanthine Efflux from the Human Heart.

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# Effects of Pacing-Induced Myocardial Ischemia on Hypoxanthine Efflux From the Human Heart

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**Inosine and hypoxanthine are useful markers of early myocardial ischemia in pigs. In this study the applicability of the products of adenosine triphosphate (ATP) breakdown as markers of myocardial ischemia in man was investigated in 25 patients undergoing diagnostic cardiac catheterization for suspected coronary artery disease. Pacing-induced myocardial ischemia resulted in elevated coronary sinus hypoxanthine levels only after the onset of angina in 18 patients (from 1.2 ± 0.2 μmolar [mean ± standard error of the mean] during the control period to 2.4 ± 0.5 μmolar during ischemia, $P < 0.05$), whereas lactate production often occurred before angina was noted. Neither hypoxanthine nor lactate levels changed in seven patients without angina. No significant pacing-induced alterations were found in plasma potassium, inorganic phosphate, glucose or oxygen saturation in either group of patients. No hemodynamic variable, except postpacing left ventricular end-diastolic pressure, served as a useful diagnostic marker separating patients with and without myocardial ischemia. Significant S-T changes during maximal heart rates occurred in only 12 of the 18 patients with angina and in none of those without angina. We conclude that release of hypoxanthine is a useful indicator of pacing-induced ischemia in the human heart.**

Protection of the ischemic myocardium with pharmacologic, mechanical or surgical interventions has been a major goal of cardiac research in recent years. Sensitive markers of the degree of ischemia are needed to evaluate the effects of subsequent interventions. Sudden hypoxia at the cell level results within seconds in increased anaerobiosis accompanied by a precipitous decrease in the high energy compound creatine phosphate and followed by a breakdown of adenosine triphosphate (ATP).[1,2] This enhanced degradation of ATP causes increased efflux of the breakdown products that are able to pass the cell membrane—inorganic phosphate, adenosine, inosine and hypoxanthine.[3] In the pig heart a sudden reduction of coronary blood flow is associated with release of myocardial inosine and hypoxanthine[4] that reaches a maximum at 5 minutes of ischemia. Because lactate production reaches its peak value somewhat earlier,[5,6] lactate appears to be preferable as an early metabolic indicator of ischemia and has been used in many clinical studies to quantitate the amount of ischemia. However, its usefulness for this purpose has been questioned[7] because lactate extraction or production patterns may depend on the nutritional status of the patient, the blood levels of free fatty acids and the existence of diabetes mellitus or alkalosis.[8,9] Especially in studies of several hours' duration, in the first hours of myocardial infarction, for example, lactate metabolism may be affected by extracardiac factors. Thus metabolic markers that are less susceptible to extracardiac influences are needed.

Fox et al.[10] have shown that small amounts of adenosine are produced during pacing-induced ischemia in the human heart. However, no experiments have been reported on the release during myocardial ischemia

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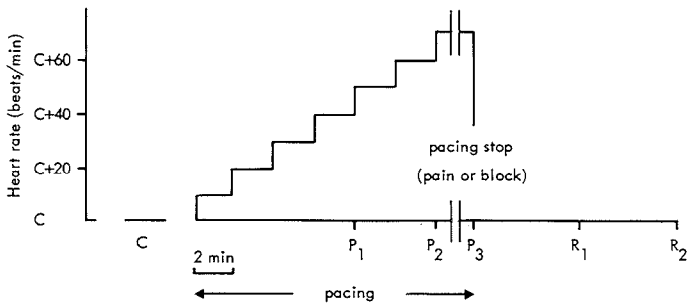


**FIGURE 1.** The pacing protocol. After control (C) determinations, pacing was started at a rate of 10 beats/min higher than control rhythm. The pacing rate was increased every 2 minutes by 10 beats/min until pacing had to be stopped (because of anginal pain or atrioventricular block). Samples obtained during the control period and during pacing ($P_1$, $P_2$ and $P_3$) and recovery ($R_1$ and $R_2$) periods were analyzed.

of the other products of ATP degradation such as inosine and hypoxanthine. We have extended our studies in the pig heart and investigated the release of hypoxanthine during pacing-induced ischemia in the human heart, comparing the possible value of hypoxanthine as an indicator of myocardial ischemia with that of other metabolic, hemodynamic and electrocardiographic variables.

## Materials and Methods

Twenty-five adults underwent cardiac catheterization for evaluation of coronary artery disease. In all patients the only manifestation of cardiac disease was angina pectoris for which they were taking nitroglycerin or beta adrenergic blocking drugs. The latter drugs were withheld 1 week before catheterization. No patient had clinical diabetes mellitus. Studies were carried out after an overnight fast and without premedication. No drug, other than 45 to 60 mg of heparin, was administered during the study.

*Right heart catherization* was performed with a no. 7F Swan-Ganz thermodilution catheter inserted by way of an antecubital vein. Measurements included pulmonary arterial and pulmonary wedge pressures, right ventricular and right atrial pressures and duplicate cardiac output measurements. After the measurements were completed the thermodilution catheter was replaced with a no. 7 bipolar Zucker catheter, which was positioned with its proximal electrode just inside the coronary sinus orifice, where it remained throughout the study. Its position was checked regularly with fluoroscopy.

TABLE I

**Summary of Clinical and Catheterization Data**

| Case no. | Age (yr) & Sex | Site of Old MI | Vessel Involved | Collateral Vessels | LV Contraction | S-T Segment Depression (mv) During Pain |
|---|---|---|---|---|---|---|
| | | | Group With Angina (no. = 18) | | | |
| 1 | 58M | — | LAD, RCA | + | ↓ | 0.2 |
| 2 | 46M | Ant | LAD, LCx | — | ↓ Ant | — |
| 3 | 46M | Inf | LAD, RCA | + | ↓ Apex | 0.1 |
| 4 | 49M | Post | LAD, LCx, RCA | + | Normal | — |
| 5 | 49M | — | LAD, LCx, RCA | + | ↓ Inf | 0.1 |
| 6 | 40M | — | LCx, diag, RCA | — | Normal | — |
| 7 | 52M | Ant | LAD, LCx, RCA | + | ↓ Inf | 0.2 |
| 8 | 41M | — | LAD | — | ↓ Ant | — |
| 9 | 56M | Ant, Inf | LMCA, LAD, LCx, RCA | — | ↓↓ Apex, Ant | — |
| 10 | 44M | — | LAD, diag, LCx, RCA | + | Normal | 0.1 |
| 11 | 51M | Ant, Inf | LAD, LCx, RCA | + | ↓↓ Apex | 0.2 |
| 12 | 51M | Inf-Post | LAD, LCx, RCA | — | ↓↓↓ | 0.5 |
| 13 | 38F | — | LAD, marg, LCx, RCA | + | Normal | 0.1 |
| 14 | 45M | — | LMCA, LAD, LCx | + | Normal | 0.2 |
| 15 | 53M | — | LAD, LCx, RCA | + | ↓↓ Apex, Ant | 0.1 |
| 16 | 40M | Ant | LAD, LCx, RCA | + | ↓↓ Ant | — |
| 17 | 46F | Ant | LAD | + | ↓ Apex | 0.2 |
| 18 | 58M | — | LAD, LCx, marg, RCA | + | Normal | 0.1 |
| | | | Group Without Angina (no. = 7) | | | |
| 19 | 49M | — | — | — | Normal | — |
| 20 | 50M | — | — | — | Normal | — |
| 21 | 42M | Post-Inf | LCx, RCA | — | ↓ Inf | — |
| 22 | 46M | — | — | — | Normal | — |
| 23 | 34M | — | LAD | — | ↓ Apex | — |
| 24 | 34M | — | — | — | Normal | — |
| 25 | 40M | — | — | — | Normal | — |

Ant = anterior; diag = diagonal branch; Inf = inferior; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; LMCA = left main coronary artery; marg = first marginal branch; MI = myocardial infarction; Post = posterior; RCA = right coronary artery.
+ = present; — = absent; ↓, ↓↓, ↓↓↓ indicate increasing degrees of abnormality of the left ventricular contraction pattern.

This catheter was used for atrial pacing and sampling of coronary sinus blood. Through a right brachial arterial cutdown procedure a tip micromanometer catheter (Dallons-Telco MMC no. 8F or Millar Instruments no. 7F) was positioned in the left ventricle for left ventricular pressure recording and sampling of arterial blood.

**Pacing procedure:** A 15 minute stabilization period was allowed after the catheters were positioned. For control measurements arterial and coronary blood samples were simultaneously drawn, three electrocardiographic leads (I, aVF and $V_2$), were recorded and left ventricular pressure and its first derivative (dP/dt) were determined. Subsequently atrial pacing was started at a rate 10 beats/min higher than the patient's own control rate. Every 2 minutes the pacing rate was increased by 10 beats/min until patient had anginal pain or the occurrence of atrioventricular (A-V) block made further pacing impossible. Blood samples and hemodynamic and electrocardiographic measurements were taken at 4 minute intervals after the onset of pacing. The last determination during pacing ($P_3$ in Fig. 1) was always made when cessation of pacing was thought necessary and thus might be obtained within 4 minutes after collection of the previous data ($P_2$ in Fig. 1). After 5 and 10 minutes of recovery ($R_1$ and $R_2$, respectively) these determinations were repeated. Cardiac catheterization was completed with left ventriculography and selective coronary angiography.

**Biochemical measurements:** Blood (1.5 ml) for hypoxanthine and lactate measurements was rapidly deproteinized with an equal volume of cold 8 percent perchloric acid ($HClO_4$) and centrifuged. In the supernatant, lactate was assayed[11] enzymatically in duplicate on an AutoAnalyzer II (Technicon, Tarrytown, New York). (Standard curves were made with lithium lactate in 4 percent $HClO_4$.) Hypoxanthine was determined according to the method of Olsson.[2] Approximately 2.5 ml of blood was collected for the assay of plasma glucose, potassium and inorganic phosphate[12] on the AutoAnalyzer II with Technicon SMA reference serum/creatine kinase as the standard. Oxygen saturation was determined in duplicate on a hemoreflectometer (American Optical Co., Framingham, Massachusetts).

**Hemodynamic measurements:** During each recording period left ventricular pressure tracings of 12 successive beats were used to determine left ventricular systolic pressure, left ventricular end-diastolic pressure and maximal rate of rise and decline of left ventricular pressure (maximal and minimal dP/dt, respectively). A modified tension-time index, the product of heart rate and left ventricular systolic pressure, was used as an indication of myocardial oxygen consumption.

**Electrocardiogram:** S-T segment depression of 0.1 mv or more was considered indicative of myocardial ischemia.

**Statistical analysis:** Values are expressed as mean values ± standard error of the mean. Comparisons between the different periods were evaluated in each patient using Student's $t$ test for paired data (two-tailed). The significance of differences between the groups with and without angina was determined using Student's $t$ test for unpaired data. A value of $P > 0.05$ was considered not significant.

## Results

In 18 of the 25 patients studied, atrial pacing was discontinued because of progressive chest pain (group with angina, Table I). All 18 of these patients had significant obstruction (more than 50 percent of luminal diameter) in one or more coronary vessels; none had single right coronary artery disease. Left ventriculography (single plane determinations in the right anterior oblique projection) showed hypokinetic or akinetic segments of the left ventricular wall in 10 patients; an aneurysm was observed in 2 others (Cases 3 and 9). No patient had mitral insufficiency. In the seven patients without angina pacing was stopped before angina developed because of the occurrence of A-V block. One of the seven (Case 21) had significant two vessel disease and one (Case 23) had one vessel disease. The remaining five patients had a slight narrowing (25 to 50 percent) of one or more vessels. The pattern of ventricular contraction was abnormal in the two patients with significant vessel disease and normal in the other five. Thus,

TABLE II

Hemodynamic Data During Atrial Pacing and Recovery (mean ± standard error of the mean)

| | HR | LVSP | TTI X $10^{-3}$ | LVEDP | LV dP/dt max | LV dP/dt min |
|---|---|---|---|---|---|---|
| | | | Group With Angina (no. = 18) | | | |
| C | 78 ± 4 | 134 ± 4 | 10.5 ± 0.6 | 13.4 ± 1.3 | 1560 ± 50 | 1720 ± 120 |
| $P_1$ | 110 ± 11 | 123 ± 10 | 14.5 ± 0.7 | 7.0 ± 1.0‡ | 1810 ± 80* | 1980 ± 160* |
| $P_2$ | 123 ± 5 | 130 ± 4 | 15.8 ± 1.0 | 9.7 ± 1.9‡ | 1840 ± 80* | 1880 ± 160 |
| $P_3$ | 133 ± 4 | 131 ± 5 | 17.1 ± 0.8 | 12.0 ± 2.0 | 1910 ± 110* | 1820 ± 160 |
| $R_1$ | 77 ± 4 | 134 ± 5 | 10.3 ± 0.7 | 11.8 ± 1.5 | 1630 ± 90 | 1680 ± 140 |
| $R_2$ | 77 ± 5 | 136 ± 4 | 10.3 ± 0.7 | 12.6 ± 1.5 | 1590 ± 80 | 1650 ± 140 |
| | | | Group Without Angina (no. = 7) | | | |
| C | 70 ± 3 | 136 ± 8 | 9.4 ± 0.5 | 12.3 ± 1.2 | 1710 ± 150 | 1790 ± 100 |
| $P_1$ | 107 ± 4 | 129 ± 10 | 13.2 ± 0.8 | 5.7 ± 1.8† | 1850 ± 170 | 1810 ± 110 |
| $P_2$ | 129 ± 5 | 126 ± 9 | 16.0 ± 0.8 | 5.5 ± 2.4† | 2000 ± 200* | 1820 ± 120 |
| $P_3$ | 143 ± 7 | 127 ± 12 | 16.6 ± 1.2 | 8.7 ± 2.6† | 2300 ± 200* | 1920 ± 160 |
| $R_1$ | 73 ± 4 | 136 ± 12 | 9.8 ± 0.6 | 12.9 ± 2.3 | 1710 ± 140 | 1860 ± 130 |
| $R_2$ | 75 ± 3 | 137 ± 12 | 11.2 ± 1.0 | 12.6 ± 3.0 | 1750 ± 140 | 2000 ± 200 |

*$P < 0.05$; †$P < 0.02$; ‡$P < 0.001$, versus control.

C = control; HR = heart rate (beats/min); LVdP/dt max and LVdP/dt min = peak positive and negative value of the first derivative of left ventricular pressure (mm Hg/sec); LVEDP = left ventricular end-diastolic pressure (mm Hg); LVSP = left ventricular systolic pressure (mm Hg); $P_1$, $P_2$, $P_3$ = three levels of pacing rates; $R_1$, $R_2$ = recovery, 5 and 10 minutes after cessation of pacing (see Fig. 1); TTI = modified tension-time index (heart rate X left ventricular systolic pressure [mm Hg/min] ).

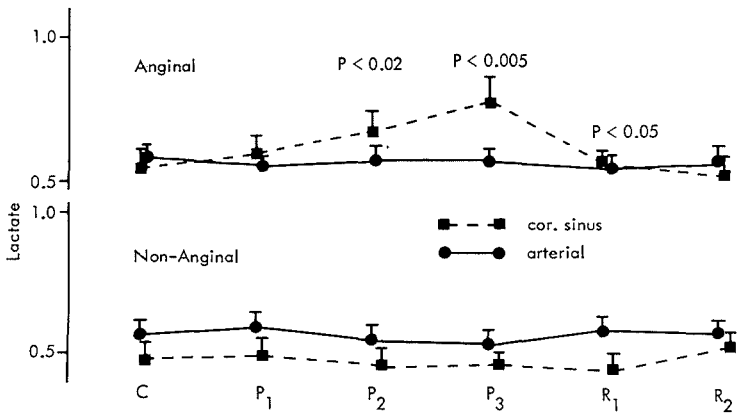


**FIGURE 2.** Arterial and coronary (cor.) sinus lactate levels (mmolar) during control (C), pacing ($P_1$,$P_2$,$P_3$) and recovery ($R_1$,$R_2$) periods. In the group without angina the arteriovenous difference in lactate remained unchanged, whereas in the group in whom pacing was stopped because of angina lactate extraction changed to lactate production. Probability (P) values indicate a significant difference between coronary sinus levels and control values.

these seven patients had less coronary arterial obstruction than the group with angina.

**Hemodynamic findings (Table II):** There were no significant differences between the groups with and without angina in the control values for any hemodynamic variable. Heart rates in the two groups of patients were similar in each of the three pacing periods. Left ventricular systolic pressure did not change throughout the study. Therefore the changes in the modified tension-time index merely reflect the changes in heart rate. Left ventricular end-diastolic pressure was decreased in both groups during pacing. Immediately after pacing was stopped there was an abrupt increase in left ventricular end-diastolic pressure to values that were different ($P < 0.05$) for the patients with (20 ± 2 mm Hg) and without (12.0 ± 1.6 mm Hg) angina. However, after 5 minutes of recovery, values returned to control level in both groups. Maximal left ventricular dP/dt increased in both groups during pacing. Although the increments were less in the group with angina, the differences were not significant. The maximal rate of decline of left ventricular pressure (minimal dP/dt) remained unchanged in the group without angina but increased during pacing period $P_1$ in the group with angina (Table II).

**Metabolic findings:** *Lactate:* Arterial lactate levels were the same in both groups of patients and remained constant throughout the study (Fig. 2, Table III). During the control period there was lactate extraction in both groups. Lactate extraction remained constant throughout the pacing and recovery periods in the 7 patients without angina. In the 18 patients with angina, there was a progressive increase in coronary sinus lactate levels during pacing, giving rise to lactate production in some patients during pacing period $P_1$. During period $P_2$ (Fig. 2) coronary sinus lactate levels of the group with angina were significantly elevated when compared with control values, but not when compared with the coronary sinus levels of the patients without angina. However, during angina ($P_3$) coronary sinus lactate increased to values that were different from those in the group without angina. Recovery values were not different from control values. A relatively large number of patients produced lactate before pain caused a cessation of pacing: Four of the 6 patients with lactate production during $P_1$ and 7 of the 11 patients who pro-

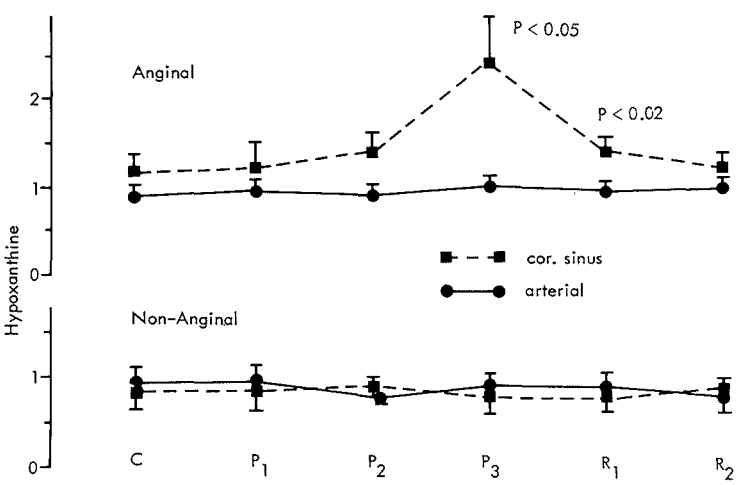


**FIGURE 3.** Arterial and coronary (cor.) sinus hypoxanthine levels (μmolar) during control (C), pacing ($P_1$,$P_2$,$P_3$) and recovery ($R_1$,$R_2$) periods. In the group without angina there were no arteriovenous differences. In the group with angina there was a sharp rise in coronary sinus levels during $P_3$. Probability (P) values indicate a significant difference between coronary sinus levels and control values.

duced lactate during $P_2$ were free of pain. The other patients felt some slight discomfort that was not considered severe enough to discontinue pacing.

*Hypoxanthine:* Arterial levels of hypoxanthine remained unchanged throughout the study in both groups (Fig. 3, Table III). Coronary sinus levels in the group without angina also remained unchanged and did not differ from arterial levels. Consequently no arteriovenous difference in hypoxanthine was observed at any time in the group without angina. By contrast, coronary sinus hypoxanthine concentrations increased gradually during pacing in the group with angina, differing significantly from the control value during the last pacing period ($P_3$, Fig. 3). At this time the coronary sinus levels in the group with angina were much higher than in the group without angina ($2.4 \pm 0.5$ versus $0.8 \pm 0.2$ μmolar, respectively, $P < 0.05$). During recovery there was a return toward control levels, although during the first recovery period ($R_1$) coronary sinus concentrations in the group with angina were still significantly higher than control levels. A large number of patients produced lactate before the occurrence of pain. However, only three patients had elevated coronary sinus hypoxanthine levels (2 μmolar or more) before pain caused cessation of pacing.

*Potassium:* The arterial potassium levels of both groups showed small but significant fluctuations (Fig. 4). This was especially true for the group without angina, who had significantly elevated levels during pacing. In the group with angina, the fluctuations were less marked and only became significant when the pacing had to be stopped because of pain. In spite of an initial increase in the potassium content of the coronary sinus blood in the group with angina there were no significant differences from the group without angina. Arteriovenous potassium differences did not provide any information that would aid in separating patients with and without angina.

*Glucose:* In both groups of patients small continuous increases in arterial and coronary sinus levels of glucose

TABLE III

**Metabolic Data Obtained in 25 Patients During Atrial Pacing**

| | Lactate (mmolar) | | | | | | | | Hypoxanthine (μmolar) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | | $P_2$ | | $P_3$ | | $R_1$ | | C | | $P_2$ | | $P_3$ | | $R_1$ | |
| Case no. | Art | CS | Art | CS | Art | CS | Art | CS | Art | CS | Art | CS | Art | CS | Art | CS |
| Group With Angina (no. = 18) | | | | | | | | | | | | | | | | |
| 1 | 0.42 | 0.40 | 0.47 | 0.41 | 0.38 | 0.47 | 0.42 | 0.38 | 1.0 | 0.9 | 0.8 | 0.7 | 1.0 | 1.4 | 0.8 | 1.3 |
| 2 | 0.40 | 0.39 | 0.48 | 0.52 | 0.45 | 0.53 | 0.48 | 0.42 | 1.6 | 1.6 | 1.3 | 1.5 | 1.2 | 3.6 | 1.2 | 1.8 |
| 3 | 0.36 | 0.25 | 0.30 | 0.37 | 0.36 | 0.46 | 0.25 | 0.41 | 1.2 | 1.2 | 1.1 | 1.2 | 1.2 | 1.4 | 1.1 | 1.8 |
| 4 | 0.53 | 0.52 | 0.58 | 0.50 | 0.52 | 0.56 | 0.58 | 0.60 | 1.4 | 1.5 | 1.1 | 1.3 | 0.5 | 1.1 | 1.2 | 1.3 |
| 5 | 0.71 | 0.87 | 0.70 | 1.01 | 0.70 | 1.14 | 0.71 | 0.94 | 1.6 | 1.1 | 1.5 | 1.9 | 1.5 | 2.7 | 1.8 | 1.8 |
| 6 | 0.75 | 0.60 | 0.75 | 0.72 | 0.76 | 0.73 | 0.75 | 0.71 | 1.3 | 1.5 | 1.1 | 1.4 | 1.4 | 1.5 | 1.2 | 1.5 |
| 7 | 0.61 | 0.56 | 0.60 | 0.59 | 0.57 | 0.69 | 0.48 | 0.64 | 0.6 | 1.3 | 0.5 | 1.0 | 0.8 | 2.2 | 0.6 | 0.9 |
| 8 | 0.52 | 0.48 | 0.51 | 0.53 | 0.39 | 0.62 | 0.46 | 0.55 | 0.6 | 0.9 | 1.1 | 0.7 | 0.8 | 2.0 | 0.7 | 1.6 |
| 9 | 0.68 | 0.62 | 0.66 | 0.64 | 0.68 | 0.84 | 0.64 | 0.61 | 0.6 | 0.8 | 0.7 | 0.6 | 0.6 | 2.0 | 0.4 | 0.8 |
| 10 | 0.54 | 0.56 | 0.52 | 0.55 | 0.55 | 0.63 | 0.54 | 0.56 | 0.8 | 1.1 | 1.0 | 1.0 | 1.1 | 1.2 | 1.0 | 1.1 |
| 11 | 0.57 | 0.57 | 0.60 | 1.15 | 0.62 | 1.04 | 0.62 | 0.75 | 0.9 | 0.6 | 0.8 | 1.3 | 0.9 | 2.4 | 1.0 | 1.3 |
| 12 | 0.98 | 1.33 | 1.09 | 1.53 | 1.03 | 1.60 | 0.98 | 1.25 | 1.8 | 4.0 | 1.3 | 2.1 | 2.3 | 3.7 | 1.2 | 3.7 |
| 13 | 0.50 | 0.57 | 0.53 | 0.66 | 0.55 | 0.68 | 0.48 | 0.55 | 1.0 | 1.3 | 0.9 | 1.5 | 0.9 | 2.4 | 0.9 | 1.4 |
| 14 | 0.47 | 0.34 | 0.52 | 1.21 | 0.64 | 1.58 | 0.60 | 0.48 | 0.6 | 0.5 | 0.8 | 3.8 | 1.2 | 10.6 | 1.2 | 1.8 |
| 15 | 0.47 | 0.45 | 0.44 | 0.47 | 0.46 | 0.31 | 0.47 | 0.37 | 0.4 | 1.1 | 0.6 | 1.2 | 1.0 | 0.8 | 0.8 | 1.0 |
| 16 | 0.99 | 0.50 | — | — | 0.89 | 0.71 | 0.89 | 0.42 | 0.5 | 0.5 | — | — | 0.4 | 1.6 | 0.5 | 0.8 |
| 17 | 0.53 | 0.44 | 0.68 | 0.67 | 0.59 | 0.74 | 0.58 | 0.60 | 0.8 | 0.9 | 0.8 | 1.2 | 0.9 | 1.2 | 0.7 | 1.2 |
| 18 | 0.54 | 0.50 | 0.54 | 0.59 | 0.55 | 0.71 | 0.55 | 0.53 | 0.6 | 0.7 | 1.0 | 1.6 | 0.9 | 1.4 | 0.7 | 1.3 |
| Mean | 0.58 | 0.55 | 0.59 | 0.71 | 0.59 | 0.78 | 0.58 | 0.60 | 1.0 | 1.2 | 1.0 | 1.4 | 1.0 | 2.4 | 0.9 | 1.5 |
| ±SEM | 0.04 | 0.05 | 0.04 | 0.07 | 0.04 | 0.08 | 0.04 | 0.05 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.5 | 0.1 | 0.2 |
| *P* value | | | | | | | | | | | | | | | | |
| vs C | | | NS | <0.02 | NS | <0.005 | NS | <0.05 | | | NS | NS | NS | <0.05 | NS | <0.02 |
| Art vs CS | NS | | <0.05 | | <0.01 | | NS | | NS | | <0.025 | | <0.02 | | <0.001 | |
| Group Without Angina (no. = 7) | | | | | | | | | | | | | | | | |
| 19 | 0.62 | 0.48 | 0.60 | 0.50 | 0.63 | 0.50 | 0.62 | 0.38 | 0.7 | 1.2 | 0.8 | 1.1 | 1.2 | 1.0 | 0.8 | 1.3 |
| 20 | 0.59 | 0.46 | 0.57 | 0.53 | 0.59 | 0.54 | 0.63 | 0.67 | 2.0 | 1.0 | 1.1 | 1.3 | 1.2 | 1.0 | 1.2 | 0.8 |
| 21 | 0.54 | 0.44 | 0.59 | 0.41 | 0.59 | 0.37 | 0.56 | 0.39 | 0.6 | 1.0 | 0.8 | 1.4 | 0.6 | 0.7 | 0.8 | 1.0 |
| 22 | 0.44 | 0.33 | 0.47 | 0.37 | 0.50 | 0.46 | 0.55 | 0.33 | 0.6 | 0.9 | 0.7 | 0.9 | 1.0 | 1.1 | 0.8 | 1.0 |
| 23 | 0.71 | 0.74 | 0.76 | 0.71 | 0.72 | 0.70 | 0.85 | 0.68 | 0.2 | 0.2 | 0.5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 24 | 0.40 | 0.30 | 0.33 | 0.33 | 0.37 | 0.35 | 0.37 | 0.37 | 0.9 | 0.6 | 0.6 | 0.8 | 0.5 | 0.4 | 1.0 | 0.4 |
| 25 | 0.70 | 0.64 | 0.68 | 0.58 | 0.53 | 0.54 | 0.69 | 0.64 | 1.3 | 1.2 | 1.3 | 1.2 | 1.2 | 1.0 | 1.3 | 0.7 |
| Mean | 0.57 | 0.48 | 0.57 | 0.49 | 0.56 | 0.49 | 0.61 | 0.49 | 0.9 | 0.9 | 0.8 | 1.0 | 0.8 | 0.8 | 0.9 | 0.7 |
| ±SEM | 0.05 | 0.06 | 0.05 | 0.05 | 0.04 | 0.05 | 0.06 | 0.06 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.0 |
| *P* value | | | | | | | | | | | | | | | | |
| vs C | | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Art vs CS | <0.01 | | <0.01 | | NS | | <0.05 | | NS | | NS | | NS | | NS | |

Art = arterial; C = control heart rate; CS = coronary sinus; NS = not significant; $P_2$ and $P_3$ = two levels of pacing rates (see Table I); *P* = probability; $R_1$ = 5 minutes after cessation of pacing; SEM = standard error of the mean.

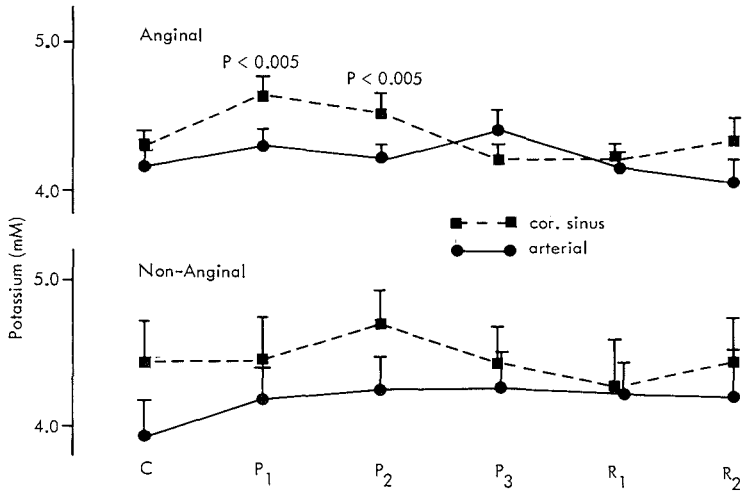


**FIGURE 4.** Arterial and coronary (cor.) sinus potassium levels during control (C), pacing ($P_1$,$P_2$,$P_3$) and recovery ($R_1$,$R_2$) periods. In spite of significant arteriovenous differences in the group with angina, there were never significant differences in the potassium levels of the two groups. Probability (P) values indicate a significant difference between coronary sinus levels and control values.

were found during the pacing and recovery periods (Fig. 5). However, arteriovenous differences remained very small and were not different in the two groups of patients.

*Oxygen extraction:* This variable remained unchanged for both groups (60 ± 2 percent) throughout the pacing and recovery periods.

*Inorganic phosphate:* Determinations were carried out in the first eight patients (Fig. 6). No significant changes in inorganic phosphate levels were observed.

**Electrocardiographic measurements:** No patient in the group without angina had S-T changes during pacing. Twelve of the 18 patients with angina showed S-T segment depression of 0.1 mv or more during pain when values were compared with control values (Table I).

## Discussion

This study was designed to determine whether ischemia induced by atrial pacing would result in the release of breakdown products of adenosine triphosphate from the human heart. The production of other metabolites and hemodynamic as well as electrocardiographic alterations were measured. The patients were separated into groups with and without angina; in 18 patients atrial pacing was stopped when angina occurred and in 7 patients it was stopped when A-V block occurred without angina.

**Electrocardiographic measurements:** S-T changes during atrial pacing have not been proved useful as a discriminant factor[13] in angina. Our study confirms this as only 12 of 18 patients manifested significant S-T segment changes during pain.

**Hemodynamics:** The hemodynamic findings in our patients compare very well with those reported by others. Maximal pacing heart rates and modified tension-time index values are comparable with those found by others, although heart rates were increased at various intervals.[14–18] Immediately after pacing a significant

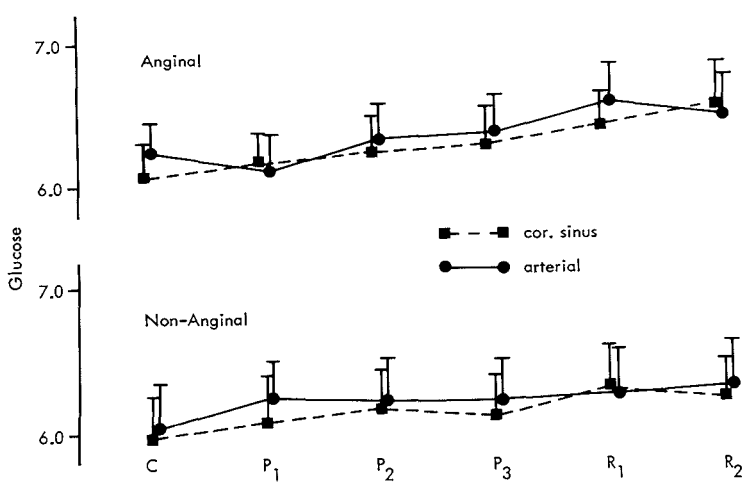


**FIGURE 5.** Arterial and coronary (cor.) sinus glucose levels (mmolar) during control (C), pacing ($P_1$,$P_2$,$P_3$) and recovery ($R_1$,$R_2$) periods.

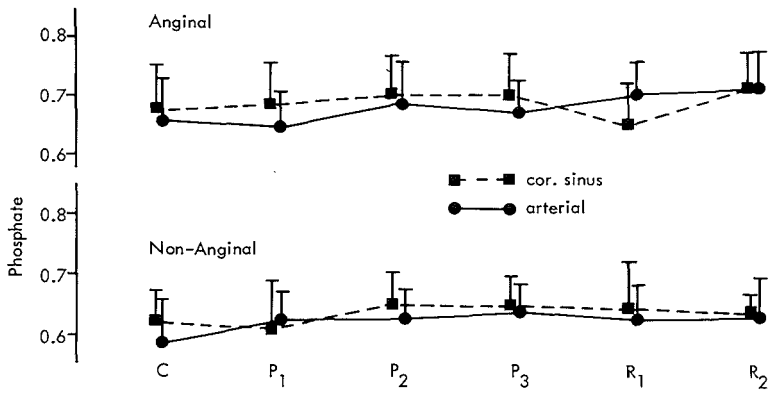


**FIGURE 6.** Arterial and coronary (cor). sinus inorganic phosphate levels (mmolar). The differences between the groups with and without angina were not significant. Abbreviations as in Figure 5.

difference in left ventricular end-diastolic pressure was observed between patients with and without angina. In the group with angina end-diastolic pressure increased above the control value, whereas in the group without angina it did not differ from the control value. This confirms the findings of others.[14,19] McLaurin et al.[20] reported a decrease in the maximal rate of decline of left ventricular pressure (minimal dP/dt) in patients with angina and suggested that this variable could be used as an index for impaired ventricular relaxation. By contrast, we found no change in minimal dP/dt in patients with or without coronary artery disease; this finding is in agreement with the data of Schwarz et al.[21]

**Metabolic markers:** *Increases in coronary sinus hypoxanthine concentrations* to values exceeding control up to 20-fold (Table III) were found during anginal pain, whereas there were no appreciable changes in the group without angina. Because arterial levels for both groups remained constant throughout the experiment, hypoxanthine production must have increased during pain in the group with angina. This increased hypoxanthine efflux probably reflects ATP degradation.[2,3] Inosine, although a very good marker of myocardial ischemia in the pig heart,[4-6] was not produced in measurable amounts in this study. Even during ischemia the inosine concentration was less than 0.4 μmolar. This may indicate a quick turnover from adenosine by way of inosine to hypoxanthine.

*Adenosine efflux* was observed by Fox et al.[10] in coronary sinus blood during pacing-induced ischemia, although the nucleoside usually was not detectable during control situations. Adenosine was a relatively insensitive marker of ischemia (only 10 of their 15 patients had significant differences during angina). In our study only 3 of the 18 patients with angina failed to show increases in venous hypoxanthine levels (more than 0.2 μmolar) during angina. Because the method of Fox et al. for measuring adenosine requires 90 ml of blood compared with only 1.5 ml for hypoxanthine measurements, the latter method is obviously preferable.

*The data on lactate metabolism* show increased anaerobic glycolysis in the course of atrial pacing in the group with angina. There was extraction during the control period, although somewhat less than in the group without angina because some patients were already producing lactate. During the pacing periods there was progressive lactate production. All but one patient with angina showed significant lactate abnormalities during the last pacing period ($P_3$). Because this patient (Case 6) also had unchanged venous hypoxanthine levels it is possible that he did not have true angina or that his coronary artery disease lay outside the area sampled by the catheter. He was the only patient in the group with angina who had no significant obstruction in the left anterior descending coronary artery. The presence of abnormal myocardial lactate metabolism before angina suggests that anaerobic glycolysis occurs very early in the course of developing myocardial ischemia and that angina is experienced somewhat later in the process.[13] Significant hypoxanthine production also developed later than lactate production. This finding is in agreement with the results of our experiments in the ischemic pig heart,[5,6] in which sharp increases in local coronary venous nucleoside concentrations were preceded by relatively larger lactate concentrations.

*None of the other metabolic variables* proved useful in identifying myocardial ischemia. There was no change in oxygen saturation in either group during pacing; this finding has been reported before.[14,22,23] Dagenais et al.[24] found no significant differences in myocardial glucose extraction in patients with and without angina. A difference was found by Most et al.,[25] but they concluded that glucose extraction could not be used as an indicator of myocardial ischemia because of a wide variability in individual subjects. No significant arteriovenous differences in glucose levels were found in our patients, although small but steady increases in arterial and venous levels were observed. The latter increases may have been the result of catecholamine stimulation in response to the stress situation, but it is doubtful that such small increases in glucose concentrations interfered with lactate metabolism in our study.

*Inorganic phosphate production* by the ischemic myocardium has been reported in dog[26-29] and pig[6] preparations. Changes depended on the extent of myocardial ischemia[27] and were usually small when

compared with changes in other metabolites.[6] Chiong et al.[30] studied the effects of pacing-induced ischemia on inorganic phosphate balance of the human myocardium. Small but significant changes were found in lactate producers. However, inorganic phosphate production was found in only 55 percent of their patients with angina. In our first eight patients no significant changes could be found (see also Ref 24) and further determinations were not carried out.

*Potassium egress* induced by atrial pacing from both the nonischemic and (to a greater extent) the ischemic heart was reported by Parker et al.[31] They suggested that the negative potassium balance was rate-dependent and apparently aggravated in the presence of myocardial ischemia. Our findings partly support these observations because early rises in venous potassium levels occurred in both groups, and were more pronounced in the group with evidence of ischemia. Changes in venous potassium were always small and accompanied by similar fluctuations in arterial potassium levels. Thus potassium efflux was an unreliable indicator of myocardial ischemia.

**Clinical implications:** Abnormal myocardial lactate metabolism occurs early in the course of developing myocardial ischemia. Therefore it remains a very important, but not always necessarily specific, metabolic marker of myocardial ischemia. In these situations the simultaneous measurement of hypoxanthine could provide additional information about abnormal myocardial metabolism.

## Acknowledgment

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# Chapter V:

# MYOCARDIAL LACTATE METABOLISM AND ISCHEMIA.

# V.

# Assessment of Myocardial Ischemia in Man. Reevaluation of the Usefulness of Myocardial Lactate Monitoring

**W.J. Remme, M.P. Look, P.A. Remme, M.D. van den Berg, X.H. Krauss, H.A.C.M. Kruyssen, D.C.A. van Hoogenhuyze.**

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*Submitted for publication in abbreviated format*

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## Abstract.

Anaerobic glycolysis is a primary event in myocardial ischemia and has provided for a valuable tool in defining ischemia under experimental conditions. However, the usefulness of myocardial lactate production as ischemic marker in man has been debated. To reexamine sensitivity and specificity of this metabolic indicator of ischemia, 232 patients, 161 with significant (≥70%) left coronary artery disease (gr LC), 48 with ≤25% coronary lesions (gr MC) and 23 patients without coronary disease (gr N) underwent an incremental atrial pacing stress test, which focussed on the direct post-pacing phase. Groups MC and N had no background of cardiac disease. Hemodynamic changes during pacing were comparable between groups, except for heart rate which was less in LC (141±2 beats/minute, versus 151±3 and 154±4 beats/minute in MC and N, resp.). In contrast, left ventricular systolic pressure fell with 5% and 12% in groups MC and N resp., but not in group LC. Consequently, the double product was similar in all groups. Pacing resulted in angina-like pain in 52% (N), 69% (MC) and in 80% (LC). Also, maximal ST-segment depression was comparable, i.e. 0.16±0.02, 0.14±0.02 and 0.16±0.01 mV in groups N, MC and LC, resp. By contrast, left ventricular filling pressure only increased in group LC. Baseline lactate extraction values were comparable between groups. Also, arterial lactate levels were similar and did not change during the test. Moreover, in groups MC and N coronary venous lactate and, hence, lactate extraction remained unaltered. By contrast, in patients with significant coronary disease coronary venous lactate rose early but progressively with marked elevations at 15 seconds post-pacing (0.82±0.03 mmol/l, compared with 0.53±0.02 mmol/l at control, $p<0.05$), resulting in lactate production during and for 2 minutes after pacing. Abnormal coronary venous levels persisted until 5 minutes post-pacing. Additional lactate measurements at 0, 30 and 45 seconds post-pacing in a subgroup of LC indicated maximal, comparable lactate production values at 15 and 30 seconds post-pacing. In 28 patients in group LC, lactate production was only observed after pacing, increasing its overall incidence from 60% to 78%, which compared favourably to ST-segment depression (60%) and left ventricular filling pressure changes (50%). Alternatively, in groups N and MC lactate production only occurred in 9% and 10% of patients, in contrast to ST-segment depression in 57% and 29% and abnormal left ventricular filling pressures in 46% and 39%, resp. Thus, myocardial lactate production is highly specific and more sensitive than commonly applied criteria, such as ST-segment changes or angina, in identifying functionally significant coronary disease, provided that lactate metabolism is evaluated between 15 and 30 seconds post-pacing.

## Introduction.

A derangement of oxydative phosphorylation is among the first intrinsic cardiac cellular alterations to follow the onset of myocardial ischemia and the subsequent lack of oxygen supply. As a result, anaerobic glycolysis is activated early during ischemia through enhanced activity of the glycolytic enzymes phosphofructokinase and glyceraldehyde-3-P-dehydrogenase. The latter follows the accumulation of ATP catabolites, such as ADP, AMP and inorganic phosphate[1]. Simultaneously, glucose uptake in the cell may improve, depending on blood supply to the ischemic area, whereas glycogenolysis will increase, both in an effort to enhance substrate availability for glycolysis and anaerobic ATP production. As the endproduct of the glycolytic pathway,

pyruvate, cannot enter the Krebs cycle, it will be transformed into lactate. Thus, a change from myocardial lactate extraction to production is found early during ischemia, enabling this metabolite to function as a potentially sensitive marker of this condition[2-4].
Moreover, as primary metabolic event, it may be more consistent as marker than secondary events, such as electrophysiologic alterations, changes in (local) contraction or relaxation or subjective symptoms, such as angina or dyspnoe. However, the usefulness of myocardial lactate production as indicator of myocardial ischemia in humans has been disputed[5,6]. Much of this depends on the model of ischemia used. Exercise increases arterial lactate levels and myocardial extraction in normal, non-ischemic areas. As a result, changes in the ischemic region may be entirely overshadowed when myocardial lactate metabolism is assessed from coronary sinus blood, as is common practice. In the resting patient, efforts to induce ischemia, for instance by rapid atrial pacing, do not invariably result in myocardial lactate production.

Reported percentages of patients with lactate production in this model range between 50 and 60% in the majority of studies[6-13]. This may be explained, to a certain extent, by technical problems, concerning the position of the sampling catheter in relation to the ischemic area, by atrial reflux[14] or by inappropriate admixture of blood from non-ischemic areas with that from the ischemic region. Alternatively, this parameter may have been underestimated. Available data on myocardial lactate production during pacing-induced ischemia are derived from small patient populations. Also, the relative presence of proximal versus distal left coronary artery disease or right coronary lesions has generally been given little attention. More importantly, lactate determinations are typically carried out during the test only, i.e. during pacing. We have previously suggested that the sensitivity of lactate as marker of ischemia may significantly improve when determined in the immediate post-pacing period[15].

In the present study the results of a large prospective investigation of the potential of myocardial lactate metabolism, determined in this way, and other objective and subjective parameters to delineate ischemia are reported in patients with significant left coronary artery disease compared to individuals without coronary lesions or with only minimal disease.

## Methods

### Patients

After the study protocol was approved by the Institutional Ethical Review Board and informed consent was obtained, 457 consecutive patients were studied prospectively. Patients were scheduled to undergo elective cardiac catheterization for the evaluation of stable, exercise-inducible angina-like symptoms and/or a previous myocardial infarction or for the presence of atypical, but persistent complaints of chest discomfort. Objective signs of myocardial ischemia were not required as the aim of this study was to acquire data on patients with coronary artery disease and individuals without coronary lesions. Eligible patients had to be normotensive without symptoms of heart failure (NYHA class III and IV). Exclusion criteria comprised the presence of conduction disturbances, clinically significant valvular disease, pulmonary hypertension or a recent myocardial infarction (less than one month old). Furthermore, patients with unstable angina, defined by the occurrence of recent episodes of angina, increased duration and/or frequency of anginal episodes during the preceding 4 weeks or recent prolonged episodes at rest, were also excluded. Moreover, patients with predominant angina at rest and/or ECG changes suggestive of variant angina were not included.
Antianginal therapy was stopped at least one day prior to the study but usually for longer periods, i.e. 36-72 hours pre-study, depending on the plasma half-life of the respective medication. Oral anticoagulants were withheld 2-3 days before the investigation and NSAIDs, if present, 10 days. Diuretics were not given on the day of investigation, whereas patients on digitalis therapy were excluded. Only short-acting nitroglycerin was allowed up to 6 hours pre-study.
Of the 457 patients studied, 232 patients were selected, who represented the 3 subgroups chosen for this investigation, i.e. patients with significant left coronary disease (group LC, n=161), individuals with normal coronary arteries (group N, n=23) and those with only minimal coronary lesions (group MC, n=48). Significant left coronary disease was defined by the presence of at least one $\geq$70% coronary diameter narrowing in either the left anterior descending, a diagonal branch, the proximal part of the left circumflex artery or a proximal marginal branch. Normal coronary arteries were considered present if the epicardial system was entirely smooth without luminal irregularities. Minimal coronary lesions were

defined as one or more coronary diameter narrowings of 25% or less.
The decision whether to include patients based on these criteria was made after catheterization following analysis of the coronary angiogram by 2 independent angiographers, experienced in the qualitative assessment of over 600 coronary angiograms annually. In case of disagreement, a third party would be involved or the patient discarded from further participation. Moreover, several precautions were taken to ensure that the groups with minimal coronary lesions or without coronary disease really represented individuals without ischemic or other forms of heart disease. Thus, patients with a history of myocardial infarction or objective signs of ischemia during stress testing were not included in these groups. Also, those with an abnormal left ventriculogram or valvular disease (mitral prolapse) were excluded. Clinical and angiographic characteristics of the 3 patient groups are given in Table I.

**Table I. Clinical and angiographic characteristics.**

| | Gr. LC (n=161) | Gr. MC (n=48) | Gr. N (n=23) |
|---|---|---|---|
| **Sex** (male/female) | 147/14 | 29/19 | 14/9 |
| **Age** (average/range), yrs | 54±0.8†/30-72 | 46±1.4/18-62 | 49±2.1/24-63 |
| **Previous infarctions** (anterior/inferior) | 45/39 | -- | -- |
| **Positive exercise test (n)** | 82 | -- | -- |
| **Medication:** | | | |
| nitrates | 86 | 10 | 4 |
| calcium-antagonists | 84 | 22 | 6 |
| ß-blockers | 103 | 18 | 9 |
| **Coronary angio (n)** (≥70% diameter stenosis) | | | |
| 1-vessel (L-CAD only) | 52 | -- | -- |
| 2-vessel | 54 | -- | -- |
| 3-vessel | 55 | -- | -- |
| **LV ejection fraction (%)** | 50±1.2† | 62±1.8 | 59±2.4 |
| **LV end diastolic volume** ($ml/m^2$) | 78±2.2 | 78±3.3 | 77±4.5 |
| **LV wall motion abnormalities (n)** | 70 | -- | -- |

*Abbreviations:*
*LC = patients with ≥70% left coronary artery disease; L-CAD = left coronary artery disease; LV = left ventricular; MC = patients with ≤25% coronary artery disease; N = patients without coronary artery disease; n = number of patients; yrs = years; † $p<.05$ vs other groups.*

*Values are $\bar{x}\pm SEM$.*

By design, groups N and MC had no appreciable abnormalities. In contrast, in group LC, previous myocardial infarctions were present in 63% of patients, objective signs of ischemia during stress testing (ST-segment depression >0.1 mV) in 50%. In the group with significant coronary lesions (LC), 52 patients had single-vessel, 54 double-vessel and 55 triple-vessel disease. Left ventricular ejection fraction was significantly lower in patients with coronary disease (50±1.2%, compared with 59±2.3% and 62±1.8% in patients with normal coronary arteries and minimal disease, resp.). Also, 70 patients in group LC had abnormal left ventricular wall motions, whereas, by design, patients in the other 2 groups had a normal ventriculogram. Furthermore, pre-existing cardiac medication differed between groups with significantly more patients on antianginal medication in group LC than in the other 2 groups. Finally, the average age in group LC was slightly, but significantly higher than in the other 2 groups.

**Study procedures**

Studies were carried out at approximately the same time in the morning without premedication. Patients had been fasting since midnight and had been supine for approximately 1.5 hours. Instrumentation was carried out after local anaesthesia with 1% lidocaine. First, coronary angiography was performed using the Seldinger technique, usually including 6 to 7 left and 3 right coronary angiograms with a total of 60 to 80 ml Renografin or Iopamidol administered.
Then, a no 7 Fr Millar microtip manometer catheter, an 8 Fr Sentron microtip manometer pigtail or a 7 Fr Cordis pigtail catheter was introduced into the left ventricle through a Desilet introducer system in a femoral artery. Next, a 7 Fr thermodilution pacing catheter (Wilton Webster Laboratories) or a 7 Fr Zucker bipolar pacing catheter was advanced into the midportion of the coronary sinus from a right antecubital vein, such that the proximal thermistor was at least 3 cm beyond the orifice of the coronary sinus. The catheter position had to be stable and allow for repetitive, fast sampling of coronary venous blood. The absence of right atrial reflux was confirmed by a bolus injection of 10 ml of saline into the right atrium under study conditions. The position of the catheters was subsequently recorded on video disc to allow rechecking of their position during the study.

After instrumentation, the fluid-filled catheters were calibrated using Statham or Bentley pressure transducers with zero reference levels set at mid-chest. The micromanometer pressure was balanced to zero and superimposed on the conventional pressure curves.

**Hemodynamic and electrocardiographic measurements:**

Measured hemodynamic parameters in this study included left ventricular systolic and end diastolic pressures (mmHg), left ventricular pressure-derived contractility and relaxation parameters [LV peak positive dP/dt (mmHg/sec), dP/dt/P at 40 mmHg, Vce40 ($sec^{-1}$), Vmax ($sec^{-1}$) and peak negative dP/dt (mmHg/sec), resp].
After calibration, left ventricular pressures and the first derivative of left ventricular pressure were recorded on paper at different paper speeds, i.e. at 10, 25 and 100 mm/sec, using a CGR 1000 cath lab system. Moreover, left ventricular pressures and pressure-derived indices were also determined and displayed on-line by a Mennen cath lab computer system. In a beat-to-beat analysis over 15-20 consecutive beats, the system averages out respiratory fluctuations. Representative beats may be chosen by the operator for further analysis.
A 3-lead electrocardiogram (leads I, II and V5) was recorded continuously on paper to determine heart rate and ST-segments. The latter were measured from 3 consecutive beats, at 80 msec after the J-point using a calibrated magnifying glass.

**Blood sampling technique and assay of lactate**

For lactate determination, simultaneous sampling of arterial and coronary venous blood was carried out. Before each sample, residual blood in the catheters was first discarded. Then, 1 ml of blood was directly transferred into chilled glass tubes, containing 2 ml of ice-cold 0.6 M $HClO_4$, vortexed and kept on ice. Immediately after the study, samples were weighed and centrifuged for 20 minutes at a speed of 2000xg and the supernatant frozen for subsequent determination. Lactate assay was carried out in triplicate by the enzymatic technique described by Guttman and Wahlefeld[16]. In our laboratory, the standard deviation of this assay carried out in approximately 2500 samples annually, is 0.012 mmol/l[17].

**Scoring of angina-like pain**

Before the study, patients were instructed to indicate any kind of chest discomfort or related pain as soon as this occurred. They were then familiarized with a pain scoring system according

to a simplified Borg scale. Also, they were asked to indicate the time of disappearance of these complaints following cessation of pacing.

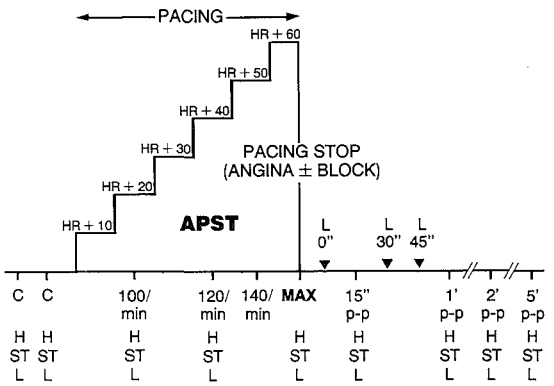



*Fig 1: Schematic representation of the atrial pacing stress test protocol. Repetitive baseline measurements are carried out at least 20 and 40 minutes after instrumentation and coronary angiography resp. Pacing is then performed with increments of 10 beats/2 minutes until significant angina-like pain, atrioventricular block or a maximal heart rate of 170 beats/minute. Hemodynamic (H), electrocardiographic (ST) and metabolic (lactate, L) variables are assessed at fixed rates during pacing, at maximal rates (MAX) just before cessation of pacing, followed by repeated measurements at 15 seconds, 1,2 and 5 minutes post-pacing (P-P). In addition, in a subgroup lactate was also assessed at 0, 30 and 45 seconds P-P (arrows).*

The study was carried out following a stabilization rest period of at least 20 minutes after instrumentation and 40-50 minutes after coronary angiography. Before pacing was initiated, multiple control hemodynamic measurements were performed and analysed on-line to ascertain stable baseline conditions. Also, the ECG was compared with recordings made at entry in the catheterization laboratory to record baseline changes. Next, lactate sampling was carried out in duplicate. This was followed immediately by the pacing stress test. The pacing protocol consists of increments in heart rate of 10 beats/2 minutes until significant angina is present, atrioventricular block occurs or a maximum pacing rate of 170 beats/minute is reached. Significant angina was defined as the level, at which the patient would usually rest or take nitroglycerin. Atrioventricular conduction disturbances were accepted as an endpoint. Atropine was not administered during the test. All measurements were repeated at fixed intervals during pacing, i.e. halfway the 100, 120, 140 and 160 beats/minute period, if applicable. All determinations were repeated at maximal heart rates, just before cessation of pacing. Next, variables were reassessed at 1, 2 and 5 minutes after pacing. In addition, left ventricular end diastolic pressures were determined at 10 seconds post-pacing. Moreover, lactate was also assessed at 15 seconds post-pacing, together with heart rate and left ventricular systolic pressure.
In a subgroup of 38 patients, lactate measurements were carried out as well at different periods after pacing, i.e. direct after cessation at 0 seconds, at 30 and/or at 45 seconds after pacing (Fig. 1). As the full procedure of sampling, including clearing of the catheters and transfer of blood, usually took 10-15 seconds, it was not always possible to evaluate all time points in the post-pacing phase in each patient in this subgroup.

### Statistical analysis

Data are presented as averages and 1 standard error of the mean. Group differences at baseline as well as for calculated changes from control were examined using a one-way analysis of variance. Within groups, the change in values between measurements during and after pacing versus baseline were calculated. Comparisons with control values were evaluated using a two-tailed paired t-test. Coefficients of correlation were calculated where appropriate. A p-value of $<0.05$ was considered significant.

## Results

At control, none of the patients in groups N and MC had clinical signs of myocardial ischemia, e.g. no anginal complaints or electrocardiographic abnormalities. Also, in group LC, none of the patients had anginal complaints at rest. However, asymptomatic ischemia at rest was documented by ST-segment depressions in 5 patients and by myocardial lactate production in 14 patients.

### Baseline hemodynamic, electrocardiographic and metabolic variables

Baseline hemodynamic, electrocardiographic and metabolic parameters were comparable between the 3 study groups. Only left ventricular end dias-

tolic pressure was significantly lower in patients without coronary (group N) disease compared with group LC (12±1.4 mmHg versus 16±0.5 mmHg, resp.). Baseline heart rates and double products were similar in all groups, as well as control ST-segment levels. Also, control lactate extraction values were identical, i.e. 17±3%, 19±2% and 19±1% in groups N, MC and LC, resp.

Table II. Hemodynamic variables before and during pacing.

| | | Control | 100/min | 120/min | 140/min | MAX |
|---|---|---|---|---|---|---|
| HR (b/min) | LC | 75±1.0 | 101±0.2* | 121±0.3* | 140±0.4* | 141±1.7*+ |
| | MC | 74±1.8 | 101±0.5* | 122±0.5* | 140±0.9* | 151±3.0* |
| | N | 75±2.4 | 100±0.4* | 121±0.8* | 140±0.8* | 154±4.3* |
| LVSP (mmHg) | LC | 140±2.2 | 138±2.1 | 137±2.4 | 134±3.3 | 138±2.0 |
| | MC | 133±3.5 | 133±3.5 | 131±3.6 | 123±4.2 | 127±3.7* |
| | N | 132±2.7 | 131±2.1 | 129±3.1 | 128±3.4 | 116±3.1*+ |
| DP ($HRxLVSPx10^{-3}$) | LC | 10.6±0.2 | 13.8±0.2* | 16.6±0.3* | 18.7±0.5* | 19.3±0.3* |
| | MC | 9.8±0.3 | 13.3±0.4* | 16.0±0.4* | 17.2±0.6* | 19.0±0.6* |
| | N | 10.0±0.4 | 13.1±0.2* | 15.6±0.4* | 17.8±0.5* | 17.9±0.7* |
| LVEDP(mmHg) | LC | 16±0.6 | 12±0.6* | 10±0.7* | 10±1.0* | 13±0.8* |
| | MC | 15±1.0 | 11±1.0 | 8.5±0.9* | 7±1.1* | 9±1.3* |
| | N | 12±0.8 | 8±0.8 | 6±0.9* | 7±1.3* | 10±2.4 |
| LV dp/dt pos. ($mmHg.sec^{-1}$) | LC | 1767±47 | 1983± 56* | 2150± 67* | 2130± 98 | 2169± 68* |
| | MC | 1788±84 | 1944±108 | 2066±116* | 2050±135 | 2153±120* |
| | N | 1614±91 | 1805± 75* | 2008±110* | 2134±159 | 2009±111* |
| VCE40 ($sec^{-1}$) | LC | 33±0.8 | 39±1.1* | 42±1.2* | 43±2.1 | 41±1.3* |
| | MC | 33±1.3 | 38±1.8* | 42±1.9* | 43±2.4 | 41±2.1* |
| | N | 33±1.9 | 39±1.4* | 44±2.0* | 45±3.0 | 43±2.3* |
| Vmax ($sec^{-1}$) | LC | 48±1.1 | 56±1.6* | 59±1.7* | 60±2.5 | 57±1.9* |
| | MC | 51±2.0 | 57±2.6* | 62±3.1* | 63±3.9 | 60±3.2* |
| | N | 50±2.6 | 59±2.2* | 65±2.6* | 67±4.1 | 65±3.5* |
| LV dp/dt neg. ($mmHg.sec^{-1}$) | LC | 1690± 51 | 1802± 60* | 1843± 61* | 1890±111 | 1799± 62 |
| | MC | 1826± 87 | 1930± 98 | 2031±101* | 1951±118 | 1932±129 |
| | N | 1809± 92 | 1905± 97* | 2012± 99* | 1968±135 | 1556±113* |

Abbreviations:
b/min = beats/minute; DP = double product; HR = heart rate; LC = patients with ≥70% left coronary artery disease (n=161); LVEDP = left ventricular end diastolic pressure;
LVSP = left ventricular systolic pressure; MAX = maximal pacing rate; MC = patients with ≤25% coronary artery disease (n=48); N = patients without coronary artery disease
(n=23); p-p = post-pacing; " = seconds; ' = minutes. All values x±SEM; *p<.05; + p<.05 vs other groups;

## Changes in hemodynamic variables during pacing (Table II and IIa)

Pacing-induced hemodynamic changes were comparable in patients without or with only minimal disease, but differed in several aspects from patients with significant coronary disease. First, groups N and MC could be paced to a significantly higher heart rate than group LC, i.e.

Table IIa. Hemodynamic variables before and after pacing.

| | | Control | 15"p-p | 1'p-p | 2'p-p | 5'p-p |
|---|---|---|---|---|---|---|
| HR (b/min) | LC | 75±1.0 | 76±1.8 | 79±1.5 | 78±1.7 | 79±2.2 |
| | MC | 74±1.8 | 78±3.1 | 80±2.4 | 81±3.0 | 78±4.2 |
| | N | 75±2.4 | 86±4.8 | 86±3.2 | 84±3.6 | 85±3.4 |
| LVSP (mmHg) | LC | 140±2.2 | 150±3.6 | 145±2.8 | 143±3.5 | 137±3.8 |
| | MC | 133±3.5 | 127±6.2 | 132±4.2 | 130±4.5 | 135±6.4 |
| | N | 132±2.7 | 133±3.8 | 125±2.8 | 123±4.0 | 120±4.9 |
| DP ($HRxLVSPx10^{-3}$) | LC | 10.6±0.2 | 11.8±0.4 | 11.4±0.3 | 11.2±0.4 | 10.9±0.4 |
| | MC | 9.8±0.3 | 9.7±0.8 | 10.6±0.5 | 10.2±0.5 | 10.8±0.9 |
| | N | 10.0±0.4 | 12.1±0.9 | 10.7±0.4 | 10.1±0.6 | 10.2±0.6 |
| LVEDP (mmHg) | LC | 16±0.6 | 21±1.1*+ | 16±0.8 | 14±1.0 | 14±1.2 |
| | MC | 15±1.0 | 14±1.6 | 14±1.4 | 12±1.1 | 15±1.9 |
| | N | 12±0.8 | 13±2.7 | 11±1.6 | 10±1.2 | 11±1.8 |
| LV dp/dt pos. ($mmHg.sec^{-1}$) | LC | 1767±47 | 1980± 70* | 1902± 67* | 1964± 93* | 1661± 65 |
| | MC | 1788±84 | 1903±210 | 1795±105 | 1857±123 | 1706±161 |
| | N | 1614±91 | 1786±190 | 1665± 83 | 1658± 95 | 1580±115 |
| VCE40 ($sec^{-1}$) | LC | 33±0.8 | 33±2.2 | 34±1.0 | 36±1.5 | 32±1.2 |
| | MC | 33±1.3 | 35±2.7 | 34±1.4 | 36±1.8 | 33±2.4 |
| | N | 33±1.9 | 37±3.2 | 36±1.4 | 35±1.6 | 30±2.0 |
| Vmax ($sec^{-1}$) | LC | 48±1.1 | 52±3.5 | 49±1.4 | 52±1.8 | 48±1.4 |
| | MC | 51±2.0 | 51±3.6 | 50±2.1 | 53±2.5 | 47±2.4 |
| | N | 50±2.6 | 55±4.2 | 53±1.9 | 54±1.5 | 50±2.5 |
| LV dp/dt neg. ($mmHg.sec^{-1}$) | LC | 1690± 51 | 1690±130 | 1702± 63 | 1634± 74 | 1625± 75 |
| | MC | 1826± 87 | 1870±161 | 1907± 98 | 1893± 96 | 2010±190 |
| | N | 1809± 92 | 1880±154 | 1853± 60 | 1932±110 | 1856±120 |

Abbreviations:
b/min = beats/minute; DP = double product; HR = heart rate; LC = patients with ≥70% left coronary artery disease (n=161); LVEDP = left ventricular end diastolic pressure;
LVSP = left ventricular systolic pressure; MAX = maximal pacing rate; MC = patients with ≤25% coronary artery disease (n=48); N = patients without coronary artery disease
(n=23); p-p = post-pacing; " = seconds; ' = minutes. All values x±SEM; *p<.05; + p<.05 vs other groups;

154±4.4 and 151±3.0 beats/minute (groups N and MC, resp.) versus 141±1.6 beats/minute (group LC).
Consequently, the change from control levels was different. However, this did not result in different double products as left ventricular systolic pressure fell progressively and significantly during pacing in groups N and MC by 12% and 5%, resp., but not in group LC. Following the greater reduction in left ventricular systolic pressure, the peak negative value of LV dP/dt decreased sharply at maximal heart rates in group N, but not in MC and LC.
Contractility parameters improved similarly in all groups. At maximal pacing rates, Vmax increased by 30%, 19% and 20% in groups N, MC and LC, resp. Thus, no significant differences were present in the various determinants of myocardial oxygen demand between groups.

**Pacing-induced myocardial ischemia. Comparison of subjective and objective criteria (Table III and IIIa)**

*Angina-like pain*

Angina-like pain, chest discomfort or related complaints were indicated by 12 patients without coronary disease (52%), by 32 patients (67%) with only minimal disease and by 129 patients (80%) with significant coronary lesions. The degree of discomfort was comparable between groups. Following pacing, angina usually subsided within the first minute in each group.

*Left ventricular end diastolic pressure*

Left ventricular end diastolic pressure decreased during the initial phase of pacing in all patients. At 120 beats/ minute, pressures were significantly lower than control in each group and decreased further in groups MC and LC during maximal pacing rates, but not in group N. Immediately following cessation of pacing, left ventricular filling pressures returned to control levels in groups N and MC. In contrast, they increased in group LC (21±1.1 mmHg versus 16±0.6 mmHg at control, $p<0.05$) (Fig. 2).

*ST-segment depression*

Significant electrocardiographic changes occurred in all groups with comparable values for ST-segment depression during maximal pacing, i.e. 1.58±0.22, 1.42±0.19 and 1.60±0.11 mV in

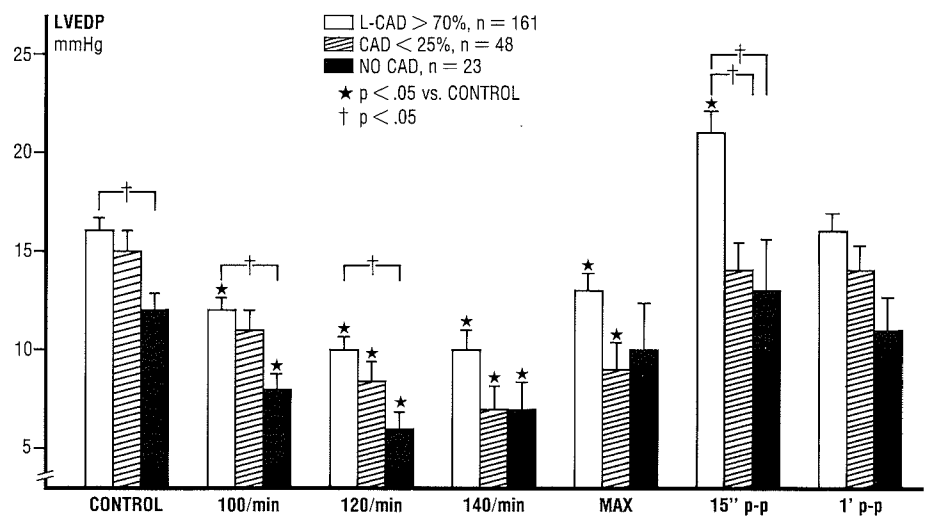



*Fig 2: Pacing-induced changes in left ventricular filling pressure. An abnormal rise immediately (10 seconds) post-pacing (P-P) is only observed in patients with significant coronary disease. MAX = maximal pacing heart rates; '= minutes; "= seconds. Values are $\bar{x}$±SEM.*

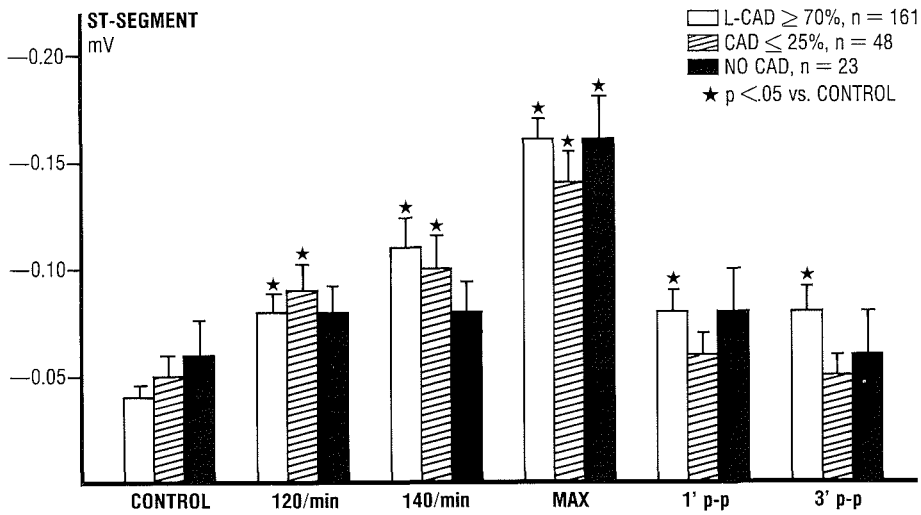



*Fig 3: Pacing-induced electrocardiographic changes. A significant and equal ST-segment depression is observed in all groups, although they persist somewhat longer after pacing (P-P) in patients with significant coronary disease. MAX = maximal pacing heart rates; ' = minutes; " = seconds. Values are* $\bar{x}\pm SEM$.

groups N, MC and LC, resp. (Fig.3).

*Lactate*

Pacing-induced alterations in myocardial lactate metabolism differed significantly between groups N and MC on the one hand and group LC on the other. Whereas arterial lactate levels did not change during the study and were comparable during and after pacing between groups, coronary venous levels were not. In groups MC and N, coronary venous levels and, consequently, lactate extraction did not change and were comparable during and after pacing. In contrast, in patients with significant left coronary artery disease, coronary venous levels progressively increased during pacing. At 100 beats/minute they were already elevated ($p<0.05$ versus control) (Fig. 4). Consequently, in this group myocardial lactate extraction decreased and changed into production at maximal heart rates (-2±2% versus 19±1 % at control, $p<0.001$). At 15 seconds post-pacing a marked rise in coronary venous levels was observed with an additional increase in lactate production (extraction -23±4%, $p<0.05$ versus maximal rates)(Fig.5).

Hereafter, coronary venous levels gradually declined. Lactate production values at 1 minute post-pacing were comparable to those during maximal pacing rates and returned to control values at 5 minutes post-pacing.

**Assessment of lactate metabolism after pacing**

In a subgroup of patients with ≥70% left coronary artery disease, additional sampling was carried out at 0 sec (n=37), at 30 sec (n=22) and at 45 sec post-pacing (n=15). Data are displayed in Fig. 6, which shows the additional increase in coronary venous lactate and lactate production values, relative to the levels observed during maximal rates. As the latter were comparable both in the subgroup and the main study group, the additional measurements are compared to the 15-second evaluation in the latter. Results indicate that lactate production is maximal at 15 and 30 seconds post-pacing with little difference in between these two time-points. However, values are less at 0 and 45 seconds. Although lactate production at 0 seconds post-pacing differed from values observed during maximal pacing, it was significantly less than the levels at 15 and 30 seconds post-pacing.

TABLE III: Electrocardiographic and metabolic changes during pacing.

| | | Control | 100/min | 120/min | 140/min | MAX |
|---|---|---|---|---|---|---|
| ST-segment | LC | -0.4±0.05 | -0.6±0.9 | -0.8±0.08* | -1.1±0.13* | -1.6±0.1 * |
| mV | MC | -0.5±0.10 | -0.7±0.11 | -0.9±0.12* | -1.0±0.16* | -1.4±0.19* |
| | N | -0.6±0.16 | -0.7±0.13 | -0.8±0.12 | -0.8±0.13 | -1.6±0.22* |
| Lactate, | LC | 0.67±0.02 | 0.69±0.02 | 0.68±0.02 | 0.67±0.03 | 0.69±0.02 |
| Arterial | MC | 0.62±0.02 | 0.58±0.02 | 0.58±0.02 | 0.56±0.03 | 0.60±0.03 |
| mmol/l | N | 0.69±0.03 | 0.72±0.04 | 0.68±0.04 | 0.64±0.03 | 0.70±0.03 |
| Lactate, | LC | 0.53±0.02 | 0.60±0.03* | 0.60±0.02* | 0.64±0.09* | 0.70±0.03* |
| Coronary venous | MC | 0.51±0.02 | 0.46±0.03 | 0.46±0.02 | 0.44±0.02 | 0.49±0.02 |
| mmol/l | N | 0.57±0.03 | 0.56±0.03 | 0.54±0.03 | 0.52±0.04 | 0.56±0.03 |
| Lactate extraction | LC | 19±1.4 | 14±2.3 | 11±1.9 | 4±2.5 * | -2±2.2 * |
| % | MC | 19±2.1 | 21±2.4 | 22±2.1 | 20±3.1 | 18±2.2 |
| | N | 17±3.2 | 22±4.8 | 19±2.8 | 19±5.1 | 20±3.0 |

Abbreviations:
LC = patients with >70% left coronary artery disease (n=161); MAX = maximal pacing rates; Min = minute; MC = patients with ≤25% coronary artery disease (n=48); N = patients without coronary artery disease (n=23); p-p = post-pacing; " = seconds; ' = minutes; * p<.05 vs control.

### Incidence of objective criteria for ischemia

To investigate sensitivity and specificity of the 3 objective criteria for ischemia used in this study, patients were categorized according to the presence or absence of abnormal changes in left ventricular filling pressure, determined as an increase of ≥5 mmHg above control values, ST-segment depression of ≥0.1 mV from baseline or by the occurrence of myocarial lactate production. In the group without appreciable coronary lesions, pacing induced abnormal elevations in left ventricular filling pressure in 46%, an abnormal ST-depression in 57%, but myocardial lactate production only in 9% of patients. Likewise, in the group with minimal disease, pacing resulted in abnormal left ventricular filling pressures in 36% of patients, in significant ST-segment depression in 29% and in lactate production in 10%. In contrast, myocardial lacate production was observed in 78% of patients with left coronary artery disease, a significantly higher incidence than that of ST-segment depression (60%) or abnormally elevated left ventricular filling pressure (50%) in this group. The pronounced sensitivity score of lactate in determining functionally significant coronary artery disease related to the fact that 28 patients in this group only demonstrated lactate production during the 15-second post-pacing sampling phase. Without these patients, sensitivity would have been reduced to 60%, equal to electrocardiographic changes.
Pacing-induced changes in heart rate as well as in maximal pacing rates and double products were significantly less in non-lactate as compared to lactate producers in group LC. No such discrimination was present for the other variables.

## Discussion

On theoretical grounds, the evaluation of myocardial lactate metabolism should be useful in assessing occurrence and degree of myocardial ischemia. Although a valuable tool in animal models of regional ischemia with the inherent easy accessibility of metabolites in the coronary venous effluent, its applicability in this respect is less obvious in man. Changes in substrate availability make lactate unsuitable as a research tool during exercise, even apart from the inability to perform sensible catheter-based metabolic studies in this model.

TABLE IIIa: Electrocardiographic and metabolic changes after pacing.

| | | Control | 15"p-p | 1'p-p | 2'p-p | 5'p-p |
|---|---|---|---|---|---|---|
| ST-segment | LC | -0.4±0.05 | -0.9±0.12* | -0.8±0.10* | -0.8±0.11* | -0.6±0.11 |
| mV | MC | -0.5±0.10 | -0.8±0.15 | -0.6±0.11 | -0.5±0.10 | -0.5±0.10 |
| | N | -0.6±0.16 | -0.9±0.21 | -0.8±0.20 | -0.6±0.20 | -0.3±0.12 |
| Lactate, | LC | 0.67±0.02 | 0.68±0.02 | 0.69±0.02 | 0.70±0.02 | 0.71±0.03 |
| Arterial | MC | 0.62±0.02 | 0.63±0.04 | 0.64±0.04 | 0.64±0.03 | 0.63±0.05 |
| mmol/l | N | 0.69±0.03 | 0.72±0.04 | 0.68±0.03 | 0.69±0.04 | 0.69±0.05 |
| Lactate, | LC | 0.53±0.02 | 0.82±0.03* | 0.73±0.03* | 0.66±0.03* | 0.61±0.03* |
| Coronary venous | MC | 0.51±0.02 | 0.50±0.03 | 0.48±0.03 | 0.49±0.03 | 0.50±0.03 |
| mmol/l | N | 0.57±0.03 | 0.55±0.04 | 0.55±0.04 | 0.56±0.04 | 0.58±0.05 |
| Lactate extraction | LC | 19±1.4 | -23±4.5 * | -4±2.8 * | 7±2.6 | 15±2.4 |
| % | MC | 19±2.1 | 20±2.7 | 24±2.5 | 23±3.0 | 19±3.5 |
| | N | 17±3.2 | 23±4.1 | 20±3.7 | 19±2.9 | 16±3.5 |

Abbreviations:
LC = patients with >70% left coronary artery disease (n=161); MAX = maximal pacing rates; Min = minute; MC = patients with ≤25% coronary artery disease (n=48); N = patients without coronary artery disease (n=23); p-p = post-pacing; " = seconds; ' = minutes; * p<.05 vs control.

However, also in the resting patient, available data from human studies do not seem to favour this metabolite as a sensitive indicator of functionally significant coronary disease. In the latter situation, nearly all studies have been based on pacing-induced stress.
Of these, the majority indicate that the incidence of myocardial lactate production ranges between 50% and 60%[6,7,9-11,13,16]. However, it should be noted that other objective parameters of myocardial ischemia also score relatively low in these studies, including ST-segment changes, the "gold standard", which was reported to vary between 50 and 60%[6,11,13].
Data on ischemia-induced lactate changes are mainly derived from studies in relatively small patient populations, which do not uniformly discriminate between type and location of vessel stenosis or compare the results from patients with significant coronary artery disease with normal individuals. Above all, these studies usually only concentrate on lactate changes during pacing.
The present study re-examines the usefulness of myocardial lactate production as marker of ischemia by taking these considerations into account in a large patient population. It clearly indicates, that by extending lactate assessments into the immediate post-pacing period, the sensitivity to indicate functionally important coronary artery lesions improves significantly and becomes superior to other objective markers, including ST-segment changes. Also, in so doing, the number of patients with demonstrable lactate production values increases significantly. Alternatively, the incidence of myocardial lactate production in patients with minimal coronary lesions or in individuals without coronary disease is low, i.e. 9-10%. In view of the relatively high incidence of other markers in these latter patient groups, this suggests that myocardial lactate production, besides being more sensitive, is also more specific in the evaluation of ischemia than commonly used objective criteria.

## Usefulness and applicability of myocardial metabolites as indicators of ischemia in man

As myocardial ischemia results in early and profound metabolic alterations in the heart, identification of these changes has been one of the diagnostic goals in cardiovascular medicine since the introduction of coronary sinus catheterization in man by Bing et al in 1947[19]. Although conceptually a sensible approach, several questions

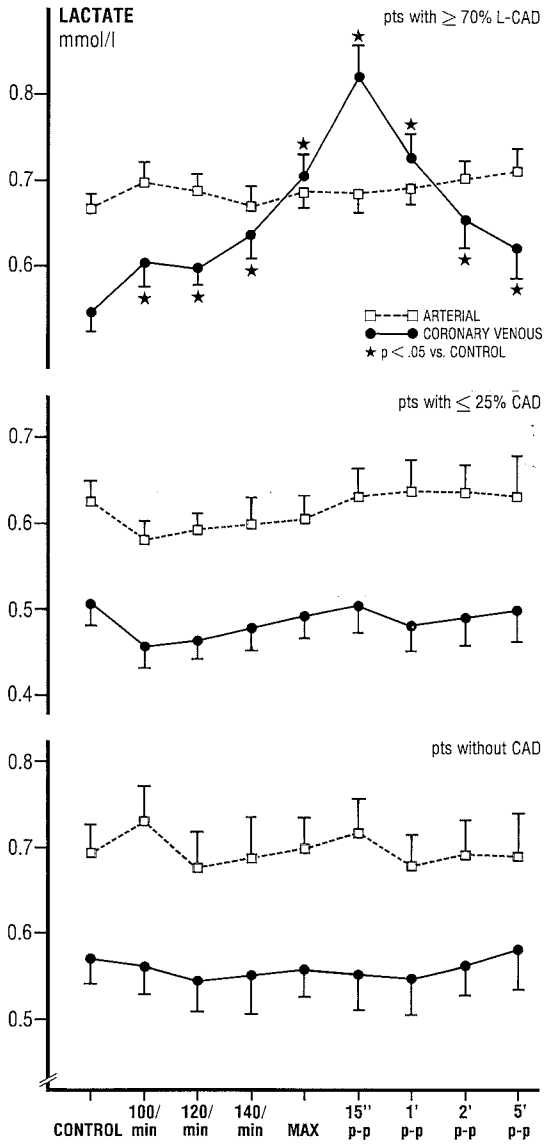


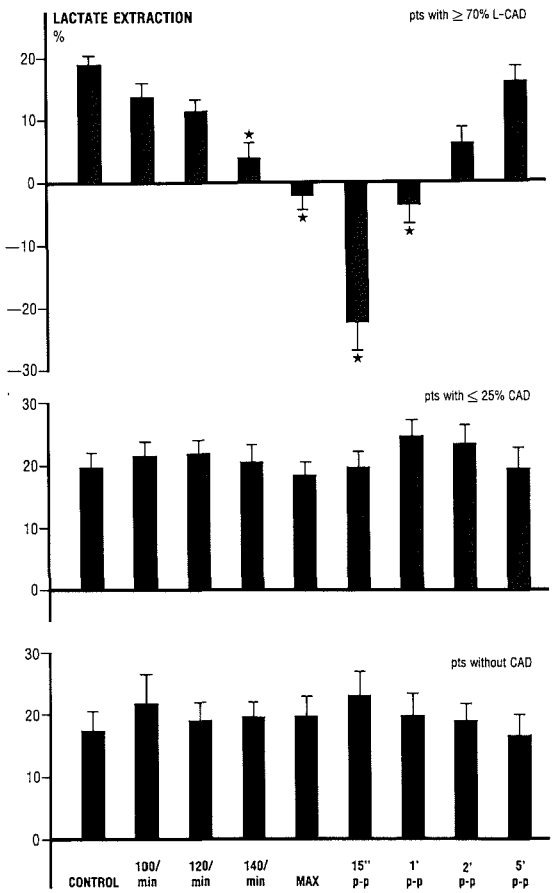

*Fig 4: Pacing-induced changes in lactate levels. Arterial values are comparable between groups and do not change during pacing. Also, coronary venous levels remain unaltered in patients without and with only minimal coronary disease. In contrast, in patients with significant lesions, coronary venous levels rise early [at 100 beats/minute (b/min)] with a marked increase at 15 seconds post-pacing (P-P). Levels are still elevated, compared with control, at 5 min P-P. MAX = maximal pacing heart rates; '= minutes; " = seconds. Values are $\bar{x}\pm SEM$.*

arise concerning the usefulness of specific metabolites as markers of ischemia, their reproducibility during repetitive periods of ischemia and the applicability of invasive techniques in general. With respect to the latter, non-invasive metabolic imaging techniques have certain apparent advantages, but, alternatively, may be limited by several technical shortcomings[20]. In general, they are not suitable for fast, sequential determination of metabolic changes, due to the relatively long half-life of applicable radio-isotopes. Fast and accurate determinations of metabolic alterations can be accomplished with catheter tip electrodes, sensitive for pH, oxygen or potassium[21-23]. The main disadvantage of this technique is, that it does not allow for the concomitant measurement of other metabolites or coronary flow. This does not apply to conventional methods where coronary venous blood is sampled through catheters, positioned in the coronary sinus, such as in the present study. Consequently, a host of metabolites have been assessed in humans in an attempt to better identify the ischemic event. Of these, citrate, alanine, glutamate and the adenine nucleoside, hypoxanthine, seem potentially useful candidates as markers of ischemia, besides lactate[16,18,24]. However, whether they are superior to lactate or whether they may improve the assessment of ischemia in addition to lactate, remains to be evaluated. In contrast, changes in pyruvate, glucose, free fatty acids, inorganic phosphate, potassium or in blood gases are either absent or insufficient to mark the ischemic event in man[12,16,24-26]. Hence, when invasive metabolic studies are considered, lactate would still seem the substrate of choice. In addition, as studies are preferentially carried out in the resting patient, the atrial pacing stress test appears the method of choice to induce ischemia[27-29].

## Potential limitations of lactate as marker of ischemia

A potential limitation of myocardial lactate metabolism as a marker of ischemia is its dependency on substrate availability. During exercise, arterial lactate levels increase, accompanied by enhanced extraction in non-ischemic areas. This is bound to affect the evaluation of lactate levels in the overall coronary effluent. In contrast, during pacing, arterial levels remain unaltered, as shown in the present study. Also, myocardial lactate extraction does not change as a result of pacing per se. Thus, this aspect may not apply to this test. In contrast, conditions of

*Fig. 5: Lactate extraction patterns during and after pacing (P-P). Lactate extraction remains unchanged in patients without or with minimal disease. Early changes occur in patients with significant lesions with production at maximal rates (MAX), followed by a significant increase in production values at 15 seconds (") post-pacing (P-P), returning to control levels thereafter. Values are $\bar{x}\pm SEM$.*




hyperventilation and neurohumoral activation, e.g. of catecholamines, may reduce lactate extraction, which may be one of the reasons that a reduction of lactate extraction under a certain level, i.e. 10%, as sometimes used in human studies, cannot be taken as an objective sign of ischemia[8,30,31]. As ischemia-induced stimulation of circulating catecholamines has been demonstrated in similar type patients and an identical study design as in the present investigation[32], the latter also emphasizes the necessity to adhere to the stringent criterion of myocardial lactate production during the evaluation of ischemia.

The major limitation in the invasive assessment of ischemia by way of metabolites, such as lactate, from coronary sinus blood in humans, lies in the inability to measure regional alterations in metabolism. To obviate atrial reflux on the one hand and sampling as well as capture problems during pacing on the other, the coronary sinus catheter should neither be too high up in the coronary sinus nor too near its orifice. Consequently, in the preferred position, the midportion of the coronary sinus, blood will invariably be sampled from ischemic and non-ischemic areas. As, during a procedure as fast atrial pacing, blood inflow in the post-stenotic area decreases and, subsequently, venous outflow diminishes, coronary sinus samples may mainly reflect blood from normal, non-ischemic regions, thus obscuring the main event to be studied.

**Improved assessment of lactate metabolism**

The present study clearly indicates that alterations in myocardial lactate metabolism, due to pacing-induced ischemia, are optimally assessed in the post-pacing period, more precisely between 15 and 30 seconds post-pacing. The latter procedure significantly enhances the sensitivity of this marker of ischemia, making it superior to other objective indicators. In so doing, the number of patients in whom lactate production is demonstrable also increases significantly. Moreover, our studies indicate that the collection of blood immediately after cessation of pacing (i.e. at 0 seconds) or at a later interval, e.g. at 45 seconds, does not lead to similar results. Already in 1981, in a preliminary report, we suggested that abnormalities in lactate metabolism during pacing-induced ischemia are optimally assessed at 15 seconds post-pacing[33]. Our findings were later confirmed by Ihlen et al.[7] Also, a study into the effect of pacing-induced ischemia on coronary venous pH, did indicate that maximal changes in pH occurred in the immediate post-pacing phase[21].

It may be speculated that the enhanced lactate release from the heart after pacing results from a diminution in coronary flow through non-ischemic areas together with a concomitant improvement in flow in the ischemic area upon cessation of pacing. As the determinants of myocardial oxygen demand are back to control levels soon after pacing, as can be seen from our data, a quick return to baseline values of coronary flow appears

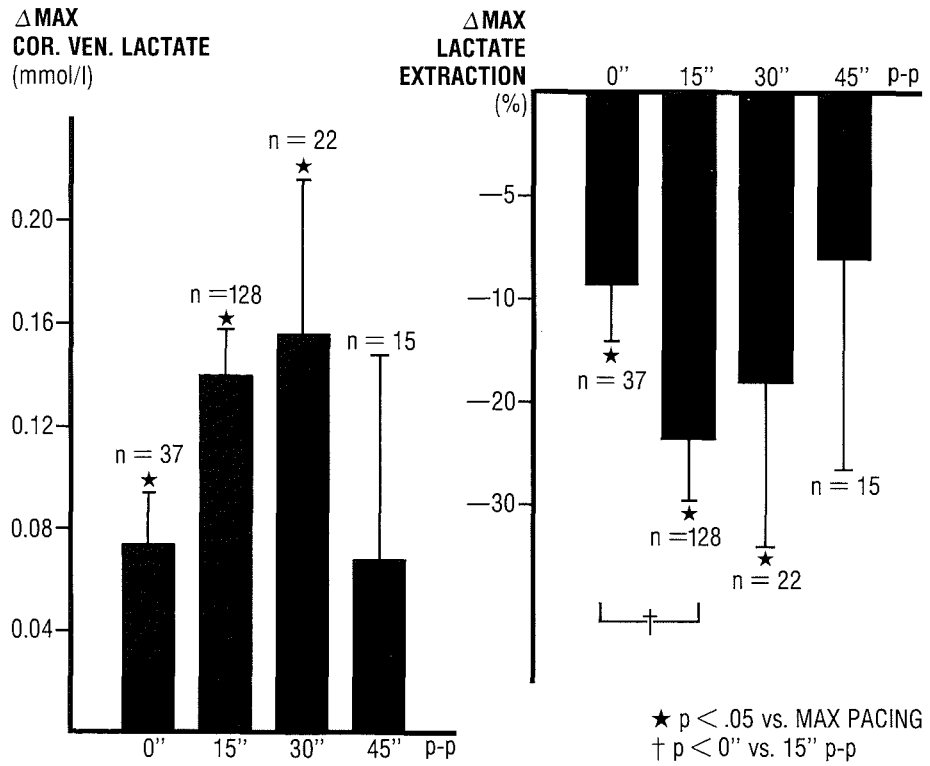



*Fig. 6: Additional evaluations of lactate in the post-pacing (P-P) phase in a subgroup of patients with ≥70% left coronary artery disease. Although at 0 seconds (") P-P lactate production values have increased compared with values at maximal pacing (MAX), maximal changes are found at 15 and 30 " P-P. Values are $\bar{x}\pm SEM$.*

likely in the non-ischemic areas. However, it is doubtful whether this also holds true for the ischemic regions. We have demonstrated that myocardial perfusion remains disturbed for a relatively long period after pacing, at least for 5 minutes, persisting after lactate metabolism has normalized[18,34]. Also, sustained reductions in perfusion for periods up to 20 minutes after ischemia have been reported[35]. Hence, a rearrangement of coronary flow immediately after pacing is unlikely and cannot explain the pronounced increase in coronary lactate levels at this point in time.

Coronary venous flow occurs predominantly in systole. During pacing, the duration of systole progressively declines and so does the time for venous outflow. In contrast, immediately after pacing, systolic periods lengthen considerably. Moreover, there is a general tendency that left ventricular peak systolic pressure also improves in the post-pacing phase. We would propose that a prolonged systolic squeezing effect is the main mechanism underlying the observed increase in coronary venous levels in the early post-pacing interval.

### Comparison of lactate production with other markers of ischemia

Lactate production, in the present study, proved to be a better indicator of ischemia than the other variables tested, both with respect to sensitivity and specificity. In patients with a normal coronary artery system and no background of ischemic heart disease, lactate production occurred in only 9% compared with 52%, 57% and 46% of patients with angina, electrocardiographic and hemodynamic abnormalities, respectively. Similar differences were present in patients with only minimal coronary diasease, but also without a background of ischemic heart disease.

The selection procedure of these study groups

makes it very unlikely that ischemic heart disease was present, but does not provide absolute proof. After all, patients were catheterized for the evaluation of angina-like chest-pain or equivalent symptoms, typical or atypical of nature. Also, as coronary flow measurements in this study were only carried out in a very limited group of patients, we have no information on coronary vascular reserve. Some patients may have had small vessel disease or the so-called syndrome X. However, these patients generally have objective signs of ischemia during exercise[36,37], which our patients had not. Thus, the absence of lactate production in the two study groups without significant coronary lesions probably did correctly indicate the absence of ischemic heart disease. It is interesting to note, that angina and ST-segment depressions apparently were less specific. Artifactual ST-segment depression during pacing in our study is unlikely as all electrocardiograms were also assessed during the first 5 complexes following cessation of pacing.

Also, it is interesting that there was no appreciable difference between patients without lesions and those with minimal disease. It has been suggested, that coronary vasomotion is disturbed already in arterial segments with minimal lesions as it is in stenosed arteries with an abnormal response to pacing[38,39]. For this reason, patients with coronary lesions and those with only minimal disease were considered separately in our study, although the response of fast atrial pacing was comparable in these two study groups.
Besides a better specificity, lactate production was also more sensitive than other markers in detecting functionally significant coronary artery disease. Its occurrence was only equalled by angina-like pain, which, as we have shown, is a very unspecific symptom. Our observation is particularly relevant in relation to (the lack of) changes in ST-segment. Not only was the incidence of lactate production greater, there was also a significant correlation between the absence of lactate changes and myocardial oxygen demand, which was not present for ST-segment changes. Patients in the coronary disease group, in whom myocardial lactate metabolism was not affected by pacing, were stressed to a significantly lower heart rate and double product than the patients with lactate production, suggesting that the sensitivity of this marker might have been significantly greater when appropriately high pacing rates were applied to all patients. In contrast, such discrimination could not be made for the other variables. Furthermore, lactate production persisted longer than ST-segment depressions following pacing. In earlier studies we suggested that certain metabolic changes, i.e. adenine nucleoside release, may be sustained after the ischemic event[18]. The present study indicates that this also holds true for lactate.
Ischemia-induced abnormalities in left ventricular ejection fraction and in regional wall motion may precede lactate production[40] and may also be more sensitive[41]. However, these studies only considered lactate production during pacing.
Preliminary data from our laboratory indicate that the occurrence of new wall motion abnormalities is comparable to myocardial lactate abnormalities, when assessed as suggested in this report (Bartels, unpublished observation).

### Implications

Our data indicate that myocardial lactate production is a useful marker of myocardial ischemia in man and sensitive as indicator of functionally important coronary artery disease. Alternatively, the parameter appears very specific. As such, it does appear more optimal than uniformly applied criteria with the exception perhaps of regional wall motion abnormalities. Also, although angina did occur slightly more often in patients with significant coronary lesions, this parameter, on the other hand, is highly unspecific. To obtain optimal information on myocardial lactate metabolism, it is necessary to assess lactate between 15 and 30 seconds post-pacing. As myocardial lactate production, assessed in this way, is both sensitive and specific, it should be useful in delineating the antiischemic effects of interventions.

## Acknowledgement.

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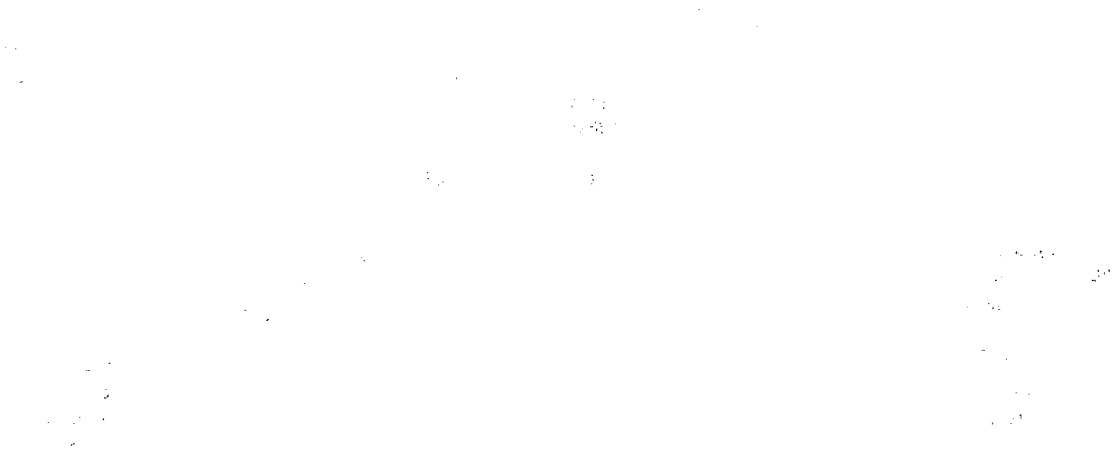

# Chapter VI:

# REPRODUCIBILITY OF MYOCARDIAL METABOLIC MARKERS DURING INTERMITTENT ISCHEMIA.

# VI.1.

# Myocardial Substrate Utilization and Hemodynamics Following Repeated Coronary Flow Reduction in Pigs.

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# Myocardial substrate utilization and hemodynamics following repeated coronary flow reduction in pigs*)

## Myokardialer Substratverbrauch und Hämodynamik während wiederholter Reduktion der Koronardurchströmung in Schweinen

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With 7 figures and 2 tables

(Received February 23, 1979)

*Summary*

The effect of repeated local ischemia and reperfusion on myocardial metabolism and ventricular performance was studied in 12 open-chested pigs fasted overnight. Myocardial ischemia was induced by reduction of the flow in the left anterior descending coronary artery to 40% of control during 30 min. After 35 min of reperfusion a second 30-min occlusion period was started, again followed by a 35-min reperfusion period. At the end of both reperfusion periods coronary flow and coronary resistance had returned to control values. During control there was lactate uptake, but no significant uptake of glucose, free fatty acids (FFA), triglycerides, glycerol and inosine. During the first occlusion period the heart released lactate and inosine, and used glucose and FFA. At the end of the first reperfusion period lactate uptake approached control values, but inosine was still released by 10 of the 12 animals. In the second ischemic period, glucose and FFA were again taken up. Lactate and inosine were released, but the production was much smaller than during the first occlusion period. Depletion of myocardial glycogen and high-energy phosphates could be responsible for this quantitatively different response. Necrosis may have played a role, although enzyme release was minimal and only observed after the second occlusion period.

Heart rate, peripheral resistance and ventricular filling pressure were virtually unchanged throughout the course of the experiments. Maximum rate of fall of left ventricular pressure (min LVdP/dt) decreased during ischemia and did not recover during reperfusion. Changes in min LVdP/dt and cardiac output were more closely related than changes in max LVdP/dt and cardiac output.

This model cannot be used for the study of interventions during myocardial ischemia in which the animal serves as its own control.

A large number of experimental studies dealing with the consequences of acute myocardial ischemia or infarction have been carried out in the dog. However, the coronary vasculature and the collateral circulation of

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the dog is quite different from that found in man (14). The pig may be more suitable for these studies, since striking similarities exist between the hearts of man and pig (4, 7, 13, 28 and 29). Consequently different aspects of myocardial ischemia have been studied in the pig heart (1, 7, 8, 11, 18, 23, 31, 34 and 36). In an earlier report from our laboratory (10), we compared myocardial lactate release with the release of the ATP-catabolites inosine and hypoxanthine, during severe ischemia and reperfusion. In a large number of investigations multiple occlusions are carried out in order to evaluate the capability of drugs to modify the degree of ischemia. ST-segment changes are frequently used as an indicator of the degree of ischemia in such studies. However, little attention has been paid to myocardial metabolism, although alterations in the oxygen demand/supply ratio is often the prime target of such interventions. We therefore studied myocardial metabolism during two periods in which the flow in the left anterior descending coronary artery (LAD) was reduced to 40% of its control value for 30 minutes. After the first partial occlusion period the LAD was reperfused until flow and coronary vascular resistance resumed control values. Subsequently the second occlusion period was started. In our previous communication (10) nucleoside and carbohydrate metabolism were evaluated. In this study considerable attention has been paid to the role of free fatty acids.

## Materials and methods

The experiments were performed on 12 Yorkshire pigs fasted overnight. The animals were sedated with 120 mg azaperone i. m. (Stresnil®, Janssen Pharmaceutica, Beerse, Belgium). Subsequently 150 mg metomidate (Hypnodil®, Janssen Pharmaceutica, Beerse, Belgium) was administered via a vein on the dorsal surface of the ear. The animals were intubated and connected to a Bird Mark 4 respirator for assisted ventilation with a mixture of 33% oxygen and 67% nitrous oxide. Ventilation was controlled by intermittent measurement of arterial blood gases (ABL1, Radiometer, Copenhagen, Denmark). The temperature of the animals was kept between 36.5 and 37.5 °C with a heating pad. ECG leads I, II and III were monitored throughout the experiment. The animals were kept anesthetized with a mixture of 2 $mg \cdot kg^{-1} \cdot hour^{-1}$ azaperone and 8 $mg \cdot kg^{-1} \cdot hour^{-1}$ metomidate, administered through the lumen of an 8 F Cournand catheter placed in the right atrium from the right jugular vein. A single 8 F Cournand catheter with its tip positioned in the thoracic aorta, was used for central aortic pressure measurements and for the collection of blood samples for the determination of blood gases and biochemical parameters. Through the left carotid artery a high fidelity 8 MMC Telco tip-manometer catheter (Thomson, Paris, France) was placed in the left ventricle for pressure measurements. Cardiac output was measured using the thermodilution technique. To this end a 7 F triple lumen balloon-tipped catheter was inserted in a femoral vein and its tip was positioned in the pulmonary artery. A 7 F Cournand catheter was positioned in the vena cava inferior for infusion purposes.

*Surgical procedure*

The left anterior descending coronary artery (LAD) was exposed by means of a midsternal thoracotomy. Parts of the fourth and fifth ribs were removed to get easy access to the LAD and its accompanying vein. The LAD was prepared free from its origin to its first branch and an electromagnetic flow probe (Skalar, Delft, The Netherlands, 20–25 mm in diameter) and a screw clamp were placed around the artery.

The accompanying vein was cannulated and a polyethylene catheter was inserted with the tip in the area to become ischemic. Care was taken for a free run-off of the myocardial venous blood, which was collected and returned to the animal via the catheter in the vena cava superior. Heparin (3000 units) was administered to prevent clotting.

*Hemodynamic measurements*

All tracings were written out on a Siemens Oscillomink B recorder. Cardiac output (CO) determinations were made in duplicate. Between the cardiac output measurements, the ECG, left ventricular pressure (LVP), its first derivative (LVdP/dt), central aortic and right atrial pressures were recorded.

*Biochemical measurements*

Arterial and local coronary venous pH, $P_{O_2}$ and $P_{CO_2}$ were determined with the ABL1 blood gas analyzer (Radiometer, Copenhagen, Denmark). Oxygen saturation was measured with an in vitro hemoreflectometer (American Optical Company, Framingham, Massachusetts).

Lactate was assayed enzymically in deproteinized samples on a Technicon AutoAnalyzer II (Tarrytown, New York, USA) as described by *Apstein* (2). Inosine was determined in neutralized samples (11). Samples containing less than 5 μM inosine were analyzed according to *Olsson* (24). Plasma glucose was assayed with the AutoAnalyzer II, using Technicon methodology. Serum free fatty acids were measured according to *Dole* and *Meinertz* as modified by *Trout* et al. (33); titration was carried out on a Titrigraph (Radiometer, Copenhagen, Denmark). Biochemica test combinations (Boehringer, Mannheim, Germany) were used to determine plasma glycerol, triglycerides, lactate dehydrogenase-1-isoenzyme (αHBDH, EC 1.1.1.27) and creatine kinase (CK activated, EC 2.7.3.2). Lactate dehydrogenase (LDH, EC 1.1.1.27) was assayed according to *Bergmeyer* (3). Blood gases at 30 °C were determined immediately after withdrawal of the blood samples. The other samples were stored at –30 °C until determinations were carried out.

*Experimental protocol*

After completion of surgery, a stabilization period of thirty minutes was allowed before control measurements were made. Subsequently the LAD flow was reduced to 40% of control by tightening the screw clamp. When necessary, the screw was adjusted to keep the flow at its reduced value. After 30 minutes the clamp was released and the LAD was reperfused. Thirty-five minutes later the LAD flow was again reduced to 40% of control for a period of 30 min and the LAD was subsequently reperfused. At fixed time intervals, arterial (a) and local coronary venous blood (cv) samples were drawn for the determination of biochemical parameters and the hemodynamic state of the animal was evaluated.

Only the anesthetics mentioned before, pancuronium (2 mg, when necessary to suppress muscle tremor) and saline (154 mM NaCl, to compensate for blood loss) were given throughout the study.

*Statistics*

The paired Student t-test (two tailed) was employed to determine probability levels. Only $p < 0.05$ was considered to be significant.

## Results

*Ventricular arrhythmias*

Ventricular ectopic activity was rare during both the flow reduction and reperfusion periods. In one animal a short lasting ventricular fibrillation was induced by the catheter in the ischemic myocardium during one

of the blood sampling periods. The results of this experiment were not different from the others and were therefore included in the study.

*LAD blood flow and myocardial $O_2$ consumption*

LAD blood flow was 47 ± 4 ml/min (mean ± SEM) during the first control period. This was reduced to 18.3 ± 1.7 ml/min (39% of control) during the first occlusion. After the clamp was released, at t = 30 minutes, there was considerable reactive hyperemia with a peak of 70 ± 5 ml/min ($p < 0.001$, compared to control) after 2 minutes of reperfusion (fig. 1). The flow gradually decreased and 35 minutes after release of the clamp LAD flow was not different from control. The coronary resistance of the vascular bed perfused by the LAD was not calculated during the occlusion period because the post-stenotic pressure was not measured. However, calculation of the resistance immediately after reperfusion was started, indicated vasodilatation had occurred (fig. 1). After 35 min of reperfusion, the coronary resistance had also returned to control values. At this time the second partial occlusion was started. In the second reperfusion period again a reactive hyperemic flow was found with a peak of 60 ± 4 ml/min after two minutes. At the end of the second reperfusion period, the LAD flow as well as the coronary vascular resistance values were not different

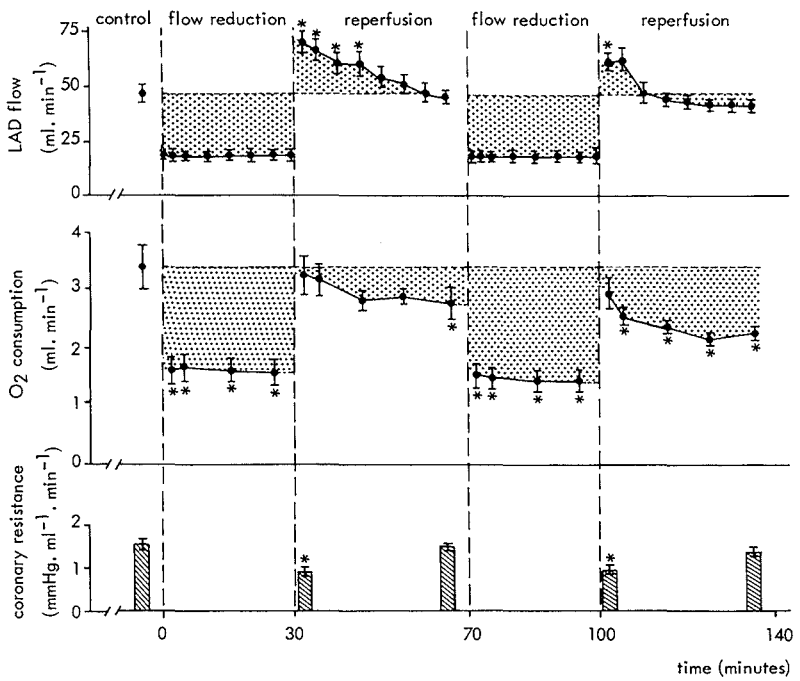


Fig. 1. From top to bottom are shown the left anterior descending coronary artery (LAD) flow, myocardial $O_2$ consumption and the resistance of the vascular bed perfused by the LAD. Because the post-stenotic pressure was not measured during flow reduction, the coronary resistance immediately after the beginning of the reperfusion was considered to be a measure of the vasodilatation occurring during the occlusion period. * stands for $p < 0.05$ vs. control.

from control and from the values found at the end of the first reperfusion period. Myocardial $O_2$ consumption which had decreased with 50% during occlusion, returned to control only in the early reperfusion period. Thereafter there was a gradual decrease and after 35 min of reperfusion $O_2$ consumption was only 80% of control. During the second occlusion and reperfusion periods a similar pattern was found.

*Hemodynamics*

No significant changes from control occurred during the course of the experiments in the following parameters: heart rate (115 ± 6 beats/min), right atrial pressure (5.1 ± 0.6 mm Hg) and peripheral resistance (1900 ± 200 $gcm^{-4}sec^{-1}$) while only a transient increase in left ventricular end-diastolic pressure was observed in the first minutes after the first flow reduction (11.5 ± 1.0 mm Hg vs. 9.0 ± 0.6 mm Hg, $p < 0.025$). Max LVdP/dt decreased by 25% during the first occlusion, but control values were restored during reperfusion (fig. 2). A small decrease in max LVdP/dt was seen in the second occlusion period, but recovery was incomplete in the following reperfusion. The most sensitive hemodynamic parameter was the maximum rate of fall of left ventricular pressure (min LVdP/dt), which showed large increases during both occlusions (20–25%), without any recovery during reperfusion (fig. 2). Cardiac output (CO) also decreased during both occlusions without improvement during reperfusion. The correlation between CO and min LVdP/dt ($r = -0.88$, $p < 0.001$) was much better than between CO and max LVdP/dt ($r = 0.40$, $p < 0.05$).

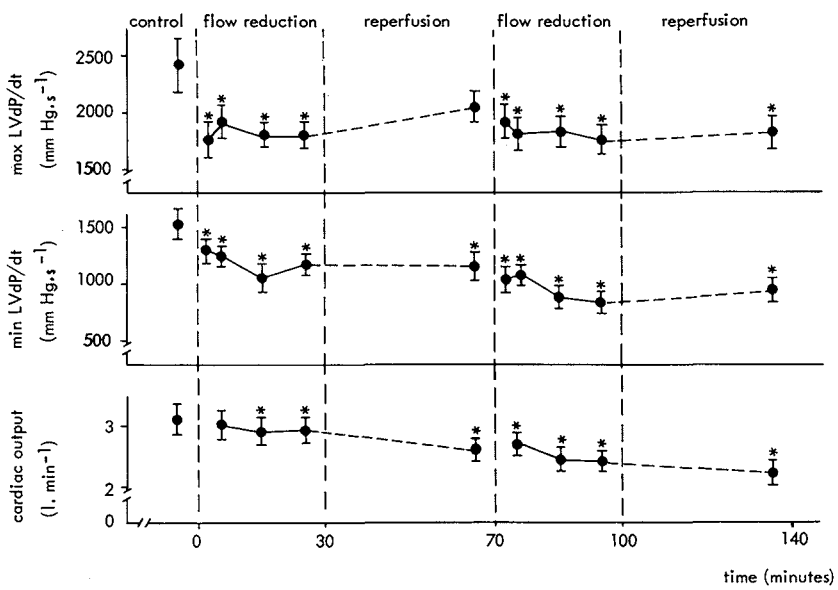


Fig. 2. From top to bottom are shown the maximum rate of rise (max LVdP/dt), and fall (min LVdP/dt) of left ventricular pressure and the cardiac output. Cardiac output and min LVdP/dt did not improve during the reperfusion periods. * stands for $p < 0.05$ vs. control.

*Blood gases*

Arterial blood gases were in the normal range throughout the entire experiments in all animals: pH: 7.35–7.45, $P_{CO_2}$: 35–45 mm Hg and $O_2$ saturation > 95%. Arterial-coronary venous difference (a-cv) in pH was 0.048 ± 0.005 during control and increased to 0.214 ± 0.021 after 5 minutes of LAD obstruction ($p < 0.001$, fig. 3). In the following 20 minutes (a-cv) pH gradually dropped. Control values had resumed at the end of the first reperfusion period. The second occlusion period gave rise to a new increase of (a-cv) pH. After 5 minutes a difference of 0.137 ± 0.016 was observed; this is smaller ($p < 0.005$) than the value measured at the comparable time in the first occlusion period. As in the first ischemic period (a-cv) pH started to decrease after 5 minutes of ischemia in the second period and after 25 minutes (a-cv) pH was again significant lower than after 5 min ($p < 0.001$). During the second reperfusion period control values returned. The (cv-a) $P_{CO_2}$ response to ischemia and reperfusion correlated very closely with that of (a-cv) pH ($r = 0.99$, $p < 0.01$, fig. 3).

*Lactate*

Arterial lactate levels increased gradually throughout the occlusion and reperfusion periods (fig. 6). This increase correlated with the decrease in cardiac output ($r = -0.92$, $p < 0.001$).

Lactate uptake (31 ± 8 μmoles/min) during control changed to lactate release in the first occlusion period, reaching a peak of 48 ± 9 μmoles/min

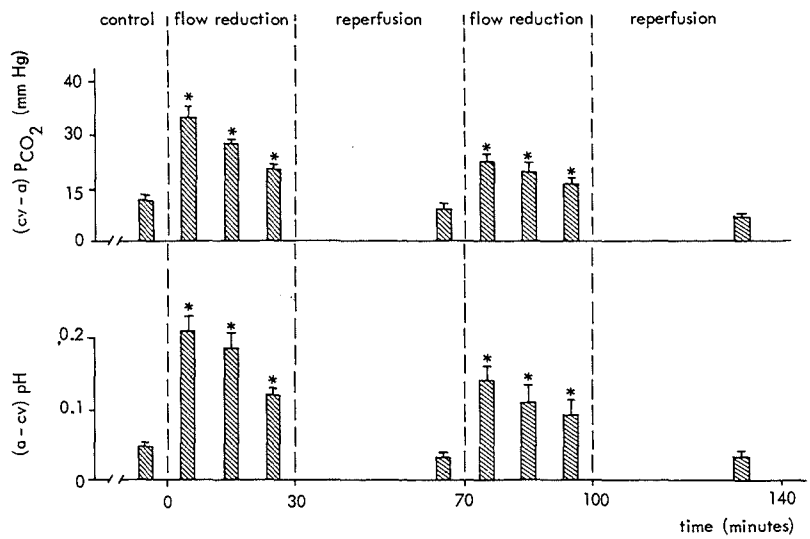


Fig. 3. Myocardial $CO_2$ (top) and proton (bottom) balance during control, flow reduction to 40% and reperfusion. Control and reperfusion values did not differ significantly. Large arterio-coronary venous differences in pH and $P_{CO_2}$ were found during the flow reduction periods. Proton and $CO_2$ release during the second occlusion period were significantly smaller than at comparable times during the first period. * stands for $p < 0.05$ vs. control.

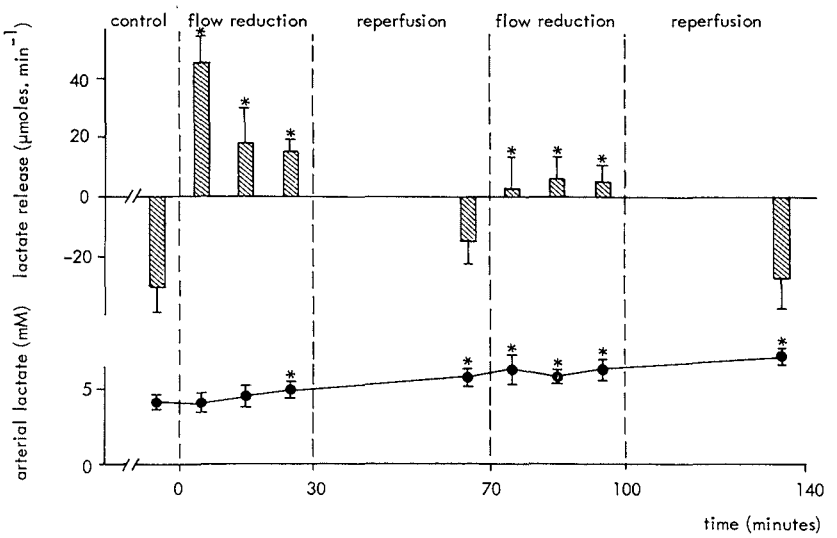


Fig. 4. Arterial lactate levels (bottom) increased gradually during the experiments. Myocardial lactate balance (top) shows that lactate release after 25 min in the first occlusion period was significantly smaller than after 15 min. The figure also indicates that at any time during the second occlusion period, a much smaller release was found than at comparable times during the first occlusion period, despite the same LAD flow (see fig. 2). Control and reperfusion values did not differ significantly. * stands for $p < 0.05$ vs. control.

($p < 0.001$) after 5 minutes. Subsequently lactate release started to decrease and after 25 minutes a value of 15 ± 4 μmoles/min was found ($p < 0.005$ when compared to 5 minutes of ischemia).

Uptake at the end of the first reperfusion period was not significantly different from control. However, myocardial lactate release in the second occlusion period was much less than that at comparable times during the first occlusion period (fig. 4).

After 5 minutes in the second occlusion period, no significant lactate release took place (3 ± 11 μmoles/min, compared to 48 ± 9μmoles/min after 5 minutes of the first occlusion, $p < 0.002$). Uptake by the heart at the end of the second reperfusion period was not different from control.

*Inosine*

Arterial inosine levels (13.3 ± 1.8 μM during control) did not change significantly during the experiments (fig. 5), which is at variance with the increase in the arterial lactate levels. Myocardial inosine uptake (0.047 ± 0.022 μmoles/min) changed into release during the first occlusion period reaching a peak of 0.923 ± 0.243 μmoles/min after 5 minutes of occlusion. Inosine release after 25 minutes of occlusion was significantly smaller than after 5 minutes ($p < 0.005$). At the end of the first reperfusion period, there was still a significant inosine release (0.128 ± 0.046 μmoles/min, $p < 0.01$ vs. control), indicative for the presence of ischemia. A remarkable observation is that 10 out of 12 animals had still inosine release, whereas

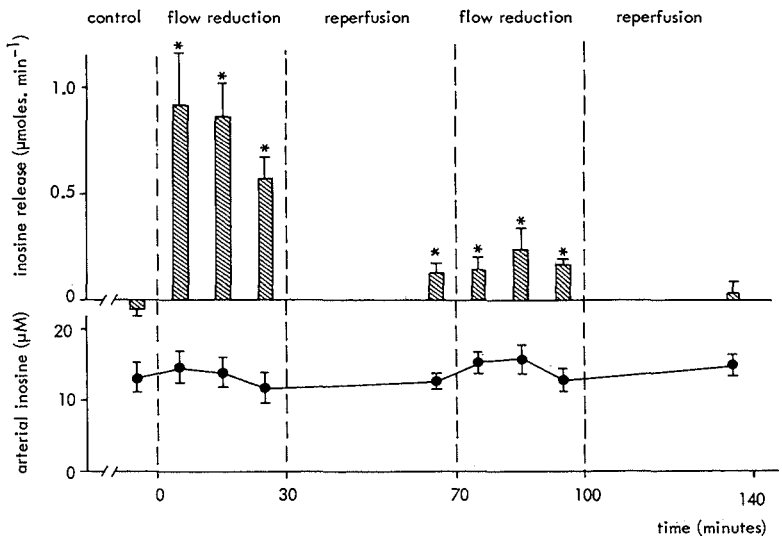


Fig. 5. Arterial inosine levels (bottom) did not change during the experiments. Myocardial inosine balance showed a similar pattern during the occlusion periods as myocardial lactate balance (see fig. 6). At the end of the first reperfusion period there was still a significant inosine release. * stands for $p < 0.05$ vs. control.

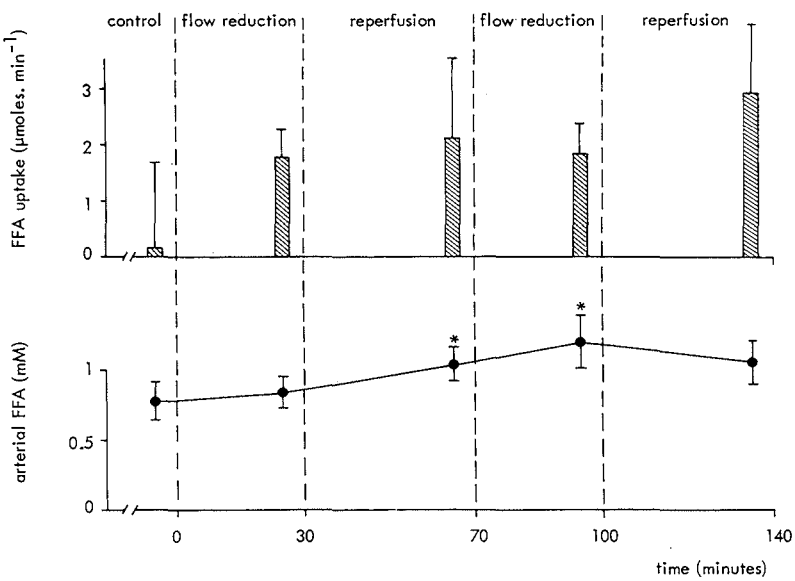


Fig. 6. Arterial glucose levels (bottom) did not change during the experiments. There was no glucose uptake during control and at the end of reperfusion but there was a similar glucose uptake at the end of both occlusion periods. * stands for $p < 0.05$ vs. control.

only 2 of the animals released lactate. During the second occlusion period inosine release increased to a maximum of 0.236 ± 0.100 μmoles/min after 15 minutes, which was again much less than that at the comparable time during the first period ($p < 0.005$). At the end of the second reperfusion (at t = 140 min), the arterial as well as the venous levels were not different from those found at the end of the first reperfusion period.

*Glucose*

Arterial glucose levels did not alter during the experiments. During control as well as at the end of the reperfusion periods, the heart did not use glucose. However, during the occlusion periods, there was a significant and comparable uptake (fig. 6).

*Free fatty acids*

The myocardium did not use any free fatty acids (FFA) during control as arterial and coronary venous levels were identical (0.77 ± 0.15 mM). Arterial levels increased gradually during the experiment. This increase became significant during the first reperfusion period (1.04 ± 0.13 mM, $p < 0.05$, fig. 7). After 25 minutes of flow reduction a significant ($p < 0.005$) uptake of FFA by the ischemic myocardium was observed: 1.8 ± 0.5 μmoles/min. Uptake at the end of reperfusion (t = 65 min) was not significant, whereas during the second occlusion period FFA uptake was the same as during the first occlusion period. No significant FFA uptake was found during the last reperfusion period.

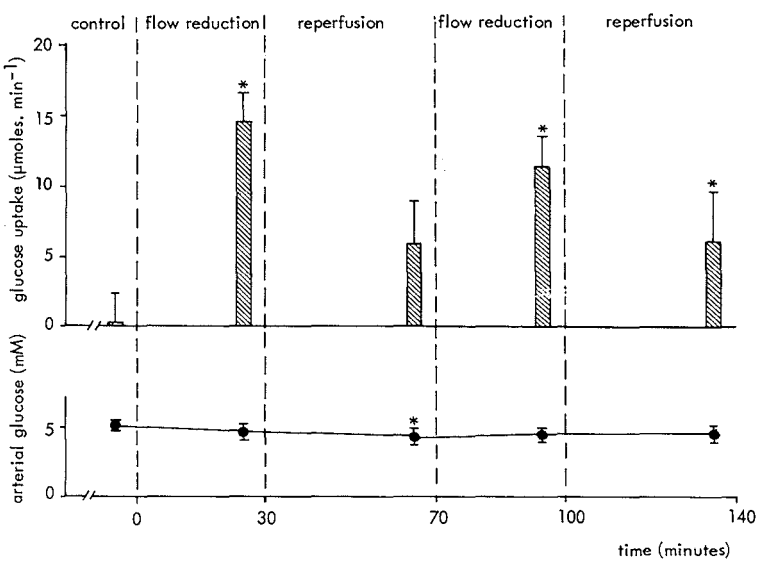


Fig. 7. Arterial free fatty acid (FFA) levels showed a slight increase during the experiment. FFA was not used by the heart during control and at the end of the reperfusion periods, but there was a significant and similar uptake during the occlusion periods. * stands for $p < 0.05$ vs. control.

Table 1. Triglyceride, glycerol and potassium release after repeated 30 min partial occlusion and after 35 min reperfusion of a coronary artery in pigs.

| | Triglycerides | | Glycerol | |
|---|---|---|---|---|
| | Arterial | Venous | Arterial | Venous |
| Control | 0.118 ± 0.019 | 0.113 ± 0.010 | 0.20 ± 0.02 | 0.19 ± 0.02 |
| First occlusion | 0.149 ± 0.012 | 0.156 ± 0.008* | 0.24 ± 0.03 | 0.26 ± 0.03* |
| First reperfusion | 0.188 ± 0.023* | 0.170 ± 0.030 | 0.31 ± 0.04* | 0.34 ± 0.04* |
| Second occlusion | 0.166 ± 0.019 | 0.143 ± 0.013 | 0.37 ± 0.06* | 0.39 ± 0.05* |
| Second reperfusion | 0.186 ± 0.019* | 0.177 ± 0.014* | 0.40 ± 0.05* | 0.41 ± 0.05* |

All values are expressed in mM; * $p < 0.05$ vs. control.

*Triglycerides and glycerol*

There was an increase in the arterial and coronary venous levels of triglycerides (up to 60%) and glycerol (up to 120%). However, no significant arterio-venous differences were observed (table 1). A significant correlation ($r = 0.61$, $p < 0.05$) between the arterial levels of FFA and glycerol was found during control. The correlation between FFA and glycerol improved gradually during the course of the experiments and at the end of the second reperfusion period ($t = 140$ min) r was 0.90 ($p < 0.001$) for the same twelve animals.

*Enzymes*

No changes in the arterial levels of total CK were oberserved during the course of the experiments (table 2). However, there was a gradual increase in the coronary venous levels and at the end of the second reperfusion period, a small but significant myocardial CK release was found. Arterial and coronary venous levels of LDH and αHBDH did not alter (table 2).

## Discussion

The effects of reperfusion of occluded coronary arteries are not fully understood. Reperfusion after a total occlusion for more than 15 minutes in pigs did not result in improvement of hemodynamic function or myocardial metabolism (1). Reperfusion after a one hour partial occlusion (flow reduced to 25% of control) resulted in arterio-venous lactate differences which were not different from control (10). However, the same authors also reported that myocardial oxygen uptake and hemodynamic function did not recover within one hour of reperfusion. In the dog, partial (15, 19) as well as complete (21) recovery have been described after complete ligation of coronary artery for more than 15 minutes. In the same species two hours of reperfusion after an one hour reduction of the LAD flow to 50% resulted in only partial recovery of contractility in the ischemic area (26). It may be expected that shorter periods of partial coronary artery occlusion may lead to a complete return towards preocclusion values. Therefore we studied myocardial metabolism and hemodynamics during 30 minutes in which coronary blood flow was held at 40% of

Table 2. Enzyme release after repeated 30 min partial occlusion and after 35 min reperfusion of a coronary artery in pigs.

| | CK | | LDH | | αHBDH | |
|---|---|---|---|---|---|---|
| | Arterial | Venous | Arterial | Venous | Arterial | Venous |
| Control | 0.40 ± 0.04 | 0.37 ± 0.03 | 0.56 ± 0.03 | 0.53 ± 0.04 | 0.276 ± 0.014 | 0.270 ± 0.017 |
| First occlusion | 0.41 ± 0.04 | 0.40 ± 0.04 | 0.55 ± 0.05 | 0.57 ± 0.07 | 0.271 ± 0.019 | 0.276 ± 0.016 |
| First reperfusion | 0.41 ± 0.03 | 0.44 ± 0.04 | 0.54 ± 0.03 | 0.47 ± 0.05 | 0.260 ± 0.014 | 0.272 ± 0.014 |
| Second occlusion | 0.45 ± 0.03 | 0.47 ± 0.04* | 0.57 ± 0.04 | 0.53 ± 0.04 | 0.278 ± 0.018 | 0.282 ± 0.014 |
| Second reperfusion | 0.44 ± 0.03 | 0.48 ± 0.04$^{+*}$ | 0.54 ± 0.04 | 0.51 ± 0.03 | 0.267 ± 0.020 | 0.271 ± 0.015 |

All values are in units/ml plasma. Abbreviations: CK, creatine kinase; LDH, lactate dehydrogenase; αHBDH, α-hydroxybutyrate dehydrogenase (LDH-l-isoenzyme).
$^{+}$ = $p < 0.05$ vs. arterial level; * = $p < 0.05$ vs. control.

control. After 35 min of reperfusion, coronary flow and resistance had resumed control values. $O_2$ consumption of the LAD perfused area was still slightly depressed (80% of control). $O_2$ consumption of the entire heart was not measured in this study, thus no conclusive data are available about the course of the $O_2$ consumption of the area which was not subject to coronary flow reduction. An indirect way of estimating myocardial $O_2$ demand is by calculation of the double product, heart rate times left ventricular systolic pressure. Control data were (10.5 ± 0.9) $10^3$ mm Hg/min. At the end of the first reperfusion it was (9.9 ± 0.6) $10^3$ mm Hg/min, which was not statistically different from control. On the basis of these data, it is assumed that the previously underperfused area was not able to use as much $O_2$ as the normal part of the myocardium.

During control the heart preferred lactate as substrate over glucose and free fatty acids. The choice of the anesthetics could explain this unexpected finding, because the combination of azaperone/metomidate yielded rather high arterial lactate levels (4 mM). This enhances myocardial lactate uptake (16), which could be at the cost of free fatty acid oxidation. This hypthesis is also supported by the findings that in pigs anesthetized with halothane, lower arterial lactate levels (1.0 mM) and uptake of free fatty acids were found (22, 35). During the course of the present experiments a gradual rise in arterial lactate levels was observed, while arterial inosine concentrations did not change. This increase in arterial lactate levels may be partially responsible for the discrepancy in the lactate and inosine data at the end of the first reperfusion period. At that time inosine is still released (an indication of ischemia), whereas lactate is extracted by the heart in amounts not different from control. Thus, lactate data in addition to coronary flow and resistance data suggest that at the end of the first reperfusion period the heart has returned to its control state. Inosine, on the other hand, reveals that return yet is incomplete. The increase in arterial lactate concentrations may have further promoted lactate uptake and consequently disturbs a proper evaluation of the metabolic state of the myocardium (6).

Lactate uptake changed into lactate release during the first occlusion period, with a peak value after 15 minutes. After 25 minutes a smaller amount of lactate was released by the ischemic heart, despite unchanged coronary inflow and unchanged hemodynamic determinants of myocardial $O_2$ demand. A decrease in lactate release after a longer period of ischemia has also been reported in the isolated rat heart (27) and could be explained by an inhibition of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) by decreased intracellular pH and increased cytosolic NADH levels (32). This would reduce the formation of lactate via a reduced glycolytic flux. It is attractive to adopt this explanation also for our data. However, this does not explain why inosine release exhibited a similar pattern as lactate release during the first occlusion period. Nucleotide and nucleoside contents, determined in biopsy specimen, are needed to eliminate a number of possible explanations: (i) decreased ATP content due to inhibition of glycolysis; (ii) reduced AMP catabolism; (iii) reduced nucleoside transport across the membrane; (iv) increased intracellular hypoxanthine conversion to AMP via inosine 5'-monophosphate.

Lactate and inosine release were smaller during the second ischemic period than in the first period at comparable times. This may be due to either reduced intracellular production or (and) to reduced removal. A number of mechanisms could be responsible for reduced production. First, the occurrence of necrosis. This would reduce the mass of ischemic myocardium involved in the metabolic processes. Reperfusion after coronary artery ligation in dogs results in an earlier appearance of CK in coronary sinus (5) and in arterial blood (15). In our experiments some CK, but no LDH or αHBDH was released at the end of the *second* reperfusion period (table 2). This could indicate that during the second occlusion a small part of the myocardium was irreversibly damaged. A second explanation might be the functioning of collateral vessels. Although collateral vessels in young swine have been observed (14) it is very unlikely that they start functioning within a couple of hours (28). Thus it is doubtful that the decrease in myocardial lactate and inosine release in the second ischemic period could be explained by activation of a collateral circulation. A third explanation could be the activation of other metabolic pathways due to depletion of glycogen and possibly ATP (17, 20, 25 and 30). The existence of such pathways is also suggested by the (a-cv) differences in pH and $P_{CO_2}$ during the second occlusion period. These differences reached values of 60–70% of the differences found during the first occlusion period, whereas lactate release in the second occlusion period was only 6–33% of that in the first occlusion period. Thus, while the close relation between lactate and pH changes in the first occlusion period suggests that the increase in the $H^+$ concentrations in the coronary venous effluent is associated with anaerobic glycolysis, the less strong relationship in the second period indicates that other factors than anaerobic glycolysis play a role in the formation of $H^+$.

Glucose extraction as well as (a-cv) differences were the same during the two occlusion periods (fig. 8). Therefore it must be assumed that during the second occlusion period, in view of the decreased lactate release, another pathway than glucose → lactate was preferred. This could be the conversion from glucose to glycerol-3-phosphate and subsequently esterification with fatty acids, leading to triglyceride and phospholipid formation. If this would be the case a larger FFA extraction during the second than during the first occlusion should be expected. However, such an increase was absent during the second occlusion. The possibility of an altered transport mechanism rather than a reduced intracellular lactate and inosine production can also not be excluded.

The changes in the hemodynamic parameters during the first occlusion period were minimal as heart rate, left ventricular end-diastolic pressure and systemic vascular resistance did not change appreciably, while cardiac output decreased slightly. During the reperfusion period cardiac output did not return to control values but decreased further (10–25%). In the second occlusion period the above mentioned parameters behaved very similar as during the first occlusion period. In this study the most sensitive hemodynamic parameter was the maximum rate of fall of the left ventricular pressure (min LVdP/dt), which changed with 57% against max LVdP/dt with 23% and cardiac output with 26% during the course of the experiment. Relatively little attention has been given to this parameter,

although its significance has been noticed before (9, 37). Another striking feature of min LVdP/dt is the finding that it did not even recover partially, during the first reperfusion period. Consequently min LVdP/dt and CO behaved more similarly ($r = -0.88$) than max LVdP/dt and CO did ($r = 0.40$). It is obvious from the results in this study that multiple coronary flow reductions to 40% of control, each of which lasts 30 minutes are not very well suited for the evaluation of interventions which aim to limit the degree of myocardial ischemia, despite a reproducible coronary flow and resistance.

The slightly elevated CK levels in the coronary venous effluent may indicate some necrosis during the second occlusion period, although arteriovenous differences were minimal. A less severe or a shorter lasting coronary artery obstruction probably prevents any necrosis. Both approaches have distinct disadvantages, when biochemical markers are used to describe the degree of ischemia. A minor obstruction could lead to undetectable changes in the metabolites (38). Since lactate and inosine release reach peak values after 15 minutes of ischemia, a shorter occlusion period appears to be unwanted. In order to establish if an initial slower rise in lactate (inosine) release after onset of ischemia is due to decreased anaerobic metabolism or due to an effect of the intervention on the transport mechanism of the metabolite across the cell membrane, a longer occlusion period appears to be desirable.

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*Zusammenfassung*

Der Effekt von wiederholter lokaler Ischämie und Reperfusion auf den myokardialen Energiestoffwechsel und Ventrikelfunktion wurde bei 12 narkotisierten Schweinen mit geöffnetem Thorax studiert. Die Schweine hatten 24 Stunden gefastet. Die myokardiale Ischämie wurde verursacht durch eine Reduktion der Blutdurchströmung in der linken Anterior Descending Koronar Arterie bis 40% vom Anfangswert während 30 Minuten. Eine zweite Reduktion wurde nach 35 Minuten Reperfusion angefangen. Die zweite Reduktion folgte wieder durch eine 35 Minuten dauernde Reperfusion. Am Ende der beiden Reperfusionen der Koronardurchströmung und Koronarwiderstand hatten sie wieder Anfangswerte angenommen. Während der Kontrolle gab es Laktat-Aufnahme, aber keine Aufnahme von Glukose, Freie Fettsäure (FFA), Triglyzeride, Glyzerol und Inosine. Während der ersten Okklusions-Periode wurde aus dem Herzen Lactat und Inosine freigemacht, es wurde Glukose und FFA aufgenommen. Am Ende der ersten Reperfusion wurde Laktat wieder aufgenommen, aber Inosine wurde noch immer an 10 von 12 Schweinen freigemacht. In der zweiten Okklusions-Periode gab es wieder Glukose und FFA-Aufnahme. Laktat und Inosine wurden freigemacht, aber die Produktion war viel kleiner als in der ersten Okklusions-Periode. Erschöpfung von myokardialen Glykogen und energiereichen Phosphaten können dafür verantwortlich sein. Nekrose könnte auch eine Rolle gespielt haben, obschon Enzym-Abgaben sehr gering waren und nur nach der zweiten Okklusions-Periode gefunden wurden. Herzfrequenz, Peripherewiderstand und ventrikulärer Füllungsdruck

blieben so gut wie unverändert. Während der Experimente wurde die maximale Druckabfallgeschwindigkeit (minLVdP/dt) weniger, während Ischämie sich bei Reperfusion nicht herstellte. Veränderungen in minLVdP/dt und Herzminutenvolumen hatten einen höheren Korrelations-Koeffizient als maxLVdP/dt und Herzminutenvolumen. Dieses Modell kann nicht für die Studien von Interventionen während myokardialer Ischämie benutzt werden.

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## VI.2.

# Reproducibility of Ischemia-Induced Changes in Myocardial Lactate Metabolism in Humans. A Prospective Study Comparing Atrial Pacing Stress Tests with Varying Intervals.

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## ABSTRACT

Despite widespread use of myocardial metabolic markers to define the efficacy of antiischemic interventions in myocardial ischemia in man, their reproducibility during successive ischemic episodes has not been evaluated consistently. Reproducibility of myocardial lactate production was studied during repeated, incremental atrial pacing stress tests, separated by intervals of 15, 30, 45 and 60 minutes (min) in 14, 15, 12 and 14 patients with left coronary artery disease, resp. By design, duration and maximal heart rates (MAX) were identical in both first (P1) and second (P2) pacing test in each group. Also, left ventricular systolic pressures and double products were similar and comparable between groups. In contrast, contractility improved significantly less during P2 compared to P1 after intervals of 15 and 30 min. Arterial lactate levels did not change during pacing. In contrast, coronary venous levels increased in each group. However, following intervals of 15 and 30 min, changes were significantly less during P2 compared to P1. Consequently, myocardial lactate production was 53% and 58% less during P2, compared to P1 ($p<0.05$). In contrast, average coronary venous lactate and lactate extraction values were similar following intervals of 45 min or more. Also, individual values were comparable. In only two patients in the 45-minute and in one in the 60-minute group was lactate production $\geq$10% less during P2. Other indices of ischemia were less reproducible. Angina was significantly less in 35% (15 min) or 50% (other groups) of patients during P2. Also, left ventricular filling pressure increased after P1, but not after P2 with 15 and 60 minutes intervals. Furthermore, ST-segment depression was either more extensive (15 min) or less (30 min) during P2 compared with P1, whereas individual values became comparable after 60 minutes only. Thus, the efficacy of interventions in (pacing-induced) ischemia should not be tested with angina or left ventricular filling pressure as criterion. Also, individual variability in ST-segment changes with intervals less than one hour, does not favour the use of this parameter. In contrast, good reproducibility of myocardial lactate production after recovery periods between ischemic episodes of 45 minutes or more allows interventions to be tested by this objective criteria within an acceptable time frame.

## INTRODUCTION

Myocardial ischemia is characterized by early and extensive metabolic alterations. Animal studies indicate that of these, anaerobic glycolysis is a consistent component, observed soon after onset of a critical reduction in myocardial oxygen supply[1]. Also, in humans, coronary flow reductions are quickly followed by myocardial lactate production together with other metabolic manifestations of ischemia, such as cardiac nucleoside release and electrocardiographic changes, but before angina occurs[2]. Thus, the determination of myocardial lactate production in the coronary venous effluent may serve as an early and, possibly sensitive marker of ischemia in man. However, as myocardial lactate metabolism is influenced by changes in arterial lactate levels, such as may occur during exercise, this assumption appears predominantly true for the assessment of ischemia at rest. Moreover, as studies on myocardial lactate changes by necessity will be invasive of nature, the atrial pacing stress test has been recognized as a useful tool to this purpose. With pacing, identification of abnormal myocardial lactate metabolism

significantly improves when measurements are carried out in the direct post-pacing period, e.g. at 15 seconds post-pacing[3,4]. In so doing, the sensitivity of lactate as marker of ischemia exceeds that of other objective indices of ischemia[5].
Consequently, both the variable, i.e. lactate, and the technique, i.e. pacing, have been frequently applied to identify ischemia in man and to assess the consequences of different interventions. The latter requires, that the metabolic changes studied during ischemia are reproducible within a time frame which in view of the invasive nature of the method is acceptable, both in logistical and ethical terms. Although myocardial lactate production is a sensitive indicator during short periods of ischemia, its effectiveness becomes less when myocardial ischemia is sustained as a result of inhibition of glycolysis, in particular at the glyceraldehyde-3-P-dehydrogenase level[6-8]. Consequently, the reduction in glycolytic flux leads to a decrease in lactate production, which may explain why myocardial lactate production values are not reproducible after a short interval between moderate ischemia[3,9] or more prolonged intervals when ischemia has been more severe[10].
Thus far, this aspect has received little attention in human studies. The vast majority of invasive metabolic investigations into the effect of interventions on ischemia have been uncontrolled. Also, the few studies on the reproducibility of ischemia-induced alterations in myocardial lactate metabolism, all carried out during pacing-induced stress, have invariably been conducted in small patient groups. Moreover, studies have focussed on only a limited number of intervals between pacing tests[4,9,11]. Usually one interval was tested. Also, different stress test protocols may affect the reproducibility of lactate production[4].
At present, there is no consensus on the obligatory time period between tests to allow for myocardial lactate metabolism to recover. As a result interpretation of the effect of interventions, based on so-called "historical" controls from the literature, are clearly not possible.
The present study addresses this aspect in a prospective investigation in 55 patients with left coronary artery disease, undergoing repetitive incremental atrial pacing with intervals ranging from 15 to 60 minutes.

## METHODS

### Patient characteristics

After the study was approved by the institutional Ethical Review Committee and informed consent was obtained, 55 patients, 51 men and 4 women, aged 57 years (range: 42 to 68 years) entered the trial. All patients were scheduled for elective cardiac catheterization because of stable, exercise-induced angina pectoris in conjunction with objective signs of myocardial ischemia and/or a previous myocardial infarction. To be eligible, patients had to be normotensive without signs of heart failure. Moreover, patients with conduction disturbances, valvular heart disease, unstable angina or a myocardial infarction of less than one month old were excluded. Antiischemic therapy, including beta-blockers, calcium-antagonists and long-acting nitrates were withheld 36-72 hours pre-study, depending on plasma half-life of the respective medication. Digoxin and diuretics, if present, were stopped 24 hours before the trial. Oral anti-coagulants were also withheld 2-3 days, and anti-platelet therapy and NSAIDs at least 10 days prior to the study. Only, short-acting nitroglycerin was allowed until 6 hours before onset of the investigation.
To participate in the study, patients had to have significant coronary artery disease ($\geq$70% diameter narrowing) in at least one of the major epicardial coronary arteries draining the anterior, anteroseptal and lateral part of the heart, e.g. either the left anterior descending, diagonal, proximal circumflex or proximal marginal branches. Eligibility for the study, based on this criterion, was first assessed on videorecordings of the left coronary angiogram during catheterization. However, the final decision regarding participation was made after two independent angiographers, experienced in qualitative assessment of over 600 coronary angiograms annually, agreed on the presence of significant lesions after viewing of the film.
Thus, 14, 15, 12 and 14 patients underwent repetitive atrial pacing stress tests after intervals of 15, 30, 45 and 60 minutes respectively. Baseline clinical and angiographic criteria, presented in Table I, were comparable in all groups.

### Procedures

Patients were studied at approximately the same time in the morning after an overnight fast, without premedication. All procedures were carried out under local anaesthesia with 1% lidocaine. First, left and right coronary angiography was performed with non-ionic contrast material using the Seldinger technique. When inclusion criteria were met, a no 7 Fr Millar pigtail microtipmanometer or a no 7 Fr Cordis pigtail catheter was introduced into the left ventricle through an arterial introducer system (Desilet) in a femoral

**Table I: Baseline clinical and angiographic criteria.**

| | 15 min Interval | 30 min Interval | 45 min Interval | 60 min Interval |
|---|---|---|---|---|
| Sex (M/F) | 11/3 | 15/0 | 11/1 | 14/0 |
| Age ($\bar{x}$ ± SEM/range) | 56±1.5 (45-65) | 57±1.9 (46-66) | 56±2.1 (45-67) | 58±1.5 (46-68) |
| Previous infarct (n) | 9 | 6 | 8 | 7 |
| Positive X-ECG (n) | 8 | 11 | 9 | 10 |
| Medication (n) | | | | |
| nitrates | 5 | 11 | 5 | 7 |
| beta-blockers | 11 | 11 | 8 | 10 |
| calcium antagonists | 5 | 11 | 9 | 9 |
| Coronary angiography (n) (>70% diameter stenosis) | | | | |
| 1-vessel | 4 | 5 | 4 | 3 |
| 2-vessel | 5 | 8 | 3 | 7 |
| 3-vessel | 5 | 3 | 5 | v |
| LV wall motion abnormality (n) | 6 | 8 | 9 | 7 |
| LV ejection fraction (%) | 52±4.9 | 59±2.1 | 56±4.0 | 55±3.7 |
| LVEDV (ml/m$^2$) | 77±7.0 | 70±6.0 | 70±6.2 | 72±7.2 |

*Abbreviations: EDV = End Diastolic Volume; F = Female; LV = Left Ventricular; M = Male; min = minutes; n = number*

artery. Next, a no 7 Fr thermodilution pacing catheter (Wilton Webster Laboratories) or a no 7 Fr Zucker bipolar pacing catheter was advanced from an antecubital vein into the midportion of the coronary sinus, such that the proximal thermistor or pacing electrode was at least 3 cm beyond the orifice of the coronary sinus. Particular care was taken to ensure a stable catheter position, which also allowed for sufficient amounts of blood to be collected over relatively short periods. Absence of reflux from the right atrium was confirmed by bolus injections of saline at room temperature. Catheter positions were then recorded on video disc to allow rechecking during the study.

**Hemodynamic and electrocardiographic measurements and calculations**

After instrumentation, fluid-filled catheters were calibrated using Statham or Bentley pressure transducers. Zero reference level was set at midchest. Micromanometer pressures were balanced to zero and superimposed on the conventional pressure curve. During the study, all pressures, the first derivative of left ventricular (LV) pressure, coronary sinus flow and 3 ECG leads were continuously recorded on paper at the appropriate paperspeed, using a CGR cath lab system.
Concomitantly, hemodynamic variables, including LV systolic and end diastolic pressures, LV pressure-derived contractility and relaxation parameters (peak positive and negative LV dP/dt, $VCE_{40}$ and $V_{max}$) were determined on-line with a Mennen cath lab system.
In a beat-to-beat analysis the system averages 15 to 20 consecutive beats to level out respiratory variations. Representative curves may be chosen for further analysis by the operator.

ST-segment changes were assessed in leads I, II and V5 from 3-5 consecutive beats, at 80 msec after the J point using a calibrated magnifying lens.

### Lactate determination

For lactate determination, simultaneous sampling from the coronary sinus and left ventricle was carried out. Exactly 1 ml of blood was quickly transferred into tubes containing 2 ml of icecold 0.6 M $HClO_4$, mixed thoroughly and kept on ice. Collecting tubes were colour-coded to obviate mistakes in the transfer proces during fast repetitive sampling. Immediately after the study, samples were weighed and centrifuged at 2000xg for 20 minutes and the supernatant frozen for subsequent determination. The lactate assay was carried out in triplicate. Technique and standard deviation of the assay in our laboratory has been reported[2].

### Scoring of anginal pain

Before the start of each study, patients were requested to indicate the moment, when they experienced the beginning of chest discomfort. Next, they were asked to score anginal pain, according to a modified Borg scale, during each test and to indicate the time of disappearance after each test.

### Study protocol

Studies were carried out at least 40 minutes after coronary angiography and 20 minutes after instrumentation. Multiple control determinations of all hemodynamic and electrocardiographic variables were performed and on-line registrations checked for baseline variations. Blood sampling for lactate was carried out in duplicate. Next, the first atrial pacing stress test was performed with increments in heart rate of 10 beats per 2 minutes until the occurrence of significant anginal pain, defined as the level of pain at which the patient would usually rest or take nitroglycerin. Other endpoints for pacing included atrioventricular block or a maximum paced heart of 170 beats/minute. At fixed intervals during pacing, e.g. halfway between the 100, 120, 140 and 160 beats/minute period, hemodynamic and electrocardiographic variables were reassessed and blood collected for lactate. All parameters were determined at maximal pacing rates just before cessation of pacing, with subsequent reassessment of all variables at 15 seconds and at 1 and 2 minutes post-pacing. This was followed by a predetermined stabilization period of 15, 30, 45 or 60 minutes. Next, control determinations conform the first atrial pacing stress test were carried out, followed by a second test, which was identical in design to the first atrial pacing stress test. Care was taken that measurements during this second test were carried out at exactly the same intervals as during the first test and that duration of the tests and maximal pacing rates were identical.

### Statistical analysis

Baseline variables in the 4 study groups were compared using an analysis of variance. Within groups, changes from control during each pacing test and changes in variables during and after pacing between both tests were evaluated, using a two-tailed t-test for paired observations. A p value $<0.05$ was regarded as significant. All variables are presented as average ($\bar{x}$) and one standard error of the mean (SEM).

## RESULTS

### Baseline period before the first atrial pacing stress test

None of the patients had objective or subjective signs of myocardial ischemia at baseline before the first pacing stress test. However, after analysis of lactate data, 8 patients proved to have lactate production values at baseline varying from -90% to -1%. Of these, 2, 3, 2 and 1 patients belonged to the 15-, 30-, 45- and 60-minute interval group, resp. Hemodynamic variables and electrocardiographic criteria, i.e. ST-segments, were comparable between the 4 groups. Moreover, average lactate extraction values did not differ at baseline.

### Baseline period before the second atrial pacing stress test

At baseline before the second pacing stress test, angina was still present in one patient in the 15-minute group. In the other patients, angina had usually subsided within the first minutes after the first test. Hemodynamic variables and ST-segments were comparable between the 4 groups at control before the second pacing test (Tables II and III). In contrast, myocardial lactate extraction was significantly higher in the 30- and 60-minute groups, compared with the 15- and 45-minute patients. Ten patients had lactate production at baseline. Of these, 5 were in the 15-minute group and none in the 60-minute group. Within groups, baseline hemodynamic variables of both pacing

**Table II: Hemodynamic changes during repetitive ischemic episodes with varying intervals.**

| | Control | 120/min | MAX | 15"P-P | 1'P-P | 2'P-P |
|---|---|---|---|---|---|---|
| | | | *15 MIN INTERVAL* | | | |
| **HR (beats/min)** | | | | | | |
| P I | 68±3.0 | 121±1.4* | 135±5.3* | 66±5.0 | 66±3.7 | 62±3.6 |
| P II | 72±3.6 | 120±1.1* | 132±4.8* | 71±4.6 | 70±5.2 | 66±1.8 |
| **DP (HRxLVSPx10$^{-3}$)** | | | | | | |
| P I | 8.9±0.90 | 17.3±1.77* | 17.6±1.17* | 10.4±1.36 | 9.6±0.92 | 8.9±1.33 |
| P II | 10.0±0.77 | 17.7±1.97* | 16.9±1.40* | 9.3±1.45 | 10.3±0.65 | 8.0±0.42 |
| **LVEDP (mmHg)** | | | | | | |
| P I | 14±1.4 | 12±2.2 | 16±2.6 | 19±2.1 | 17±1.5 | 17±2.2 |
| P II | 15±1.8 | 10±2.0 | 15±2.5 | 19±3.4 | 17±2.6 | 15±1.5 |
| **LV dp/dt pos (mmHg.sec$^{-1}$)** | | | | | | |
| P I | 1578±132 | 1979±262 | 2128±219* | 1801±193 | 1723±118 | 1580±144 |
| P II | 1740±232 | 1945±450 | 1975±232 | 1682±173 | 1584±109 | 1451±79 |
| **Vmax (sec$^{-1}$)** | | | | | | |
| P I | 46.7±3.5 | 56±4.8 | 67±6.5* | 45.2±3.6 | 46.7±2.4 | 44.6±2.1 |
| P II | 45.7±2.1 | 54±5.9 | 54±3.9 | 42.2±3.4 | 43.5±2.7 | 49.0±3.8 |
| | | | *30 MIN INTERVAL* | | | |
| **HR (beats/min)** | | | | | | |
| P I | 76±3.0 | 122±0.9* | 126±5.6* | 75±5.1 | 74±3.3 | 73±3.9 |
| P II | 72±3.6 | 122±0.9* | 127±3.7* | 71±4.4 | 73±4.4 | 78±6.7 |
| **DP (HRxLVSPx10$^{-3}$)** | | | | | | |
| P I | 11.6±0.6 | 18.4±0.8* | 19.0±1.3* | 12.4±1.2 | 11.7±0.8 | 11.3±0.9 |
| P II | 10.1±0.6 | 17.9±1.1* | 18.7±1.4* | 10.9±1.2 | 10.2±1.1 | 11.0±1.4 |
| **LVEDP (mmHg)** | | | | | | |
| P I | 15±1.2 | 11±1.9* | 14±2.7 | 18±3.1 | 17±2.3 | 17±1.9 |
| P II | 14±1.6 | 6±1.4* | 11±2.0 | 16±2.1 | 14±2.1 | 13±2.1 |
| **LV dp/dt pos (mmHg.sec$^{-1}$)** | | | | | | |
| P I | 1988±166 | 2597±246* | 2991±330* | 2258±281 | 2148±296† | 2310±320 |
| P II | 1753±238 | 2010±116 | 2244±203 | 1741±237 | 1657±97 | 1772±156 |
| **Vmax (sec$^{-1}$)** | | | | | | |
| P I | 53.3±4.8 | 75±7.1*† | 73±8.2*† | 63±8.6† | 54±6.1 | 57±7.6 |
| P II | 48.1±2.9 | 57±3.3 | 56±4.4 | 43±4.6 | 46±1.8 | 53±5.5 |

*Abbreviations: DP = double product; HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; MAX = maximal pacing rates; min = minute(s); P I = atrial pacing stress test I; P II = atrial pacing stress test II; P-P = post-pacing; sec = seconds; ' = minutes; " = seconds.*
*All values $\bar{x}$ ±SEM; * $p<.05$ vs control; † $p<.05$ P I vs P II.*

tests were also comparable with the exception of contractility parameters in the 60-minute group, which had improved at control before the second test compared to the first one. Within each group, electrocardiographic parameters and arterial and coronary venous lactate values were also similar at baseline before both pacing tests.

### Reproducibility of hemodynamic variables during pacing (Table II, IIa)

By design, maximal heart rates were identical during both atrial pacing stress tests within each group. As left ventricular systolic pressures did not change during either test in each group, the double

**Table IIa: Hemodynamic changes during repetitive ischemic episodes with varying intervals.**

| | Control | 120/min | MAX | 15"P-P | 1'P-P | 2'P-P |
|---|---|---|---|---|---|---|
| | | | *45 MIN INTERVAL* | | | |
| **HR (beats/min)** | | | | | | |
| P I | 74±2.7 | 120±1.1* | 131±4.5* | 77±4.3 | 77±4.8 | 74±3.6 |
| P II | 74±3.1 | 121±0.8* | 134±4.5* | 79±4.3 | 82±5.3 | 80±6.9 |
| **DP (HRxLVSPx$10^{-3}$)** | | | | | | |
| P I | 11.1±0.8 | 17.5±1.2* | 19.2±0.9* | 11.9±1.7 | 11.9±0.9 | 11.8±1.5 |
| P II | 10.4±0.9 | 16.3±0.9* | 18.3±1.2* | 11.9±1.0 | 11.7±1.3 | 10.6±2.8 |
| **LVEDP (mmHg)** | | | | | | |
| P I | 14±1.5 | 10±1.6 | 14±3.3 | 18±4.4 | 19±3.2 | 13±3.4 |
| P II | 13±1.4 | 7±1.2* | 11±2.1 | 16±2.8 | 14±3.1 | 13±1.8 |
| **LV dp/dt pos (mmHg.$sec^{-1}$)** | | | | | | |
| P I | 1554±108 | 2120±123* | 1964±99* | 1554±213 | 1596±93 | 1778±100 |
| P II | 1701±209 | 2117±410 | 2250±221* | 1891±313 | 2033±272 | 1949±228 |
| **Vmax ($sec^{-1}$)** | | | | | | |
| P I | 46±2.6 | 55±3.0* | 56±5.2* | 51±3.4 | 44±3.9 | 49±6.3 |
| P II | 47±3.8 | 58±6.0 | 60±5.3* | 53±4.4 | 48±6.9 | 50±3.0 |
| | | | *60 MIN INTERVAL* | | | |
| **HR (beats/min)** | | | | | | |
| P I | 72±3.2 | 119±0.8* | 137±4.3* | 79±5.2 | 73±3.2 | 74±3.7 |
| P II | 75±4.0 | 120±0.7* | 135±4.6* | 76±4.9 | 74±4.1 | 78±4.5 |
| **DP (HRxLVSPx$10^{-3}$)** | | | | | | |
| P I | 11.3±0.7 | 17.8±0.8* | 19.6±1.0* | 11.8±1.4 | 11.3±0.9 | 11.6±0.8 |
| P II | 11.5±0.6 | 18.3±0.9* | 19.5±1.1* | 12.3±1.3 | 11.6±1.0 | 12.1±0.9 |
| **LVEDP (mmHg)** | | | | | | |
| P I | 13±1.2 | 7±1.0* | 9±1.0* | 20±2.1* | 13±1.4 | 13±1.6 |
| P II | 14±1.4 | 7±1.2* | 9±0.9* | 19±2.6* | 13±1.8 | 11±1.9 |
| **LV dp/dt pos (mmHg.$sec^{-1}$)** | | | | | | |
| P I | 1738±122 | 2015±156 | 2394±183* | 2120±163* | 1916±151* | 1805±84 |
| P II | 1662±105 | 1978±174 | 2442±177* | 2195±178* | 2018±190* | 1755±107 |
| **Vmax ($sec^{-1}$)** | | | | | | |
| P I | 51±3.0 | 60±2.7* | 65±3.1* | 55±4.3 | 51±3.6 | 50±2.5 |
| P II | 49±2.1 | 59±3.1* | 65±3.0* | 56±3.7 | 52±4.0 | 50±1.7 |

*Abbreviations: DP = double product; HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; MAX = maximal pacing rates; min = minute(s); P I = atrial pacing stress test I; P II = atrial pacing stress test II; P-P = post-pacing; sec = seconds; ' = minutes; " = seconds.*
*All values $\bar{x}$±SEM; *p<.05 vs control; † p<.05 P I vs P II.*

product, an index of myocardial oxygen demand, inceased similarly during both tests. Maximal heart rates and the double product were also comparable between groups during both pacing tests. In contrast, pacing-induced alterations in contractility varied considerably within patient groups. Following the 15-minute interval, all indices improved significantly during the first pacing test, but no longer during the second. Likewise, with an interval of 30 minutes all but one contractility indices did not improve either during the second test. In contrast, following a 60-minute interval the improvement in contractility was reproducible during both tests and persisted for 1 minute after pacing. Only the 45-minute group did not show consistent improvement of contractility during either test.

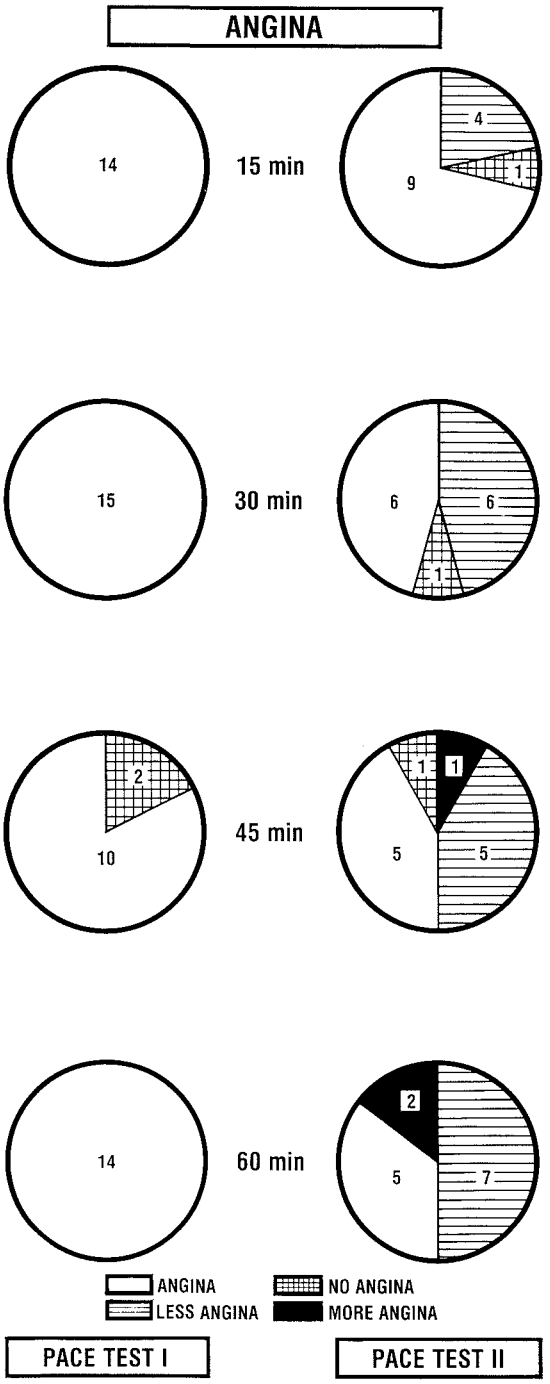
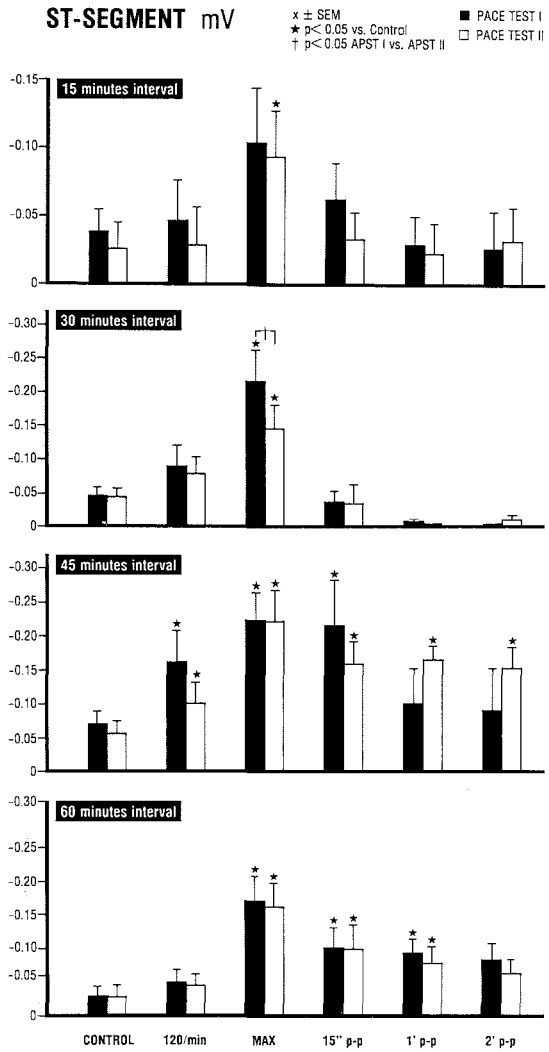



*Fig. 1: Anginal complaints during the first and second pacing stress test. Whereas during the first test angina occurs in nearly all patients, this was clearly less or absent during the second pacing test, irrespective of the interval between pacing.*

*Fig. 2: Average values of ST-segment depression during repetitive pacing tests with variable intervals. Changes are not reproducible following intervals of 15 and 30 minutes. In contrast, values are comparable during maximal pacing rates (MAX) after intervals of 45 minutes upwards. Min = minutes; P-P = post-pacing.*




## Reproducibility of indices of myocardial ischemia during pacing.

*Angina*

During the first test, pacing-induced anginal symptoms did occur in all patients except for 2 patients in the 45-minute group. However, during the second test, pacing-induced angina proved not to be reproducible in neither group (Fig. 1).

**Table III: Reproducibility of electrocardiographic changes during successive ischemic periods with different intervals - ST-segment depression.**

| | Control | 120/min | MAX | 15" P-P | 1' P-P | 2' P-P |
|---|---|---|---|---|---|---|
| | | | *15 MIN INTERVAL* | | | |
| P I | -.04±.01 | -.05±.03 | -.10±.04 | -.06±.03 | -.03±.02 | -.03±.03 |
| P II | -.03±.02 | -.03±.03 | -.09±.03* | -.03±.02 | -.02±.02 | -.03±.02 |
| | | | *30 MIN INTERVAL* | | | |
| P I | -.04±.01 | -.09±.03 | -.22±.05*† | -.04±.02 | -.01±.01 | .00±.01 |
| P II | -.04±.01 | -.08±.02 | -.14±.05* | -.04±.03 | .0 ±.01 | .01±.01 |
| | | | *45 MIN. INTERVAL* | | | |
| P I | -.07±.02 | -.16±.04* | -.24±.04* | -.21±.06* | -.1 ±.05 | -.09±.06 |
| P II | -.06±.02 | -.10±.03* | -.24±.04 | -.16±.03* | -.16±.02* | -.15±.03* |
| | | | *60 MIN. INTERVAL* | | | |
| P I | -.03±.02 | -.05±.02 | -.17±.04* | -.10±.03* | -.09±.02* | -.08±.03 |
| P II | -.03±.02 | -.04±.02 | -.16±.04* | -.10±.04* | -.08±.02* | -.06±.02 |

*Abbreviations: MAX = maximal heart rates; min = minutes; P-P = post-pacing; P I = atrial pacing stress test I; P II = atrial pacing stress test II; ' = minutes; " = seconds.*
*all values $\bar{x}$ ± SEM; *p<.05 vs control; † p<.05 P I vs P II.*

In general, patients had significantly less angina, which started later and was of shorter duration. In the groups with 30-, 45- or 60-minute intervals, angina was either absent or less in 50% of patients and in 35% of patients in the 15-minute group during the second pacing test. Also, 3 patients experienced more angina during the second pacing test, 1 in the 45- and 2 in the 60-minute group.

*ST-segment changes (Table III)*

Ischemic electrocardiographic changes were not uniformly reproducible either. In the 15-minute group, significant changes only occurred during the second test but not during the first one. In contrast, ST-segment depressions were significantly less during the second test, compared to the first, in the 30-minute group (Fig. 2). Average values for ST-segment depressions were only comparable with intervals as long as 45 and 60 minutes. Even so, individual values for maximal ST-segment alterations varied considerably in all groups, although this was less after 60 minutes as shown in figure 3.

*Left ventricular end diastolic pressure (Table II)*

Left ventricular end diastolic pressure did not change in the 30- and 45-minute groups during either test. An ischemia-induced rise in left ventricular filling pressure was only observed in the 15- and 60-minute interval groups, but in both situations only after the first test. Individual values varied significantly also (Fig.4).

*Lactate (Table IV)*

Arterial lactate values remained unchanged during and after pacing in all groups (Fig. 5). Arterial lactate levels were also similar during both pacing tests, irrespective of pacing intervals. Coronary venous lactate levels increased significantly during the first test in all patient groups. In the second test, levels were similar to those during the first one, following the 45- and 60-minute period.

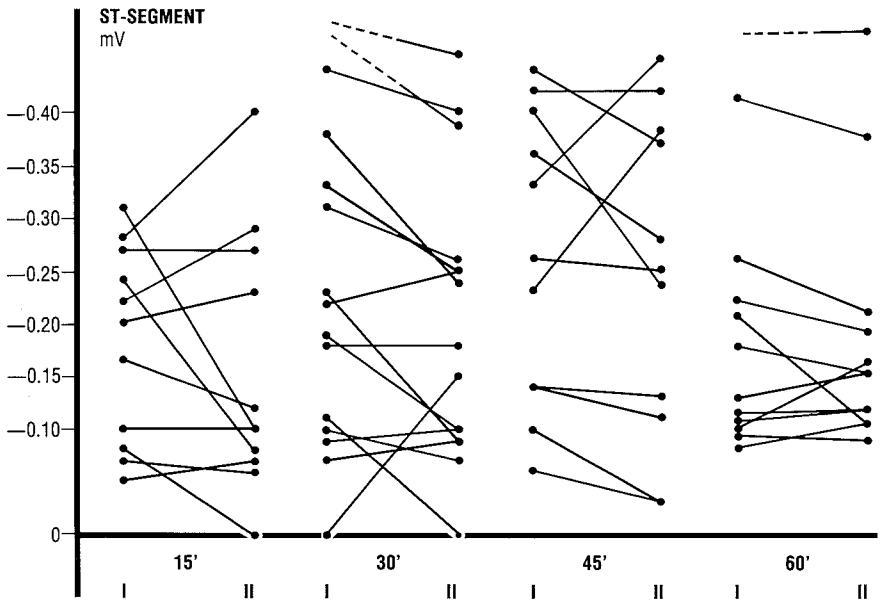



*Fig. 3: Individual ST-segment changes during two successive pacing stress tests (I and II resp.) following intervals of 15, 30, 45 and 60 minutes (').*
*There is a marked individual variability with intervals of 45 minutes or less, but a somewhat more homogeneous response after 60 minutes.*

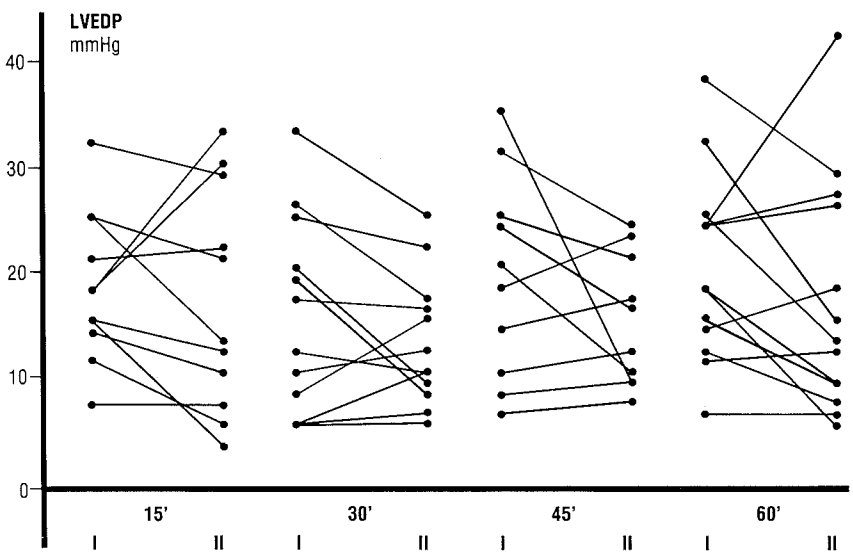



*Fig. 4: Individual values for post-pacing left ventricular end diastolic pressure during two successive pacing stress tests, 15, 30, 45 and 60 minutes apart. Irrespective of the interval between tests, pressures, measured immediately after pacing, vary considerably.*

**Table IV: Reproducibility of myocardial lactate metabolism during successive ischemic periods.**

| | Control | 120/min | MAX | 15" P-P | 1'P-P | 2'P-P |
|---|---|---|---|---|---|---|
| | | | *15 MIN INTERVAL* | | | |
| art. lactate (mmol/l): | | | | | | |
| P I | 0.63±0.04 | 0.60±0.03 | 0.62±0.04 | 0.59±0.04 | 0.60±0.05 | 0.60±0.06 |
| P II | 0.57±0.04 | 0.61±0.04 | 0.61±0.05 | 0.63±0.04 | 0.64±0.05 | 0.63±0.07 |
| cor. ven. lactate (mmol/l): | | | | | | |
| P I | 0.54±0.04 | 0.61±0.06 | 0.82±0.07*† | 0.88±0.09*† | 0.77±0.09* | 0.67±0.05* |
| P II | 0.54±0.04 | 0.56±0.06 | 0.63±0.04* | 0.77±0.05* | 0.66±0.05* | 0.57±0.06 |
| lactate extraction (%): | | | | | | |
| P I | 13±4.7 | -9±11.3 | -17±6.5*† | -53±13.1*† | -23±10.1*† | -18±13*† |
| P II | 6±3.4 | 12±5.6 | -1±4.8 | -25±9.4* | -6±6.9* | 3±9.2 |
| | | | *30 MIN INTERVAL* | | | |
| art. lactate (mmol/l): | | | | | | |
| P I | 0.54±0.05 | 0.51±0.07 | 0.54±0.06 | 0.52±0.07 | 0.54±0.07 | 0.54±0.07 |
| P II | 0.54±0.05 | 0.53±0.05 | 0.53±0.05 | 0.49±0.06 | 0.54±0.06 | 0.53±0.06 |
| cor. ven. lactate (mmol/l): | | | | | | |
| P I | 0.47±0.06 | 0.52±0.07 | 0.72±0.10* | 0.74±0.09* | 0.59±0.07 | 0.52±0.06 |
| P II | 0.43±0.06 | 0.53±0.09 | 0.58±0.08* | 0.62±0.08* | 0.61±0.11 | 0.55±0.09 |
| lactate extraction (%): | | | | | | |
| P I | 12±8.3 | -3±11.1 | -33±13*† | -55±15*† | -18±9.2* | 1±8.3 |
| P II | 21±4.8 | -8±9.1* | -8±8.8* | -23±14* | -9±11* | 0.5±8.2 |
| | | | *45 MIN INTERVAL* | | | |
| art. lactate (mmol/l): | | | | | | |
| P I | 0.63±0.06 | 0.60±0.05 | 0.66±0.06 | 0.64±0.04 | 0.66±0.06 | 0.65±0.05 |
| P II | 0.65±0.05 | 0.62±0.06 | 0.67±0.09 | 0.67±0.09 | 0.73±0.11 | 0.73±0.10 |
| cor. ven. lactate (mmol/l): | | | | | | |
| P I | 0.59±0.06 | 0.64±0.07 | 0.88±0.16* | 1.03±0.17* | 0.94±0.16* | 0.77±0.12 |
| P II | 0.61±0.07 | 0.66±0.06 | 0.87±0.15* | 1.07±0.18* | 0.96±0.15* | 0.75±0.16 |
| lactate extraction (%): | | | | | | |
| P I | 11±3.7 | -8±5.7* | -22±10.3* | -49±13.1* | -31±12.3 | -12±11.4 |
| P II | 8±3.5 | -10±2.5* | -23±6.9* | -57±17.3* | -38±16.1* | -4±7.7 |
| | | | *60 MIN INTERVAL* | | | |
| art. lactate (mmol/l): | | | | | | |
| P I | 0.76±0.11 | 0.73±0.14 | 0.80±0.12 | 0.81±0.12 | 0.84±0.13 | 0.82±0.12 |
| P II | 0.84±0.14 | 0.78±0.15 | 0.79±0.13 | 0.80±0.12 | 0.84±0.12 | 0.75±0.11 |
| cor. ven. lactate (mmol/l): | | | | | | |
| P I | 0.61±0.11 | 0.64±0.13 | 0.77±0.14* | 0.85±0.14* | 0.96±0.16* | 0.67±0.12 |
| P II | 0.60±0.11 | 0.63±0.12 | 0.77±0.14* | 0.87±0.15* | 0.89±0.15* | 0.63±0.11 |
| lactate extraction (%): | | | | | | |
| P I | 21±3.8 | 16±3.9 | 6±4.3* | -22±15* | -15±13.1 | -6±7.1* |
| P II | 28±4.1 | 17±4.0 | 3±6.1* | -20±13.1* | -4±12.6* | -9±7.3* |

*Abbreviations: art. = arterial; cor. ven. = coronary venous; MAX = maximal heart rates; MIN = minutes; P-P = post-pacing; P I = atrial pacing stress test I; P II = atrial pacing stress test II.*
*All values $\bar{x}$ ± SEM; *p<.05 vs control; †p<.05 P I vs P II.*

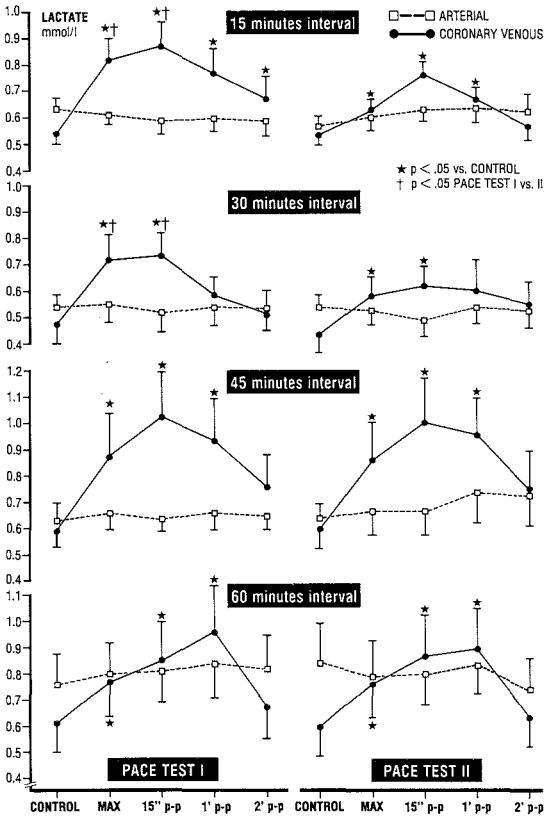



*Fig. 5: Arterial and coronary venous lactate levels during and after 2 successive pacing stress tests with different intervals. Whereas arterial levels do not change during pacing and are reproducible, coronary venous levels increase significantly with maximal levels at 15 seconds (") or 1 minute (') post-pacing (p-p). Following intervals of 15 and 30 minutes coronary venous levels rise significantly less during the second test. In contrast, they are comparable after 45 and 60 minutes.*
*MAX = maximal pacing rates.*
*Values are* $\bar{x} \pm SEM$.

In contrast, coronary venous lactate levels increased significantly less during the second test after 15 or 30 minutes (Fig. 5). Consequently, in the latter groups, the values for myocardial lactate production were also significantly less during the second pacing test, compared to the first one (Fig. 6). Alternatively, the arterial - coronary venous lactate difference and the lactate production values were comparable with intervals as great as 45 and 60 minutes. Individual lactate production values were also significantly less in the 15- and 30-minute groups during the second pacing test, compared to the first one (Fig. 7). However, they were generally comparable in the 45- and 60-minute groups. Although some fluctuations were still present, only 2 patients in the 45-minute and one in the 60-minute group had $\geq$10% less lactate production during the second test.

## DISCUSSION

Besides general indices of ischemia, such as angina or ST-segment changes, metabolic markers, in particular myocardial lactate production, are preferentially used in the invasive assessment of pharmacological interventions. Also, in these circumstances, atrial pacing has been the stress test of preference. However, despite a large volume of published material concerning such intervention studies, information on the reproducibility of lactate changes during repetitive periods of ischemia is scarce, fragmentary and inconclusive. Consequently, studies have either not been controlled or have referred to "historical" controls, often derived from the results of different laboratories. The present study addresses the question of reproducibility, not only of lactate, but also of symptomatic, electrocardiographic and hemodynamic sequelae of ischemia, in a prospective, randomized fashion. It investigates various intervals between stress tests over a period, which would appear acceptable in ethical and logistical terms, in adequate numbers of patients.
Our study clearly indicates that, after relative short intervals of 15 to 30 minutes, changes in myocardial lactate metabolism are not reproducible with significantly less lactate production during the second test. However, after 45 and 60 minutes, excellent reproducibility is achieved without significant individual variability. In contrast, whereas average values for ST-segment changes were comparable after intervals as long as 45 minutes, individual variability was still considerable at this point of time, but disappeared after a 60-minute interval between pacing periods. Likewise, contractility parameters were only comparable after 60 minutes had elapsed, whereas left ventricular filling pressure either did not change or show marked individual variability. Moreover, angina was clearly not reproducible, irrespective of pacing intervals.

### Usefulness of myocardial lactate production as marker of ischemia

The usefulness of myocardial lactate metabolism as marker of ischemia during pacing-induced stress in humans has been questioned[13]. When myocar

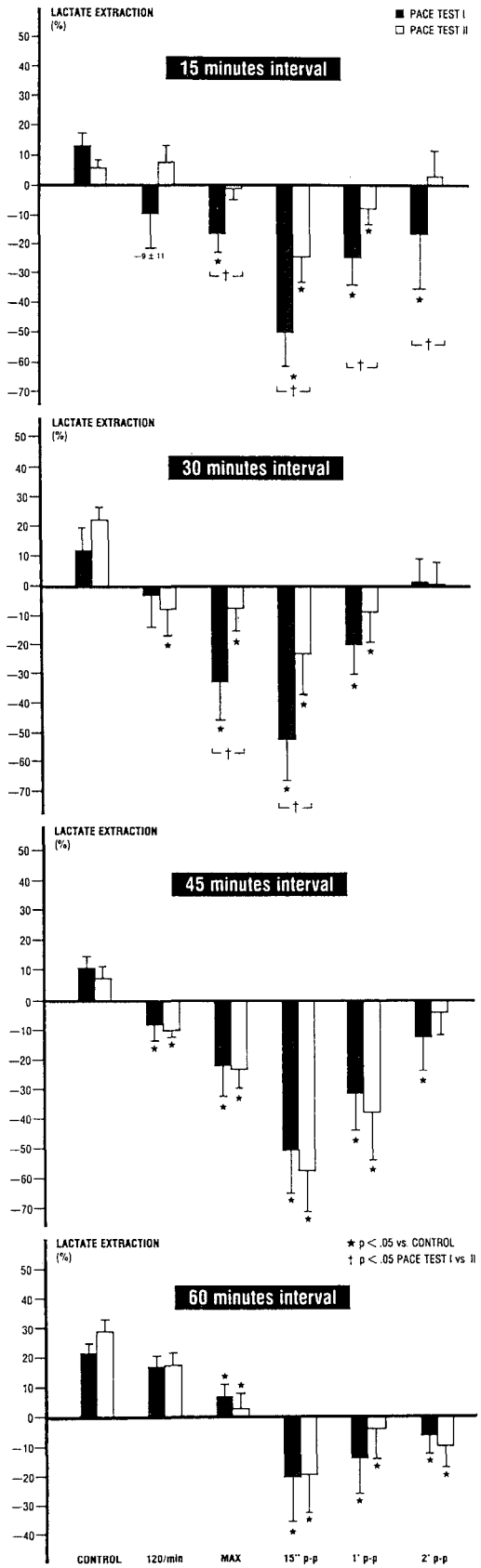



*Fig 6: Average lactate extraction values during two successive pacing stress tests (I and II) with different intervals. Following intervals of 45- and 60 minutes lactate production values are comparable. However, after shorter periods, i.e. 15 and 30 minutes, lactate production is significantly less during the second pacing test. Max = maximum pacing rates; min = minutes; p-p = post-pacing.*

dial lactate production, rather than a change in extraction is considered necessary to objectively indicate myocardial ischemia[14,15], the sensitivity of this criterion does indeed appear low as well as variable, ranging from 35% to 69% during pacing-induced stress in humans[4,16-21]. However, from the same studies it would not appear that other objective criteria of ischemia, such as electrocardiographic changes or alterations in left ventricular filling pressure are superior in this respect. Also, it should be pointed out here, that in almost all investigations which have applied atrial pacing as stress, lactate measurements were carried out during maximal pacing and not thereafter. We and others have shown, that coronary venous lactate levels increase significantly when measured in the immediate post-pacing period[3,5]. When assessed at this point of time, the sensitivity of lactate metabolism as indicator of ischemia improves significantly, compared with evaluations made at maximal pacing only. Also, in so doing, we have found that, regarding sensitivity, it becomes superior to other objective markers of myocardial ischemia (5). Thus, in considering the effect of pharmacological interventions, not only the reproducibility of lactate changes during, but also after pacing, should be taken into account.

**Reproducibility of myocardial lactate metabolism during repetitive episodes of pacing-induced ischemia**

In humans, ischemia-induced changes in myocardial lactate metabolism are not reproducible when the interval between ischemic periods is 30 minutes or less. The results of our study indicate that ischemia-induced lactate production is significantly diminished during the second stress test compared with the first. Several explanations may apply.

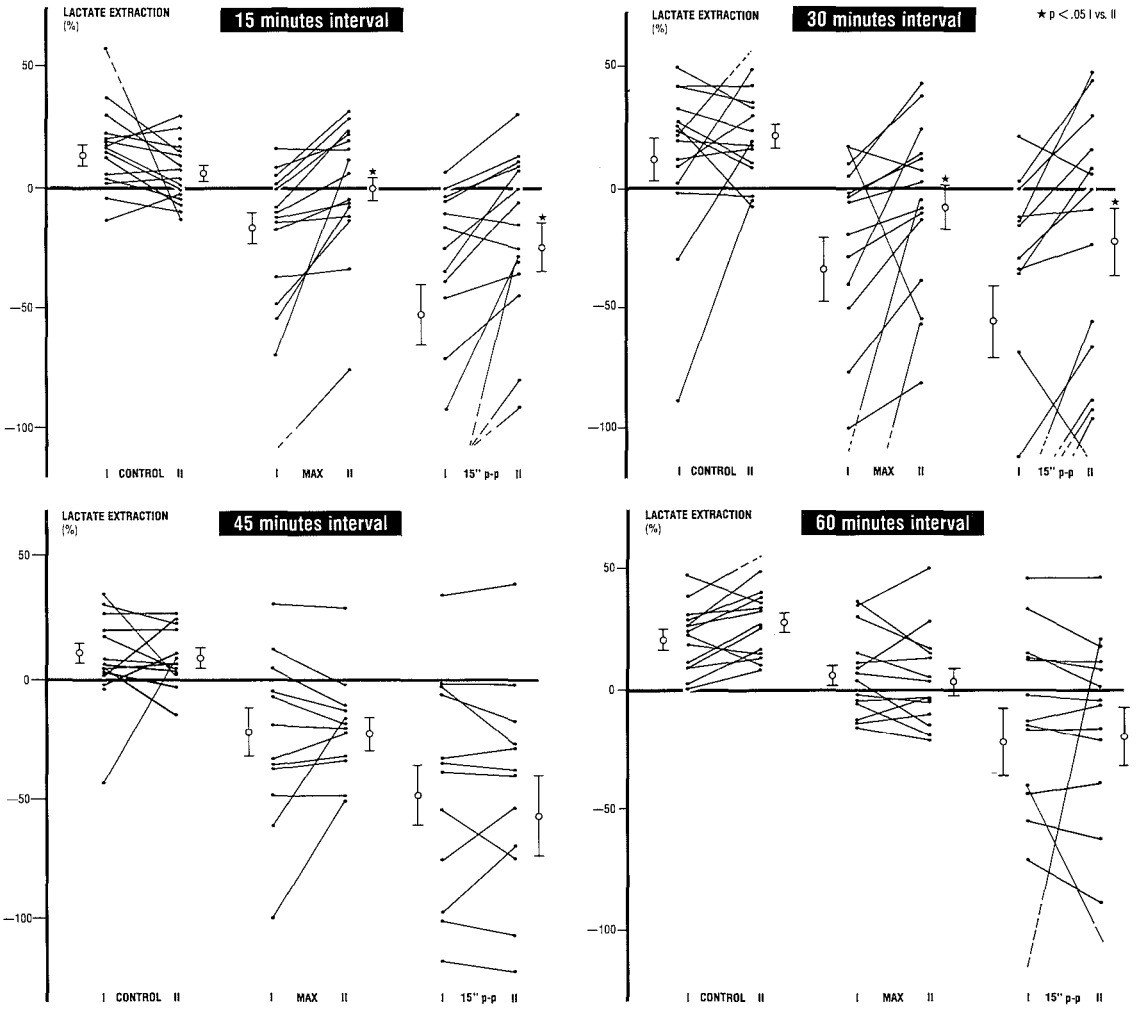



*Fig. 7: Individual values for lactate extraction during 2 successive pacing stress tests (I and II) with different intervals, measured at control, maximal rates (MAX) and at 15 seconds (") post-pacing (p-p). Individual values are significantly more positive during the second test following the 15- and 30-minute interval, indicative of less lactate production compared with the first test. In contrast, values are comparable with intervals of 45 minutes upwards, except for some fluctuations in the 60-minute interval group.*

Firstly, it could be argued, that ischemia was less during the second atrial pacing stress test and, hence, lactate production values reduced. This would seem unlikely in the event that myocardial oxygen demand was reproducible and other objective signs of ischemia did not follow a similar pattern. Thus, ST-segment depression was actually more pronounced during the second pacing test, following an interval of 15 minutes. Less ischemia, for instance due to persistent collateral flow after the first ischemic period is more likely to play a role after short intervals between successive ischemic periods. Also, previous studies in the pig heart demonstrate significantly reduced lactate production during repetitive 30-minute ischemic periods separated by 35 minutes of reperfusion[10]. In these experiments, coronary flow reductions were comparable during each ischemic period. Moreover, in this study, collateral blood supply was unlikely to have played a role[22] after the relatively short ischemic periods.

Alternatively, a reduction in myocardial lactate production during a second episode of ischemia following relatively short recovery periods, may

be explained by insufficient repletion of intracellular glycogen stores following the first ischemic period. Besides glucose uptake in the cardiocyte, which during ischemia depends critically on instantaneous coronary flow, glycogenolysis may provide for an additional substrate for glycolysis. The latter reserves are limited, however, and may be exhausted within several minutes during severe anoxia or ischemia[23]. Still, depending on the regenerating potential following ischemia, which will be time-dependent, a limited availability of glycogen at the onset of the second ischemic episode cannot be discarded.

A third and attractive mechanism to explain reduced lactate production following repetitive ischemic episodes with relatively short intervals, relates to a progressive inhibition of glycolysis in the course of ischemia. During early ischemia, glycolysis is predominantly regulated by the activity of phosphofructokinase[24,25]. Inhibition of glycolysis during more prolonged periods of ischemia occurs at the level of glyceraldehyde-3-P-dehydrogenase[26]. Both enzymes are sensitive to pH changes and depend on the ratio ATP versus ADP and AMP[27]. Sustained inhibition of glycolysis after an ischemic episode may be the explanation as to why myocardial lactate production values are not reproducible when only relatively short periods of recovery from ischemia are allowed. Persistent reductions in coronary flow and sustained release of cardiac nucleosides following a similar protocol of pacing and subsequent ischemia as in the present study, have been reported[2,28]. Selwyn et al.[29] also have reported sustained reductions in myocardial perfusion for as long as 20 minutes after stress testing. Hence, on-going residual coronary flow reductions and ischemia post-pacing may lead to persistent inhibitions of glycolysis and, subsequently, less lactate production during a second atrial pacing stress test.

Apparently, a minimum interval of 45 minutes is needed to nullify the effects of persistent ischemia on glycolysis and/or replete glycogen stores.

**Reproducibility of lactate - comparison with other studies in humans**

As mentioned, few human studies are available on the reproducibility of myocardial lactate changes during (pacing-induced) ischemia. Of these, none examined the differential effect of varying intervals between stress tests. Also, studies were usually carried out in relatively small patient groups. These investigations do not provide consensus as to the optimal time frame between tests with respect to the reproducibility of myocardial lactate production. Jackson and coworkers were the first to suggest good reproducibility after a 45-minute interval between pacing stress tests[11]. However, their observations were based on placebo studies in 5 patients, 3 of whom did not produce lactate. Several years later we confirmed their findings in a preliminary report concerning larger patient numbers and more frequent intervals[30]. Nevertheless, thus far there has been no agreement on individual reproducibility, not even after recovery periods of 45 minutes[12,31]. Likewise, whether average lactate production values are comparable following shorter periods between pacing tests has also been debated[4,9,32]. Whereas Ihlen and coworkers[4] suggested that lactate extraction may be reproducible after a 20-minute interval, this could not be confirmed in a recent study by Drobinski and coworkers[9]. Our investigations strongly suggest that average lactate production values are not reproducible following recovery periods of 30 minutes or shorter, whereas individual values only become comparable following intervals of 45 minutes upwards. The discrepancy in results from different human studies may, to a certain extent, relate to different study protocols. Although the pacing model, i.e. incremental versus continuous pacing at high rates, may not be important in this respect, the duration of pacing could affect the results. Thus, the study by Ihlen et al. suggests that very short pacing periods (20 seconds per interval) may allow for lactate changes to be reproducible after relatively short intervals (20 minutes). In contrast, longer pacing periods and, hence, more extensive ischemia, result in a suppression of lactate production following similar recovery periods. However, the very short pacing period applied by these authors is not practical, particularly not when other sequelae of ischemia have to be determined.

**Usefulness and reproducibility of other objective markers of ischemia**

In the present study, other markers of ischemia were not very reproducible. Electrocardiographic changes, i.e. ST-segment depression, is usually considered the gold standard in the objective assessment of myocardial ischemia. However, its sensitivity during pacing-induced ischemia is not impressive[21]. Although the occurrence of significant pacing-induced ST-segment depression has been reported to vary between 52% and 95%[4,16-21,33], in average, electrocardiographic abnormalities during pacing are found in 50% and 60% of patients. Also, its specificity has been

questioned[21,34]. We have recently shown that, during a similar pacing protocol as used in the present study, ST-segment depressions of ≥0.1 mV occur in 57% and 39% of patients without coronary artery disease or with only minimal lesions, resp[5]. Moreover, in patients with significant coronary artery disease, ST-segment changes were considerably less sensitive than myocardial lactate alterations.
Little information is available on the reproducibility of ST-segment changes in the context of fast atrial pacing. Thadani et al reported good reproducibility of average values after only 10-minute intervals, however, they did not provide data on individual values[19]. In contrast, Lau and coworkers found good individual reproducibility after short intervals; their study population was small, however[35].
In the present study, we were unable to detect any reproducibility in group values for this parameter for recovery periods as long as 30 minutes, whereas reasonable individual comparability was only attained after 60 minutes. Together with the relative lack of sensitivity, this suggests in our opinion that electrocardiographic changes are not as useful as a criterion whereby to judge the antiischemic efficacy of interventions during pacing as alterations in myocardial lactate metabolism.
Similarly, changes in left ventricular end diastolic pressure are not as useful as lactate in this context. Aagin, reported sensitivity may vary but on the whole appears relatively low[5,21]. This potential marker of ischemia is also not reproducible.
Thadani et al. observed less increase following a second pacing test after an interval of 20 minutes[19]. In the present study we confirmed this but also showed, that average values were still less following the second pacing test when the recovery period was at least 60 minutes. Furthermore, individual values varied considerably. Hence, changes in left ventricular filling pressure should not be taken into account when assessing the effect of interventions unless control comparisons are used.

**Reproducibility of angina**

Angina is often used as the main endpoint to assess the effect of pharmacological interventions during pacing. Either a reduction in the level of pain or lengthening of the time to reproducible angina during a second pacing test is taken to provide proof for the efficacy of the intervention. Our study clearly indicates that this is not justified. A considerable number of patients had less angina despite the absence of any intervention. Consequently, a lengthening of the time to comparable anginal pain as during the first test was present. Although this lack in reproducibility was slightly less after the shortest recovery period (15 minutes) there was no significant difference compared to the other intervals. The percentage of patients, who did not develop a similar level of angina during the second test after 15 minutes, compares well with values from other studies following comparable recovery periods, which vary from 20-43%[12,19]. That angina is even less reproducible following longer intervals has not been reported yet. Jackson et al. suggested that it was comparable after a 45-minute interval[10]. However, these observations are derived from a study of only 5 patients. In view of our findings, we do not recommend angina to be used as criterion to delineate the efficacy of therapeutic interventions.

**Limitations of the study**

An important limitation of this study is, that coronary flow was only measured in a small number of patients and not coincidental with the other measurements. Thus, it is not possible to examine the effect of varying recovery periods during successive pacing tests on coronary flow. Also, no consistent information is available on the reproducibility of cardiac lactate uptake. Comparable average values for coronary flow have been reported following an interval of 45 minutes[12] with moderate individual varations in the range of 10-20%. Preliminary data from our laboratory also indicate, that both average and individual values are comparable with an interval of 60 minutes (unpublished observations). Thus, it may be anticipated, that with the longer intervals between pacing, myocardial lactate uptake and release values may have been equally comparable in our study.
Secondly, in this study only one metabolite was studied, lactate. The overall assessment of the myocardial metabolism during ischemia could have been improved by measuring additonal metabolites. Using pacing as stress test, it has been shown, that free fatty acids are not reproducible after intervals varying between 20 and 45 minutes[9,31,32]. In contrast, amino acid and citrate fluxes may be more reproducible after recovery periods of 45 minutes[12]. Comparable increases in coronary venous hypoxanthine levels have also been demonstrated following successive coronary occlusions during angioplasty[36]. However, as it is not proven yet that any of these metabolites are better indicators of ischemia than lactate, further studies should be carried out to justify the addition

of these, in part elaborate, procedures.

### Implications

Our data indicate that the occurrence and degree of ischemia should not be asssessed during successive atrial pacing stress tests with relatively short intervals, i.e. of 30 minutes or less, at least not when myocardial lactate metabolism is considered the main criterion. However, intervals of 45 minutes or more between the end of the first and the onset of the second pacing test are sufficient to establish reproducibility of this metabolite. As more than 10% reduction in lactate production only occurred in very few patients during the second test after these intervals, a greater effect following interventions could be taken to assess individual responsiveness. With respect to ischemia-induced electrocardiographic changes, our studies suggest a minimum interval of 60 minutes, again between the end of the first and the onset of the second pacing test. Furthermore, our data do not suggest that changes in left ventricular filling pressure should be used. Finally, angina should not be taken as criterion to assess the effect of interventions in view of the significant placebo effect, which occurs during repetitive pacing, irrespective of the recovery period.

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# Chapter VII:

# REGIONAL CORONARY FLOW CHANGES DURING ISCHEMIA AND RELATION WITH METABOLIC ALTERATIONS.

# VII.1.

## Visualization of Myocardial Blood Flow Changes with Intracoronary $^{81m}Kr$.

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# VISUALIZATION OF MYOCARDIAL BLOOD-FLOW CHANGES WITH INTRACORONARY $^{81m}Kr$

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## INTRODUCTION

The decision to undertake agressive therapy of a coronary artery lesion, be it PTCA or surgery, depends entirely on the functional significance of the particular coronary artery stenosis present. Although more factors than the stenosis resistance alone determine the outcome of myocardial ischemia (see chapter 1, this volume), this can be expected to occur in high-grade lesions (>70% diameter stenosis). In smaller lesions, however, the determination of regional myocardial blood-flow may be necessary to justify a therapeutic decision.

Noninvasive myocardial perfusion studies with $^{201}Tl$ can be of particular value in single vessel lesions. In multivessel disease the sensitivity to delineate the smaller lesion may be less and overlapping of the various coronary flowbeds may prohibit the assessment of the exact localization of diminished myocardial perfusion.

Myocardial blood-flow studies in the intact subject can either be performed by the thermodilution method (1,2), inert gas washout techniques (3,4) or precordial mapping of radionuclides (5-9). In the thermodilution and inert gas washout techniques the coronary sinus has to be catheterized, which makes it an invasive procedure, allbeit of the right side of the heart. Although these methods allow for accurate measurements, even at high flowrates (4-10) an essential drawback is the fact that only overall left ventricular flow is measured. This limits its value in CAD which is essentially a regional disease. A recent modification in the thermodilution technique now allows for 2 areas of the left ventricular outflow

to be measured (11,12). However, the specific measurement of coronary flow through the smaller subregions of the left ventricle is not possible. In addition the obligatory positioning of the catheter in the mid coronary sinus to prevent atrial reflux interference with the measurements, usually precludes proper sampling from postero-lateral regions.

Labelled microspheres of approximately 15μ in diameter reflect transmural distribution of myocardial blood-flow very well as has been demonstrated in animal studies (13,14). Studies in man were carried out during heartcatheterization with macroaggregated albumin, usually employing a dual isotope technique. These investigations performed at rest and after some form of stress did show the technique to be quite reliable and safe (15-18).Obviously its potential use in humans is however limited to only a few investigations per patient.

Inert radioactive gases administered either directly into the coronary circulation or non-selectively in the aortic root have been used for the precordial mapping of coronary blood-flow. Of these $^{133}Xe$ has had the widest application (5-9). During catheterization the gas is injected in solution as a bolus into the coronary artery and the regional wash-out curves are measured. Several successive studies with a minimal interval of 6-8 min can be performed (6). It has been claimed that quantitative measurements of regional coronary blood-flow can be made. To what extent this really is possible, when measuring essentially 3-dimensional blood-flow changes in a 2-dimensional way is questionable. This criticism however, applies to any kind of precordial mapping technique. Other potential difficulties with the $^{133}Xe$ method is a (small) percentage of recirculation (± 5%) and its affinity for fat (tissue participation coeeficient ($\lambda$) in myocardium assumedly 0,72 versus $\lambda$ in fat tissue of 8 (19)),which can give background accumulation during repetitive studies. Finally its relatively low energy spectrum (gamma rays of 81 keV 37%) facilitates Compton scatter.

Several years ago a Krypton isotope, $^{81m}Kr$, was introduced (20-22), which allowed the continuous measurement of

regional myocardial blood-flow changes (23-26). In this chapter our experiences with the continuous intracoronary administration of $^{81m}Kr$ will be discussed.

Characteristics of $^{81m}Kr$

$^{81m}Kr$ is formed by isometric transition from unstable $^{81}Rb$ to stable $^{81}Kr$, emitting 190 keV gamma rays (65% abundance). The isotope is chemically and biologically inert and has a very short physical halflife of 13.6 sec. After intracoronary administration it diffuses readily through the capillary membranes and equilibrates rapidly with the extracellular myocardial fluid.

During continuous and constant administration $^{81m}Kr$ is distributed in relation to regional coronary blood-flow. Stabilization between local supply and decay of the isotope is reached within 30-60 sec after the commencement of intracoronary administration. Any change in $^{81m}Kr$ distribution hereafter depends on its regional supply rate and hence on local myocardial blood-flow. When $^{81m}Kr$ is continuously administered at a constant rate alterations in local myocardial blood-flow can be measured as the percentage change in $^{81m}Kr$ distribution in relation to the control situation. Due to its very short physical halflife $^{81m}Kr$ is not measurable in the venous effluent in the right atrium. This together with its 190 keV radiation spectrum ensures imaging with a negligable background.

$^{81m}Kr$ production. During our studies $^{81m}Kr$ was eluted from a sterile, pyrogen-free $^{81}Rb/^{81m}Kr$ generator. $^{81}Rb$ is formed during proton bombardment of natural Krypton gas, which results in a $^{81}Rb$ production rate of 3 mCi/μ A.h.; over 95% of which is recovered in aqueous solution (27). The $^{81}Rb/^{81m}Kr$ generator was calibrated to deliver 20-25 mCi at the time of the study. The unstable $^{81}Rb$ decays to $^{81m}Kr$, which emits 190 keV gamma rays (65% abundance). Elution of the generator with 5% glucose yields a solution containing only $^{81m}Kr$. Even at high perfusion rates of 25 ml/min only a negligeable break-through of $^{81}Rb$ occurs.

The eluate is then passed through a sterile millepore filter into the coronary artery catheter. Optimal perfusion rates from the generator in order to achieve sufficient and constant build-up of $^{81m}Kr$ on the $^{81}Rb$ column are in between 12-15 ml/min. In our studies a constant perfusion rate with 5% glucose of 13,3 ml/min was achieved with the use of a persistaltic infusion pump, resulting in 15 mCi total radioactivity per min with a 20 mCi generator.

Instrumentation. Contrary to studies with $^{133}Xe$, where multicrystal cameras were preferable due to their fast count rate possibilities, the single crystal camera is better for the $^{81m}Kr$ studies described in this chapter. Very fast count rates as can be realized with multiple crystal cameras (up to 200.000 - 500.000 counts per sec) are unnecessary in view of the limited production of $^{81m}Kr$ by the generator even at a higher calibration (up to 35 mCi). On the other hand the better spatial resolution of the single crystal camera enables flow changes to be determined in relatively small areas of the left ventricle, which is less optimal with the multicrystal camera due to its poor pictorial resolution. Count rates are improved using a ½-inch crystal instead of the ¼-inch crystal gamma cameras currently used for nuclear cardiology purposes. In these studies we have tried out both crystal sizes.

Total counts over the heart/min averaged ± 250.000 for the ½ inch crystal gamma camera (General electric porta camera) and ± 180.000 for the ¼ inch (Siemens LEM portable camera) using similar data processing techniques. Data given in this chapter are obtained with the ½-inch crystal gamma camera, connected on line to a Medical Data Systems - A2 computer and energy detection set on 190 keV ± 20%. Throughout the study imaging was visualized both on a persistence scope and on a monitor connected to the computer.

Images were acquired in 15 sec frames with the camera in 45° LAO position. In some studies an additional investigation was carried out in the 30° right inferior oblique position (RIO) to achieve optimal separation between the marginal branches of the left circumflex coronary artery.

Changes in $^{81m}Kr$ distribution were measured in various regions of interest. over the left ventricle, including both normal regions and areas with CAD, over the ascending aorta, total left heart and background regions, including the right atrium. Areas of interest were constructed using an electronic lightpen on the visual display unit.

Movement artefacts during the study were avoided and care was taken that successive regions of interest did not move out of their originally constructed areas. Further calculations were made of both total counts per area as well as of total counts per pixel per area. In order to correct for possible fluctuations in $^{81m}Kr$ delivery counts per area per frame were always correlated with and given as percentages of simultaneous total counts over the left heart.

Patient studies: Methodology and materials. Studies were carried out in patients with suspected or confirmed coronary artery disease in whom catheterization was believed necessary either to confirm the diagnosis or to investigate the possibility of angioplasty or by-pass surgery.

Patients were studied without premedication after an overnight fast because of concomittant metabolic investigations.

Using the Seldinger technique introducer systems were inserted in the right femoral artery and in the right brachial vein. This allowed the positioning of a 7F Judkins or Amplatz left coronary artery catheter in the ostium of the left coronary artery and a 7F Zucker bipolar pacing catheter in the mid-coronary sinus for pacing purposes and sampling of coronary venous blood. The length of the left coronary main stem and the position of the catheter tip in it were then examined in the 30° RIO position. Next, possible selective injections in one of the branches of the left coronary artery were studied by both slow and rapid manual injection of contrast material in the 45° LAO and 30° RIO positions and registered on videotape for continuous replay. When the possibility of streaming was present different catheters and catheter sizes were tried. Finally, any fluctuation in $^{81m}Kr$

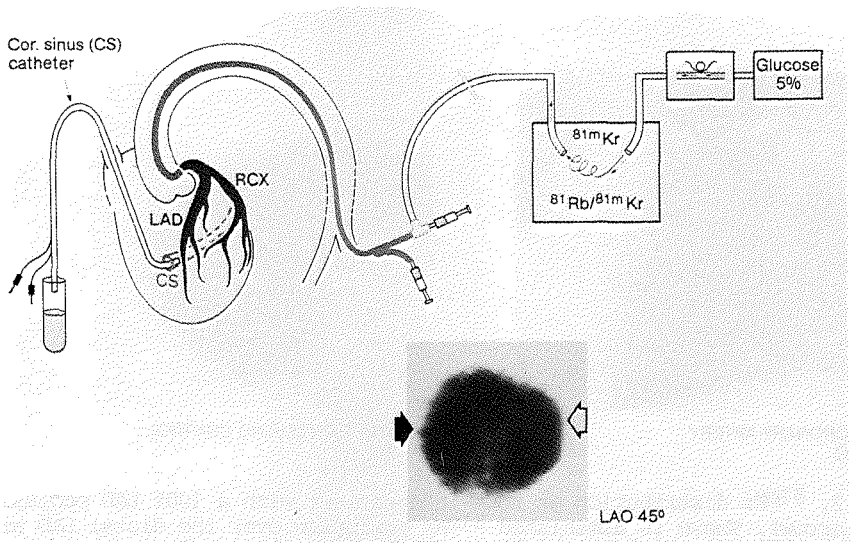
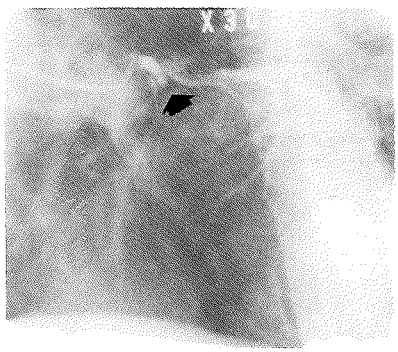


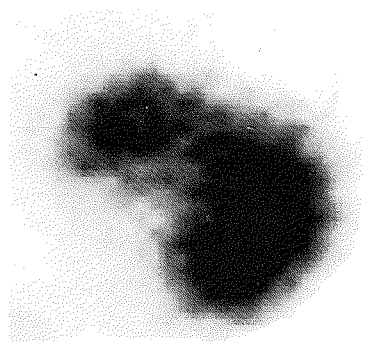

Fig. 1. Schematic representation of the study design. $^{81m}Kr$ is eluted in 5% glucose from the $^{81}Rb/^{81m}Kr$ generator and infused directly into the left coronary artery. At the bottom of the figure normal $^{81m}Kr$ distribution over the LAD and RCX area is shown in a patient without coronary artery disease. LAD = left anterior descending artery. RCX = left circumflex artery.

distribution over the left ventricle was excluded during a 10 min control period before initiation of the study.

When the possibility of streaming still existed or in the case of a short mainstem either a super-selective perfusion was carried out in the coronary branch of interest or the patient was excluded from the study altogether. A schematic representation of the investigational set-up and normal $^{81m}Kr$ distribution at rest is given in fig 1. A stabilization period of 20 min was allowed before initation of a stress test by way of incremental atrial pacing. During this test the heart rate was increased by 10 beats every 2 min until either anginal pain and/or atrio-ventricular block occurred. Throughout the study precordial imaging was carried out in successive 15 sec frames. Results are given for the control situations,



LEFT CORONARY ARTERY
45° LAO



$^{81m}$Kr DISTRIBUTION DURING CONTROL
45° LAO

Fig. 2. $^{81m}$Kr distribution at rest in a patient with a 100% LAD occlusion (see arrow). There is diminished $^{81m}$Kr perfusion over the distal LAD area and normal distribution over the RCX and proximal LAD region. LAD = left anterior descending artery. RCX = left circumflex artery.

at 100, 120, 140, 160 and 180 beats/min and during anginal pain or atrio-ventricular block, followed by determinations 15 sec, 1, 2 and 5 min after pacing. Onset and progression of anginal pain was correlated with $^{81m}$Kr distribution changes in the simultaneous 15 sec imaging frames.

Results

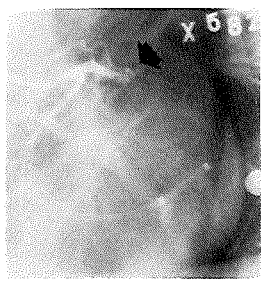
In 2 patients a selective infusion in the diseased branch of the left coronary artery was carried out because of a short mainstem.

One patient had to be excluded due to a possible streaming artefact.

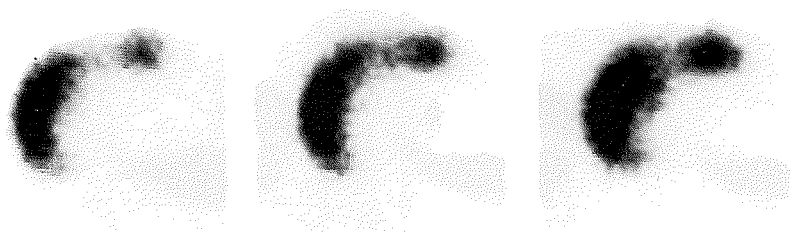
$^{81m}$Kr distribution changes in patients with normal coronary arteries. In 4 patients no significant coronary artery disease was present (CAD <50%). In these patients $^{81m}$ distribution over the left ventricle was normal during the control period and did not change during and after atrial pacing.

$^{81m}$Kr distribution changes in patients with CAD. In 16 patients with >50% CAD 12 areas with ≥90% and 6 areas with

Fig. 3a



LEFT CORONARY ARTERY
45° LAO



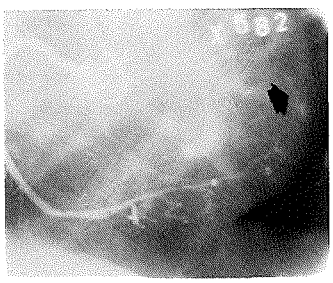
CONTROL | ANGINA PECTORIS 130 B/MIN | 5 MIN P-P

Fig. 3a. Absent $^{81m}Kr$ distribution in the RCX region at rest and during pacing in a patient who was catheterized twice within one week. Within this period a 99% RCX stenosis had progressed to a total occlusion without anterograde collaterals. Halfway the LAD a 50-70% stenosis is present, which however does not result in any change in $^{81m}Kr$ distribution in this area during pacing. LAD = left anterior descending artery. RCX = left circumflex artery.

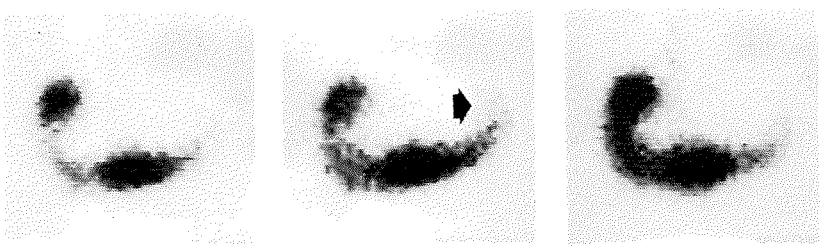
70-90% were present. Wallmotion abnormalities during left ventriculography at rest (30° RAO and 45° LAO projections) were observed in 9 patients: 8 in >90% and 1 in 70-90% left CAD areas, varying from hypo- to dyskinesia. Anterograde collaterals were present in 7 areas: in 6 with >90% and 1 with a 70-90% stenosis.

$^{81m}Kr$ distribution at rest. During the 10 min control period the $^{81m}Kr$ distribution pattern remained stable with only small fluctuations (<5%). $^{81m}Kr$ perfusion was normal in most areas. In only 5 an obviously diminished, however un-

Fig. 3b



RIGHT CORONARY ARTERY
45° LAO



CONTROL

ANGINA PECTORIS
130 B/MIN

5 MIN P-P

Fig. 3b. $^{81m}Kr$ perfusion of the right coronary artery in the same patient. During the interval between the two catheterizations a retrograde collateral circulation to the RCX area has developed. $^{81m}Kr$ perfusion is now shown to improve markedly in the RCX area during atrial pacing.

changed distribution was observed, all in ⩾90% left CAD regions. In 4 of these anterograde collaterals were present with decreased, however measurable radioactivity (fig 2). In 1 patient, who was reinvestigated within 1 week after a previous catheterization in order to perform a Krypton study, a 99% left marginal branch had closed without signs of a recent infarction. However also without the formation of anterograde collaterals (fig. 3a). No $^{81m}Kr$ perfusion of the marginal area occurred, either at rest and during pacing. Retrograde collaterals from the right coronary artery had developed in between the 2 successive catheterizations with

Table 1. $^{81m}Kr$ distribution changes during and after pacing in patients with >70% L-CAD

| Lesion: | control | 100 b/min | 120 b/min | AP | 15 sec P-P | 1 min P-P | 5 min P-P |
|---|---|---|---|---|---|---|---|
| >90% N = 12 | 100% | 86 ± 6* | 77 ± 7* | 67 ± 6.3+* | 74 ± 6+ | 83 ± 6.2* | 84 ± 6.8* |
| 70-90% N = 6 | 100% | 92 ± 4.6 | 87 ± 3.9* | 80 ± 5.6* | 80 ± 3.6* | 88 ± 6.5* | 90 ± 4.7* |
| normal areas | 100% | 112 ± 3.7* | 118 ± 3* | 125 ± 5.6* | 120 ± 3.9* | 118 ± 5.7* | 110 ± 5.1 |

Abbreviations: L-CAD = left coronary artery disease
b/min = beats/minute
AP = anginal pain
P-P = post-pacing
* = $p < 0.05$ vs control
+ = $p < 0.001$ (AP ≥90% vs AP 70-90%)

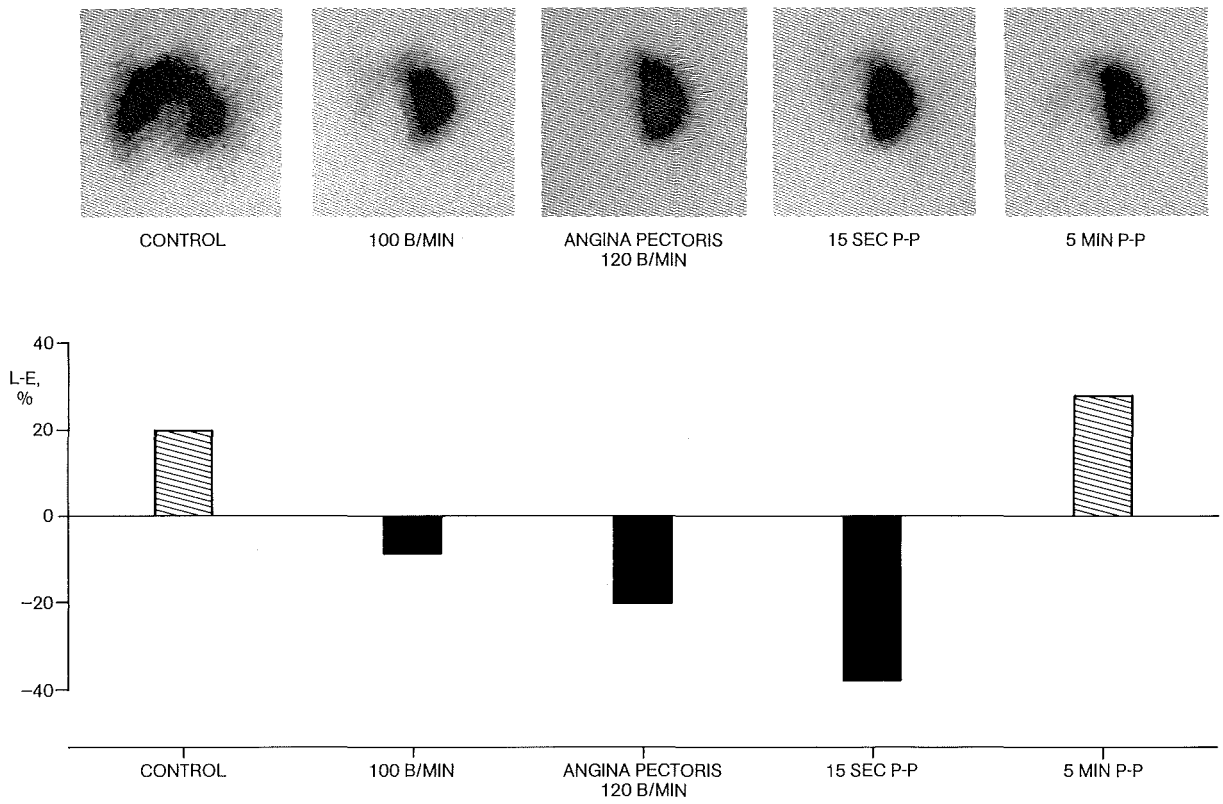
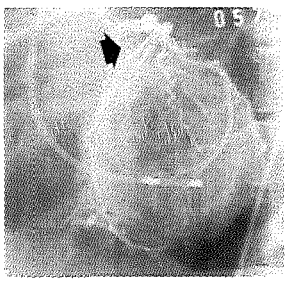
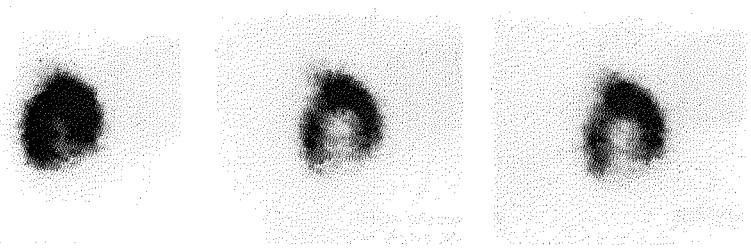



Fig. 4. Typical example of early and progressive decrease of $^{81m}Kr$ distribution in a 90% stenosis area (LAD). There is simultaneous increase in the normal RCX region. Changes occur before anginal pain and in this case, toegether with lactacte production and have not returned to control values 5 min after cessation of pacing. P-P= post pacing. B/min= beats/min. SEC= sec. RCX= left circumflex artery. LAD= left anterior descending artery.

Fig. 5a



LEFT CORONARY ARTERY
45° LAO



CONTROL | 110 B/MIN | ANGINA PECTORIS 130 B/MIN

Fig. 5a. 45° LAD view of $^{81m}Kr$ distribution at rest and during pacing in a patient with proximal 90% lesions in the LAD (closed arrow, 5a) and obtuse marginal artery (open arrow 5b). During angina pectoris there is diminished $^{81m}Kr$ distribution over the LAD area, which however is not so evident in the obtuse marginal region, presumably due to normal perfusion of postero-lateral branches which in this view are partially overlying the diseased coronary artery.

some retrograde filling at rest; which however improved during the atrial pacing stress test (fig 3b). In none of the patients was the diminished $^{81m}Kr$ distribution at rest accompanied by signs of myocardial ischemia, such as anginal pain, ECG changes or myocardial lactate production. However, abnormal wall motion at rest was found in all and documented old myocardial infarcts in 3 patients.

$^{81m}Kr$ distribution during pacing (table 1). During atrial pacing $^{81m}Kr$ distribution decreased in all >70% left-CAD areas with simultaneous increases in the normal areas. In the ≥90% lesions this fall in $^{81m}Kr$ perfusion developed at an

Fig. 5b

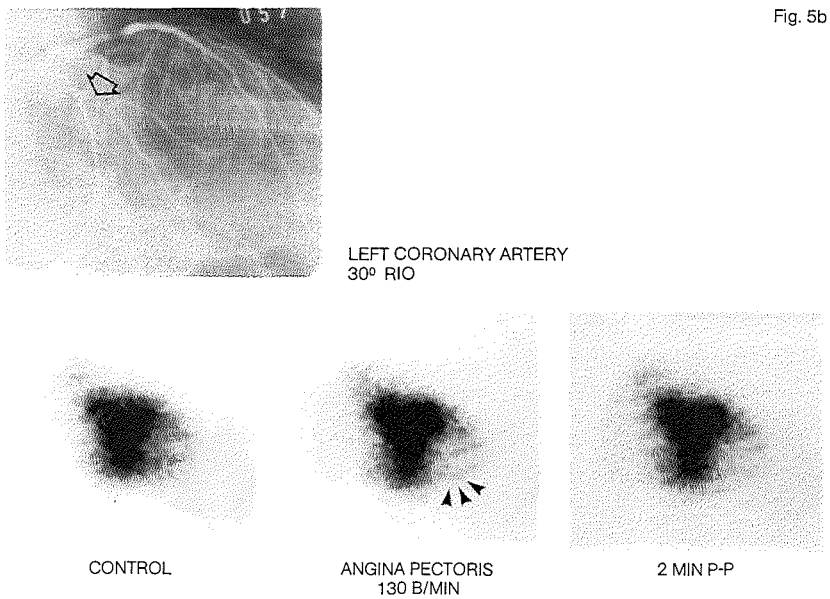



Fig. 5b. During a second atrial pacing stress test imaging was performed in the 30° RIO position. The obtuse marginal artery is in this view separated from the postero-lateral branches and a $^{81m}Kr$ perfusion defect is now clearly visible in this area during pacing (arrows). $^{81m}Kr$ distribution is still abnormal 2 min after pacing in both obtuse marginal and LAD regions.

early stage and was progressive to the end of pacing.

An example is given in fig 4. In this patient with a proximal 90% stenosis of the LAD artery $^{81m}Kr$ perfusion diminished at an early stage before the development of anginal pain, however, together with lactate production. During anginal pain an impressive shift in $^{81m}Kr$ distribution from the diseased to the normal area is seen. This progressive decrease of $^{81m}Kr$ perfusion of early onset was found in all ≥90% lesions. In the 70-90% stenosis group a change in $^{81m}Kr$ distribution usually only occurred halfway or towards the end of pacing.

Further, the magnitude of the changes differ between the 2 groups with significantly lower $^{81m}Kr$ perfusion during anginal pain, 67 ± 6.3% of control in the >90% lesions as compared to

a decrease to 80 ± 5.6% in the 70-90% stenosis group ($p<0.001$). The fact that $^{81m}Kr$ changes are found in all >70% areas and not in the <50% lesions indicates its possible use to discriminate between significant and non-significant CAD.

$^{81m}Kr$ distribution changes after pacing. In our studies usually late return to the control situation was found long after general signs of ischemia had subsided. Given the fact that the overall myocardial blood-flow is back to normal within the first 1-2 min after pacing, this implies an absolute flow reduction in the ischemic area. As can be seen from table 1 this flow reduction lasted more than 5 min and in some patients was still found to exist as long as 15 min after pacing. In only 6 of the 18 areas with >70% CAD did $^{81m}Kr$ distribution return to normal within 5 min after pacing. An explanation for this unexpected long-lasting flow reduction after atrial pacing induced ischemia is difficult to give. Prolonged post-stenotic vasodilatation with lowering of peripheral perfusion pressure may result in an increase in stenotic resistance and a reduction in regional flow (28). A continuing adenosine release from the ischemic area is likely in view of the fact that myocardial venous hypoxanthine levels are still increased 5 min after pacing in analogue studies (29). Regional myocardial blood-flow which is still abnormal 15 min after pacing is possibly the reason for a non-reproducible lactate pattern during repetitive atrial pacing stress tests with this interval (30).

Repetitive $^{81m}Kr$ distribution imaging in various positions. $^{81m}Kr$ imaging in our studies was always started in the 45° LAO position, separating the LAD and RCX regions. In addition perfusion studies of the right coronary artery were performed from this angle. This than allows a clear view of the right coronary perfusion areas in the infero-apical and postero-lateral regions (fig 3) without the problems of overprojection of the left coronary artery blood-supply in these areas. In single vessel lesions and in most of the patients with multi-vessel disease one left coronary artery in the 45° LAO view study was usually sufficient to obtain all the information

needed. However, in some instances a second study was necessary from a different imaging angle in order to separate the branches of the left circumflex artery. The 30° right inferior oblique position was chosen for this purpose separating the various marginal branches of this artery. The second study was performed after a 30 min interval. An example is given in fig 5. This patient had both a >90% stenosis of the LAD and of the obtuse marginal branch. During atrial pacing in the 45° LAO projection there is an obvious decrease of $^{81m}Kr$ distribution over the LAD area, however, not so apparent over the RCX area (fig 5a). Due to overprojection of the normal postero-lateral branch over the stenotic obtuse marginal artery a decrease in $^{81m}Kr$ perfusion in the latter could be compensated by hyperemia and an increase in $^{81m}Kr$ activity in the normal postero-lateral area.

The functional significance of the obtuse marginal stenosis is now demonstrated in the second study with the gamma camera in the 30° RIO position (fig 5b). The marginal branches and their perfusion area are now separated and diminished $^{81m}Kr$ distrubution is clearly seen in the peripheral obtuse marginal region, as well as in the LAD area.

Selective intra-coronary $^{81m}Kr$ infusion versus nonselective administration. Due to its very short half-life $^{81m}Kr$ must be administered either directly into the coronary artery (selective) or in the immediate vicinity of the coronary artery ostia (non-selective). Though the possibility of a streaming artefact with the intracoronary method, imposes an important theoretical drawback to the procedure its occurence is in our experience easily recognizable and preventable.

In the great majority of patients this type of study can be carried out without streaming artefacts after selection of the appropriate type of catheter. Also a change in catheter-tip position during pacing and fast heart rates, giving rise to a more superselective infusion and hence a distribution artefact, is unlikely in view of the fact that in most patients $^{81m}Kr$ distribution did not return to the control situation immediately after cessation of pacing, which would be expected in case of artefacts. The consistent observation of a decreased

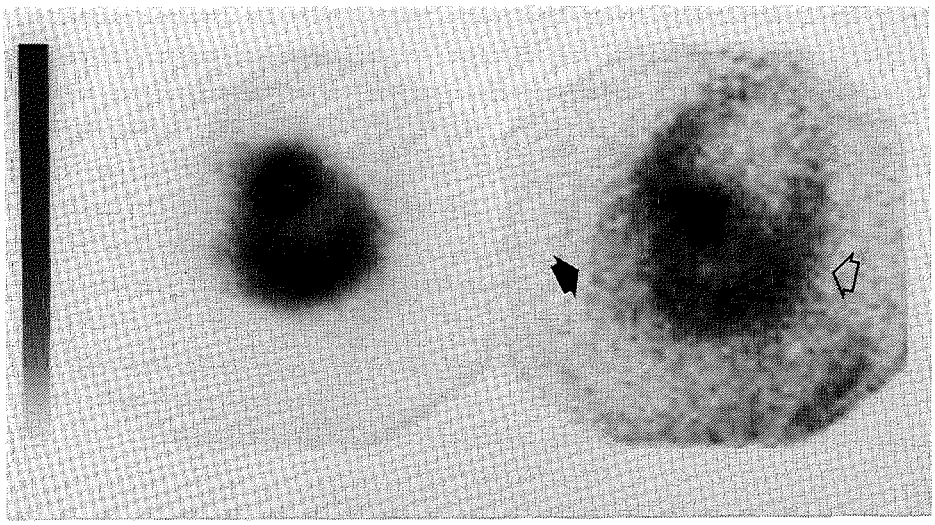


Fig. 6. Direct intracoronary administration of $^{81m}Kr$ (left image) compared with an infusion of the same amount into the aortic sinuses (right image). The difference in activity over the heart is obvious. Also, perfusion of the right coronary bed can be seen (closed arrow), which may overlap in the infero-apical region. The same problem may occur in the RCX area by counts from the descending aorta (open arrow) during intra-aortic administration. RCX = left circumflex artery.

$^{81m}Kr$ perfusion pattern during episodes of spontaneous anginal pain and normal resting heart rates found in the same areas as during pacing induced ischemia is also a strong argument against streaming artefacts (data not given in this chapter).

In our experience the selective intracoronary infusion of $^{81m}Kr$ provided acceptable information about the functional significance of the coronary artery disease present.

On the other hand we were unable to obtain reliable data when applying nonselective methods of administration of $^{81m}Kr$ in the aortic root and/or aortic sinuses both with "normal" or specially designed catheters.

Invariably the total amount of counts over the heart was far too low to allow proper statistical analysis. This is not surprising taking into consideration the relatively small portion of cardiac output which enters the coronary circulation. Even using special catheters, designed for delivery of $^{81m}Kr$ directly in the coronary sinuses, the total amount of

of radioactivity over the left ventricle is proportionally small compared with the direct intracoronary administration. An example of the best nonselective $^{81m}Kr$ imaging we were able to manage is shown in fig 6 as well as an intracoronary study in the same patient. The difference in activity is obvious. Also demonstrated is the obligate simultaneous perfusion of the right coronary artery with overprojection in the infero-apical region. Both this overprojection and the relatively high background activity of the descending aorta, usually underlying the circumflex area present problems defining $^{81m}Kr$ perfusion changes in these regions. Problems were not encountered with the direct intracoronary administration.

Clinical implications

As the investigation is necessarily invasive it is not suitable for use as a routine procedure. However, valuable information can be derived from this kind of study especially with reference to the functional significance of the various lesions in multivessel disease. This information is not always available from conventional, noninvasive techniques.

Due to the short half-life of $^{81m}Kr$ a multitude of successive studies can be performed in the same patient without extra exposure to radiation other than that necessary for the instantaneous study. The effect of repetitive stress tests and pharmaceutical intervention on regional myocardial blood-flow and myocardial ischemia can be evaluated. Further, the localization of perfusion disturbances during spontaneous anginal attacks or ergonovine-induced coronary spasm can be investigated as well as the influences of vaso-active drugs in these situations. Presumably the most important reason however, to subject the patient to this kind of investigation will be the situation where a moderate coronary artery lesion of 50-70% exists and the decision whether to perform surgery or angioplasty is debatable. The clinical significance of this stenosis will be adequately demonstrated during an atrial pacing stress test with continuous intracoronary administration of $^{81m}Kr$.

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## VII.2.

# Continuous Determination of Regional Myocardial Blood Flow with Intracoronary Krypton-81m in Coronary Artery Disease.

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# Continuous Determination of Regional Myocardial Blood Flow with Intracoronary Krypton-81m in Coronary Artery Disease

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**Pacing-induced changes in regional coronary flow were studied continuously with krypton-81m by intracoronary infusion in 25 patients: 21 with 50% or greater diameter narrowing of 1 or more left coronary arteries (group I) and 4 with less than 50% diameter reduction of a left coronary artery (group II). No changes occurred in group II. In group I, krypton-81m perfusion decreased progressively in all areas with more than 70% diameter narrowing, with a simultaneous increase in normal regions. At the end of pacing during angina, krypton-81m perfusion was reduced to 81 ± 4% of control in areas with 71 to 90% diameter reduction (n = 8) and to 69 ± 6% in areas with more than 90% diameter narrowing (n = 15). In contrast, in regions with 50 to 70% diameter reduction changes were variable (decrease in 4 regions, increase in 2 and an unchanged distribution in 1 region). Krypton-81m perfusion decreased early, before general signs of ischemia in areas with more than 90% diameter reduction, whereas this decrease occurred later in regions with 71 to 90% diameter narrowing, concurrently with ST-segment changes but before anginal pain. Although all signs of ischemia had disappeared between 2 and 5 minutes after pacing, changes in krypton-81m distribution persisted in most areas for 5 to 15 minutes after pacing. It is concluded that the functional significance of coronary arterial narrowing can be assessed with a continuous intracoronary infusion of krypton-81m. Changes in regional distribution persisted after cessation of pacing-induced ischemia, indicating an ongoing decrease in regional myocardial blood flow.**
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Regional changes in myocardial blood flow during ischemia have been studied in humans with various scintigraphic techniques. After the introduction of the rubidium-81/krypton-81m generator,[1-4] it was subsequently shown in animal experiments that krypton-81m also had the potential to indicate regional myocardial flow changes.[5-7] Selwyn et al[8,9] were the first to demonstrate coronary flow reductions in areas with a significant coronary stenosis in humans, with krypton-81m administered continuously into the aortic sinuses. The relation between the magnitude of regional coronary flow changes and degree of coronary artery stenosis, however, was not studied. Also, although the radionuclide is used more optimally during continuous intracoronary infusion, such studies in humans have not been reported, apart from preliminary data from our laboratory.[10,11] This study investigates the applicability of this technique and the relation between regional myocardial blood flow changes during ischemia and the degree of coronary artery stenosis.

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## Methods

All patients gave informed consent. Studies were conducted in patients with either documented signs of myocardial ischemia (positive results with bicycle ergometry test or positive results on thallium scintigraphy), a previous myocardial infarction or persistent angina-like complaints without objective signs of ischemia.

**Catheterization procedures:** Catheterization was performed after an overnight fast without premedication using the Seldinger technique. Studies were carried out before routine coronary and left heart angiography. After 5,000 IU of heparin and 250 mg of Aspégic® (acetylsalicylic acid) were

given intravenously, a No. 7Fr Judkins or Amplatz left coronary artery catheter was positioned in the left coronary artery.

The occurrence of selective injections in 1 of the branches of the left coronary artery was then determined during slow and rapid manual contrast injections in both the 30° right inferior oblique and 45° left anterior oblique position. All angiographic information was stored on videotape to enable rechecking of the catheter-tip position during the study.

After catheterization the coronary angiograms were analyzed by 2 independent cardiologists, experienced in reporting more than 700 angiograms annually. If there was disagreement, a quantitative determination was carried out.[12] Significant left coronary artery disease (CAD) was taken to be present with a diameter reduction of 50% or greater in 1 or more left coronary arteries. The left coronary artery lesions were separated into 5 categories: normal (0 to 20% diameter reduction), insignificant left CAD (20 to 49% diameter narrowing), 50 to 70%, 71 to 90%, and more than 90% diameter reduction, respectively. Patients with left main disease or collateral supply from the right coronary artery were excluded from the study. Concomitant right CAD was accepted only if it did not interfere with left coronary flow.

**Scintigraphic procedures:** During the study krypton-81m was continuously eluted in 5% glucose from a sterile, pyrogen-free rubidium-81/krypton-81m perfusion generator, calibrated to deliver 20 mCi.[13] The eluate was then passed through a 200-nm membrane filter and continuously infused into the left coronary artery at a constant rate of 800 ml/hour. After a 20-minute stabilization period, precordial scanning was started in the 45° left anterior oblique position using a ½-inch crystal General Electric Portacamera connected on line with a Medical Data System A² computer. Energy detection was set on 190 keV ± 20%. Images were acquired over 15-second periods and krypton-81m distribution changes measured in the various regions of interest, including areas with normal coronary arteries, regions with significant left CAD (50% or greater diameter reduction), total heart, ascending aorta and background areas. Regions of interest were constructed using an electronic lightpen on a visual display unit. Movement artifacts were avoided and care was taken that successive regions of interest did not differ from the originally constructed areas. Calculations were made of total counts per area and changes in krypton-81m distribution expressed as percentages of control. Fluctuations in krypton-81m supply to the left myocardium were accounted for by dividing total counts per region by total counts over the heart per time frame. When areas with coronary lesions were superimposed, a second study in the 30° right inferior oblique position was performed after 30 minutes.

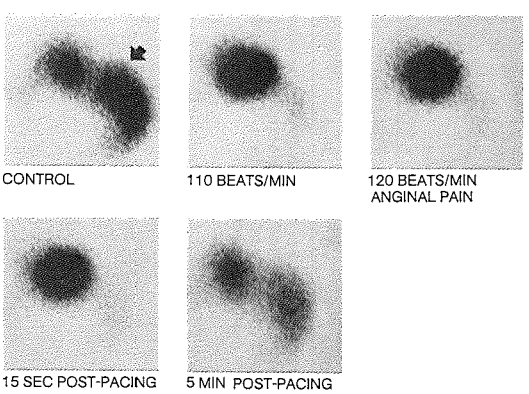


**FIGURE 1.** Selective infusion of krypton-81m in the left circumflex artery, which has a 71 to 90% diameter reduction (**arrow**). During pacing, krypton-81m perfusion decreases clearly in the poststenotic area before angina occurs with a simultaneous increase in the prestenotic region.

**Electrocardiographic measurements:** Leads I, II and $V_5$ were continuously monitored for determination of heart rate and ST-segment changes.

**Study protocol:** Approximately 60 seconds after initiation of the infusion, an equilibrium was reached in the regions studied. During the following 10-minute control period, krypton-81m distribution was continuously monitored and the distribution pattern measured during several 15-second periods. With the occurrence of streaming, i.e., distribution changes of more than 5%, the catheter was either repositioned or replaced, or a superselective perfusion of the coronary branch of interest was carried out. When this was unsuccessful the study was terminated.

After the control period, atrial pacing was carried out with increments of 10 beats/min until angina, atrioventricular block or a maximal pacing heart rate of 170 beats/min occurred. Krypton-81m distribution was monitored continuously during the pacing period and until 5 minutes after pacing. However, in most patients repeated studies were carried out and monitoring usually was performed until 15 minutes after pacing.

Statistical analysis was performed using the Student $t$ test for unpaired and paired values. All values are expressed as mean ± 1 standard error of the mean.

## Results

In 3 patients, streaming artifacts occurred during the control period. One patient was excluded. In the other 2 patients, a superselective infusion was carried out in a stenosed coronary artery branch. In both studies, a normal area and an area of stenosis were present, which allowed for satisfactory information about krypton-81m distribution changes during pacing (Fig. 1).

Complete studies were performed in 25 patients, 21 men and 4 women, aged 36 to 66 years (mean 54), either with significant left CAD (50% or greater stenosis, n = 21, group I) or no left CAD (less than 50% stenosis, n = 4, group II). Patient data and angiographic findings are listed in Table I. In group I, 15 areas with more than 90%, 8 with 71 to 90% and 7 with 50 to 70% diameter reduction of a left coronary artery were present. Ten patients had electrocardiographic signs of an old myocardial infarction. In another 5 patients, the left ventriculogram showed wall motion abnormalities at rest in areas corresponding with significant left CAD. In 3 patients, an inferior myocardial infarction with a 100% occlusion of the right coronary artery and collateral supply from the left coronary artery were present.

**Krypton-81m distribution at rest:** Krypton-81m distribution was stable throughout the control period with a count rate of 250,000 to 300,000 counts/min. Total counts over the heart and aorta showed minor fluctuations (less than 5%), and background counts were negligible. The distribution pattern over the left heart was normal in 17 patients in group I and all patients in group II (Table I). Although none of the other 4 patients in group I had signs of myocardial ischemia, a stable

**TABLE I Clinical and Angiographic Data and Krypton-81m Distribution at Rest**

| Pt | Age (yr) & Sex | AP | Healed MI | Angiographic Findings: Coronary % Diameter Reduction LAD | LC | Right | Collat. | Left Ventricular EF (%) | Left Ventricular Wall Motion | Krypton-81m Distribution at Rest |
|---|---|---|---|---|---|---|---|---|---|---|
| Group I: Left Coronary Artery Disease*—50% or Greater Diameter Reduction (n = 21) | | | | | | | | | | |
| 1 | 49F | + | 0 | 0 | 71–90 | 0 | 0 | 60 | N | N |
| 2 | 53M | + | 0 | 0 | 71–90 | 0 | 0 | 66 | N | N |
| 3 | 58M | + | Post | >90 | 100 | 0 | +LC | 45 | Post Ak/Ant Hyp | ↓LAD, ↓LC |
| 4 | 50M | 0 | Ant | 100 | 0 | 0 | +LAD | 47 | Ant Dys | ↓LAD |
| 5 | 60F | + | 0 | >90 | 50–70 | 0 | 0 | 62 | N | N |
| 6 | 61M | + | 0 | 71–90 | 71–90 | 0 | +LC | 58 | N | N |
| 7 | 64M | + | 0 | >90 | 0 | 0 | +LAD | 51 | Ant Hyp | N |
| 8 | 53M | + | Ant | 100 | 71–90 | 0 | +LAD | 62 | Ant Hyp | ↓LAD |
| 9 | 47F | + | 0 | 0 | 100 | 0 | +LC | 64 | Post Hyp | N |
| 10 | 41M | + | 0 | >90 | >90 | >90 | 0 | 59 | Ant Hyp | N |
| 11 | 58M | + | 0 | 71–90 | 71–90 | 0 | 0 | 77 | N | N |
| 12 | 59M | + | Ant | >90 | 0 | <50 | 0 | 51 | Ant Hyp | N |
| 13 | 37M | + | Ant | 71–90 | 0 | <50 | 0 | 56 | Ant Hyp | N |
| 14 | 40M | 0 | Ant | >90 | 50–70 | 0 | 0 | 74 | Ant Hyp | N |
| 15 | 62M | + | 0 | >90 | 50–70 | 71–90 | 0 | 63 | N | N |
| 16 | 51M | + | Ant | >90 | >90 | >90 | +LC | 42 | Ant Hyp/Inf Ak | N |
| 17 | 63F | + | 0 | 50–70 | 0 | 0 | 0 | 80 | N | N |
| 18 | 59M | + | 0 | >90 | 0 | <50 | 0 | 62 | Ant Hyp | N |
| 19 | 57M | + | 0 | 50–70 | 0 | 0 | 0 | 62 | N | N |
| 20 | 66M | + | 0 | 50–70 | 0 | 0 | 0 | 85 | N | N |
| 21 | 61M | + | Post | 50–70 | 100 | 0 | 0 | 45 | Post Hyp | Absent LC |
| Group II: Left Coronary Artery Disease*—Less than 50% Diameter Reduction (n = 4) | | | | | | | | | | |
| 22 | 60M | + | Post | <50 | 0 | 100 | +Right | 72 | N | N |
| 23 | 49M | + | 0 | <50 | 0 | 0 | 0 | 74 | N | N |
| 24 | 48M | + | 0 | <50 | 0 | 0 | 0 | 70 | N | N |
| 25 | 36M | 0 | Post | <50 | 0 | 100 | +Right | 53 | Post Hyp | N |

* Diameter reduction or 1 or more left coronary arteries.

Ant = anterior; Ak = akinesia; AP = preexisting angina pectoris or anginalike symptoms; Collat. = collateral filling from left coronary artery; Dys = dyskinesia; EF = ejection fraction; Hyp = hypokinesia; Inf = inferior; LAD = left anterior descending artery; LC = left circumflex artery; MI = myocardial infarction; N = normal; Post = posterior; + = present; 0 = absent; ↓ = decrease.

**TABLE II Krypton-81m Distribution Changes During and After Pacing (% of Control)**

| Areas with LCAD | Pacing Periods | | | | | |
|---|---|---|---|---|---|---|
| | 100 beats/min | 120 beats/min | AP | 15 sec P-P | 1 min P-P | 5 min P-P |
| >90% (n = 15) | 81 ± 4* | 77 ± 7.5* | 69 ± 6* | 79 ± 6* | 81 ± 7* | 84 ± 4* |
| 71–90% (n = 8) | 97 ± 2 | 87 ± 4* | 81 ± 4* | 81 ± 3* | 83 ± 5.5* | 87 ± 2* |
| Normal (n = 21) | 110 ± 3* | 116 ± 3* | 132 ± 7* | 121 ± 7* | 119 ± 7* | 113 ± 5* |

* $p < 0.05$ vs control values.
AP = anginal pain; LCAD = left coronary artery disease; P-P = postpacing.

pattern of decreased krypton-81m distribution was found in 5 areas with more than 90% diameter reduction, of which 4 corresponded with documented old myocardial infarctions.

**Pacing study period:** Maximal paced heart rates were similar in both groups (137 ± 4 beats/min in group I and 132 ± 8 beats/min in group II). In 19 patients in group I and in all patients in group II, pacing was discontinued because of chest pain. It was was first noted at a mean heart rate of 122 ± 4 beats/min in group I and at 114 ± 10 beats/min in group II (difference not significant), and lasted for 2.2 ± 0.47 minutes after pacing in the group with left CAD and for 2.5 ± 0.65 minutes in group II (difference not significant). Significant ST-segment changes occurred in 18 patients in group I, but the ST segment returned to baseline values between 2 and 5 minutes after pacing in all but 2 areas. In group II, ST-segment changes occurred in 2 patients with a 100% occlusion of the right coronary artery.

**Krypton-81m distribution during and after pacing:** Background activity remained unchanged during pacing, and significant reflux of krypton-81m in the aorta did not occur. In some patients administration of total krypton-81m decreased slightly during the study period with a decrease in total counts over the heart and the aorta of ±5%.

**Areas with less than 50% diameter reduction:** In group II patients, krypton-81m distribution did not change in the normal areas or in the regions with less than 50% diameter narrowing of a left coronary artery, although small fluctuations (less than 5% of control) were observed.

**Areas with more than 90% left coronary artery disease:** In areas with more than 90% diameter narrowing a continuous decrease in krypton-81m perfusion was found, starting early after the onset of pacing, before angina or ST-segment changes. An example is shown in Figure 2. Mean values of distribution changes in areas with more than 90% diameter reduction are listed in Table II, as well as the measured values over normal areas. The temporal relation with the occurrence of angina and ST-segment changes is displayed in Figure 3. At 100 beats/min, krypton-81m perfusion decreased to 81 ± 4% of control ($p < 0.05$ vs control), gradually decreasing thereafter to 69 ± 6% of control at the end of pacing during anginal pain. This progressive decline in krypton-81m distribution of early onset during pacing was found in 12 areas with more than 90% diameter narrowing. In 2 other regions, a late decrease in perfusion was observed toward the end of pacing (Table III). A late increase in krypton-81m perfusion during pacing was observed in an area with 100% oc-

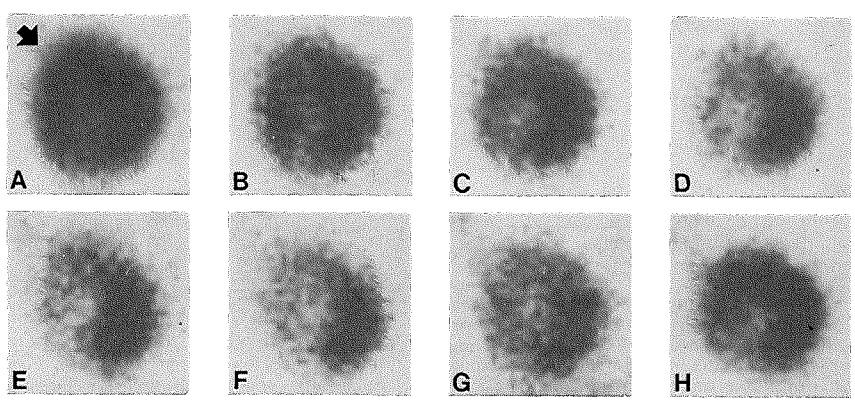


**FIGURE 2.** Krypton-81m distribution changes in a patient with a proximal (more than 90%) diameter reduction in the left anterior descending artery (**arrow**). At rest there is equal perfusion of both normal and abnormal areas (**A**). During pacing a progressive decrease in krypton-81m perfusion of the poststenotic area is observed with significant changes already at 100 and 120 beats/min (**B** and **C**), before the occurrence of ST-segment changes (140 beats/min, **D**) and angina (160 beats/min, **E**). No change 15 seconds after pacing (**F**), and, thereafter, a very slow return to the control situation, which is still not reached 15 minutes after pacing (**G**, 8 minutes after pacing; **H**, 15 minutes after pacing).

**TABLE III Number of Areas with Krypton-81m Distribution Changes During and After Pacing**

| | Krypton-81m Distribution | | | | | | |
|---|---|---|---|---|---|---|---|
| | During Pacing | | | | After Pacing | | |
| | | | | | | Return to Control | |
| Areas with LCAD | Normal Unchanged | Early Decrease | Late Decrease | Increase | Unchanged | Early (<5 min) | Late (>5 min) |
| >90% (n = 15) | 0 | 12 | 2 | 1 | 0 | 4 | 11 |
| 71–90% (n = 8) | 0 | 4 | 4 | 0 | 0 | 2 | 6 |
| 50–70% (n = 7) | 1 | 2 | 2 | 2 | 1 | 3 | 3 |
| <50% (n = 4) | 4 | 0 | 0 | 0 | 4 | 0 | 0 |

LCAD = left coronary artery disease.

clusion and anterograde collaterals with diminished krypton-81m uptake at rest. In the other areas with diminished krypton-81m distribution at rest, perfusion decreased even more during pacing with a late return to the control situation after pacing (Fig. 4).

Although krypton-81m perfusion had increased slightly 15 seconds after pacing, it was still diminished 5 minutes after pacing. In some patients, krypton-81m distribution was still abnormal, even 15 minutes after pacing. In only 4 of the 15 areas with more than 90% diameter reduction did krypton-81m distribution normalize within the 5-minute period after pacing.

**Left coronary artery disease (71 to 90% stenosis):** Krypton-81m perfusion decreased in all regions with 71 to 90% diameter narrowing of a left coronary artery during pacing. Changes occurred later than in the group with more than 90% diameter reduction (Table II), in some areas only halfway or toward the end of pacing. However, a significant decrease to 87 ± 4% of control (p <0.05 vs control) was found at 120 beats/min. During anginal pain, krypton-81m distribution had further decreased to 81 ± 4%, remained unchanged 15 seconds after pacing and thereafter slowly increased to 87 ± 2% of control 5 minutes after pacing (p <0.05 vs control [Fig. 3]). At this period krypton-81m perfusion was still diminished in 6 areas (Table III).

**Left coronary artery disease (50 to 70% stenosis):** The response to pacing was variable in areas with 50 to 70% diameter reduction (Table III). In 4 patients with a 1-vessel lesion, krypton-81m perfusion diminished during pacing in 3 and remained unchanged in 1 patient. In the multivessel group either a decrease (1 patient) or an increase (2 patients) was found.

## Discussion

Krypton-81m is a biologically and chemically inert radionuclide with a 13-second half-life, which is short in relation to myocardial transit time. This implies that during continuous administration the process of equilibrium is affected only in a very small percentage by tracer washout and that background counts are negligible. In eluted form, krypton-81m is soluble in plasma and diffuses freely through capillary membranes.[14] During continuous administration into the coronary

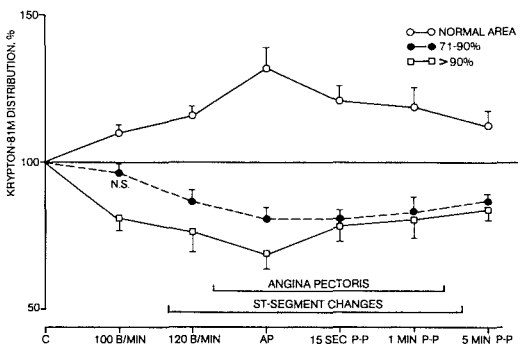


**FIGURE 3.** Mean values of krypton-81m distribution changes in areas with 71 to 90% and more than 90% diameter reductions and in normal regions. Temporal relation with the occurrence of angina and ST-segment changes is seen. Krypton-81m perfusion is significantly reduced already at 100 beats/min in areas with more than 90% diameter narrowing before signs of ischemia. In regions with 71 to 90% diameter reduction this occurs later, at 120 beats/min, concurrently with ST-segment changes, but before angina. Krypton-81m distribution is still reduced 5 minutes after pacing, in areas with both 71 to 90% and more than 90% diameter reduction. N.S. = not significant; P-P = post-pacing.

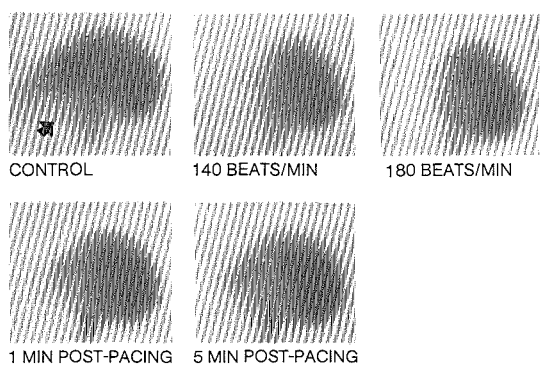


**FIGURE 4.** Krypton-81m distribution in a patient with a proximal 100% occlusion of the left anterior descending artery, anterograde collaterals and anterior and septal dyskinesia. At rest, krypton-81m perfusion is already diminished over the peripheral area of the left anterior descending artery (**arrow**) and becomes almost nonexistent during pacing, although angina does not occur. Krypton-81m distribution does not return to control 5 minutes after pacing.

arterial system, the radionuclide will readily equilibrate itself in the myocardial vascular and extravascular compartments,[15] and a stable distribution pattern is obtained approximately 60 seconds after the onset of administration.

**Sources of misinformation:** During intracoronary administration, streaming may occur because of improper mixing of the radionuclide with blood in the left mainstem, which results in an uneven and usually unstable distribution pattern. This phenomenon was observed in 3 patients in this study during the control period. In another 5 areas, krypton-81m perfusion was abnormal and was reduced as well during control; however, these abnormalities in perfusion were stable and only found in regions with more than 90% diameter reduction, corresponding with an old myocardial infarct or with an abnormal left ventriculogram. They were classified as true decreases in myocardial blood flow. Another possible source of false information could be a change in catheter-tip position caused by the fast movements of the heart during pacing. An immediate return to the control position after pacing with a prompt normalization of the krypton-81m distribution pattern would then be expected. This only happened in 1 patient. In most patients krypton-81m distribution normalized only late after pacing, which makes this hypothesis very unlikely.

**Krypton-81m distribution changes in relation to the degree of left coronary artery disease:** Abnormal krypton-81m distribution was found at rest or during pacing in all areas with more than 70% diameter narrowing, and in none of those with less than 50% diameter reduction. This finding signifies the usefulness of the radionuclide as an indicator of coronary flow changes when given through the intracoronary route. The fact that distribution changes occur early in severe CAD (more than 90% diameter reduction), but later with moderate lesions (71 to 90% diameter narrowing), also suggests that the remaining vasodilatory reserve capacity is detected adequately with this technique.

Although coronary diameter reductions of 50 to 70% are generally believed to be significant, krypton-81m distribution changes were variable in areas with 50 to 70% diameter narrowing. In 3 of the 7 regions krypton-81m perfusion either remained unchanged (1-vessel CAD) or increased (multivessel CAD) during pacing, indicating an intact vasodilatory reserve capacity. Although these findings suggest that the functional significance of a 50 to 70% diameter reduction can be assessed correctly with this technique, more observations are needed to reach definite conclusions as to its usefulness.

**Krypton-81m distribution changes after pacing:** A persistent decrease in krypton-81m perfusion was found in 17 of 23 areas with more than 70% diameter narrowing lasting more than 5 minutes after pacing. To our knowledge, this finding has not been described in comparable studies of pacing-induced changes in myocardial blood flow. Changes are reported to normalize without specification of the time required or to return to control values within a few minutes after pacing.[9] The pathophysiologic characteristics underlying the long-lasting reductions in regional krypton-81m perfusion after pacing are difficult to understand. Because total left coronary flow returns to control values within 2 minutes after pacing,[16] the implication is that a persistent reduction occurs in regional flow. A possible explanation could be persistent poststenotic vasodilation due to continuous adenosine release in the ischemic areas after pacing. This could lead to a decrease in perfusion pressure and an increase in stenosis resistance,[17] which then may result in a continuous redistribution of krypton-81m from the area of stenosis to normal, low resistance areas. Previous observations that coronary sinus hypoxanthine levels are elevated 5 minutes after pacing in patients with angina,[18] strongly suggest this continuous adenosine release to occur. Persistent changes in krypton-81m activity may occur also as a result of dyskinesia and local wall thinning in the ischemic area. However, because ischemic wall motion abnormalities are reported to normalize within 5 minutes after pacing,[19] this seems rather unlikely.

**Comparison with other imaging techniques:** In the present study, a decrease in krypton-81m perfusion was found in all areas with more than 70% coronary diameter reduction. This finding indicates that the method has a greater sensitivity for detecting functionally significant lesions than noninvasive methods like quantitative thallium-201 planar or tomographic imaging.[20,21] It also compares favorably with myocardial imaging, using labeled macroaggregated albumen by intracoronary injection. Although with this technique, perfusion abnormalities were observed in 37 of 39 patients with significant CAD, imaging defects did not occur in all of the areas with more than 70% diameter reduction.[22] Precordial mapping with xenon-133 resulted in a near complete separation between functionally significant and insignificant coronary lesions.[23] Some of the characteristics of xenon-133 (e.g., low-energy spectrum, some recirculation and affinity for fat tissue) and the time needed for the determination of washout curves[24,25] are limiting factors, particularly in repeated studies with short intervals in the same patient.

Digital coronary angiography provides a new, alternative technique for the evaluation of coronary flow reserve by measuring myocardial contrast appearance time. With this technique, an abnormal transit time and thus, presumably, a decrease in regional flow can be detected in areas with significant CAD.[26] Changes are also found, however, with 30 to 40% diameter reductions, which do not agree with our results. Whether this means that digital coronary angiography has a greater sensitivity in the estimation of coronary flow reserve has to be investigated further in comparative studies, which includes the determination of ischemia as well.

Selwyn et al[8] originally reported on the use of krypton-81m, given nonselectively in the aortic root with a specially designed catheter. Although regional perfusion abnormalities were found in practically all patients with significant CAD, various areas with more than 70% diameter narrowing were not described as abnormal in patients with multivessel disease.[9] This difference with our results may be attributable to a somewhat slower

perfusion of an otherwise similar generator. Combined with the relatively low influx of the radionuclide into the coronary system, when administered into the aortic sinuses, this may have resulted in a significant lower precordial count rate. Also, their studies do not clarify whether all significant coronary lesions or only scintigraphically abnormal areas were studied.

**Limitations of the technique:** Contrary to other precordial mapping techniques,[9,23] only distributional changes and not coronary flow changes in absolute quantities can be measured when intracoronary infusion of a constant amount of radionuclide is given. Whether the observed changes really represent a regional decrease in coronary blood flow is impossible to know. A redistribution of radioactivity from areas with limited vasodilatory reserve and diminished increase in flow during pacing to normal areas can result in the observed changes in krypton-81m perfusion as well.

In 7 of the 25 patients, spontaneous anginal attacks occurred, usually late after the first pacing stress test—a large percentage in our experience. This result could be due to the relatively long periods of continuous, low-speed intracoronary infusions. Intermittent administration, alternating with flushing of the catheters, should therefore be considered in subsequent studies.

**Clinical implications:** The intracoronary administration of krypton-81m ensures the delivery of sufficient radioactivity to measure distribution changes at short time intervals. This is important in situations in which myocardial blood flow varies continuously, e.g., during pacing or administration of vasoactive agents. Its short half-life ensures selective imaging of the target organ without background activity, whereas radiation of the patient is limited to the investigation period only. The major drawback of the technique is that it is invasive and depends on proper mixing with blood in the left coronary mainstem. It is our opinion that during a 10-minute observation period before pacing, improper distributional changes and streaming artifacts can be recognized and corrected. Changes thereafter, during pacing, reflect real alterations in myocardial blood flow, whether this is a true decrease or only an impaired increase in flow compared with normal regions. This technique may enable the clinician to better understand the functional significance of the lesion and may facilitate a proper decision on the right therapy, especially in areas with 50 to 70% coronary diameter reduction.

Finally, the observation in this study of perservering, long-lasting perfusion abnormalities after pacing emphasizes the importance of a sufficiently long interval between repetitive atrial pacing stress tests in order to obtain reproducible pacing-induced myocardial ischemia.[27]

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## VII.3.

# Temporal Relation of Changes in Regional Coronary Flow and Myocardial Lactate and Nucleoside Metabolism During Pacing-Induced Ischemia.

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# Temporal Relation of Changes in Regional Coronary Flow and Myocardial Lactate and Nucleoside Metabolism During Pacing-Induced Ischemia

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**The temporal relation between myocardial lactate and hypoxanthine metabolism and regional changes in krypton-81m perfusion during pacing-induced ischemia was studied in 17 patients with coronary artery disease (CAD). During incremental atrial pacing, lactate production and hypoxanthine release occurred early and simultaneously, accompanied by ST-segment changes, but before angina and only few minutes after a significant (17%) reduction in krypton-81m perfusion in areas with more than 90% luminal diameter reduction. During maximal pacing heart rates, krypton-81m distribution decreased to 68 ± 7% of control in areas with more than 90% diameter reduction and to 80 ± 4% in 70 to 90% reduction (both p <0.05 vs control). Maximal lactate production occurred 15 seconds after pacing (extraction −15 ± 7% vs 16 ± 2% during control, p <0.05) and peak hypoxanthine release 1 minute after pacing (Δ arteriovenous −2.64 ± 0.8 μM vs 0.08 ± 0.21 μM during control, p <0.05). Krypton-81m perfusion decreased in 20 of the 21 CAD areas. Angina, ST-segment changes, hemodynamic alterations and lactate production occurred in 15, 14, 9 and 15 patients, respectively. In contrast, hypoxanthine release was found in all cases. After pacing, lactate production and all general indexes of ischemia persisted for only 2 to 3 minutes. In contrast, krypton-81m perfusion was still significantly reduced 5 minutes after pacing and was only accompanied by hypoxanthine release (Δ arteriovenous −1.41 ± 0.6 μM, p <0.05 vs control). Therefore, although lactate production and hypoxanthine release occur early and simultaneously during pacing-induced ischemia, closely following coronary flow changes in high-stenotic areas, hypoxanthine appears to be more sensitive and consistent as indicator of ischemia. Persistant reductions in krypton-81m perfusion and hypoxanthine release strongly suggest prolonged ischemia after cessation of pacing.**

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In humans, regional coronary flow changes are observed at an early stage during incremental atrial pacing, in areas with significant coronary artery disease (CAD) before general signs of ischemia, such as angina, electrocardiographic changes or hemodynamic abnormalities occur.[1–4] Changes in myocardial lactate metabolism have been used for decades to indicate myocardial ischemia during pacing-induced stress in humans,[5,6] and compared with angina or ST-segment changes, myocardial lactate production appears to be a better indicator when assessed at the appropriate time, e.g., 15 seconds after pacing.[7] Myocardial ischemia also causes a significant release of nucleosides and oxypurines from the heart[8–16]—in humans predominantly of hypoxanthine.[14,16,17] Although results of animal and human studies suggest that lactate production precedes nucleoside release during ischemia,[11,14,18] the temporal relation between these metabolic changes and alterations in regional coronary flow in man is unknown. Also, the relative sensitivity of both metabolites as indicators of myocardial ischemia in humans is controversial.[14–16] We investigated the incidence of lactate production and hypoxanthine

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release in humans during pacing-induced ischemia and the temporal relation of these metabolic changes to alterations in regional coronary flow.

## Methods

**Patients:** Seventeen patients (14 men, 3 women), aged 37 to 64 years (mean 53), were included after they gave informed consent. Only patients with exercise-induced myocardial ischemia or previous myocardial infarction were studied. Cardiac medication was withheld 24 hours before study and none of the patients received drugs that can influence nucleoside metabolism, e.g., dipyridamole. Studies were performed without premedication after an overnight fast and carried out before routine coronary and left ventricular angiography, using the Seldinger technique. Immediately before the study, 5,000 IU of heparin and 250 mg of acetylsalicylic acid were administered intravenously. A No. 7Fr Zucker bipolar pacing catheter was advanced from a brachial vein into the midportion of the coronary sinus, such that its position was stable and blood could be drawn easily from the coronary sinus. Its position was established by way of a coronary venous angiogram, using 3 ml of Renografin 76®. Next, a No. 7Fr Swan Ganz thermodilution catheter was advanced from a femoral vein into a pulmonary artery for the recording of pulmonary wedge pressure. Finally, a No. 7Fr Amplatz or Judkins left coronary artery catheter was positioned in the ostium of the left coronary artery via a Desilet® introducer system in a femoral artery. The sidearm of the Desilet system was used to sample arterial blood for lactate and hypoxanthine determinations and to record arterial pressures. After positioning of the coronary artery catheter, slow and rapid manual contrast injections were recorded in different views to determine whether selective injections in 1 of the branches of the left coronary artery occurred. All angiographic information was stored on videotape to allow rechecking of the various of catheter positions throughout the study. After catheterization, coronary angiograms were analyzed by 2 independent cardiologists. Diameter reductions of more than 70% in 1 or more left coronary arteries were considered to indicate significant left CAD. This stenosis group was separated in 2 categories: patients with 70 to 90% and and those with more than 90% diameter reduction. A coronary artery was considered normal if 0 to 25% diameter reduction was present. Areas with 25 to 70% diameter reduction were not included in the study. Concomitant right CAD was not an exclusion criterion if it did not interfere with left coronary flow. Patients with left main disease or collateral supply from the right coronary artery were excluded from the study.

**Scintigraphic procedures (Fig. 1):** Scintigraphic procedures have been described elsewhere.[4] Briefly, a sterile, pyrogen-free rubidium-81/krypton-81m perfusion generator was used, calibrated to deliver 20 mCi, perfused with glucose 5% at a rate of 800 ml/hour with the eluate administered continuously into the left coronary artery. Planar imaging was performed in the 45° left anterior oblique position during 15-second acquisition periods, using a General Electric Portacamera®, connected on line with a Medical Data System A[2] computer with energy detection set at 190 ± 20% keV. In this study, krypton-81m distribution changes were measured in areas with significant CAD, regions with normal coronary arteries, total heart and background areas. Calculations were made of total counts per area, divided by total counts over the heart per time frame to account for possible fluctuations in krypton-81m supply to the heart. Changes in krypton-81m distribution were expressed as a percentage of control.

**Metabolic determinations:** Blood (1 ml) was sampled simultaneously from the coronary sinus and femoral artery, transferred directly into precooled tubes containing 2 ml of ice-cold 0.6 *M* HCl04, mixed thoroughly and kept on ice. Immediately after the study, the samples were weighed and centrifuged for 20 minutes at a speed of 2,000 × g and the supernatant frozen for the assay of hypoxanthine. Lactate was immediately determined in triplicate by the enzymatic technique as described by Guttman and Wahlefeld.[19] The standard deviation of the determination in our laborato-

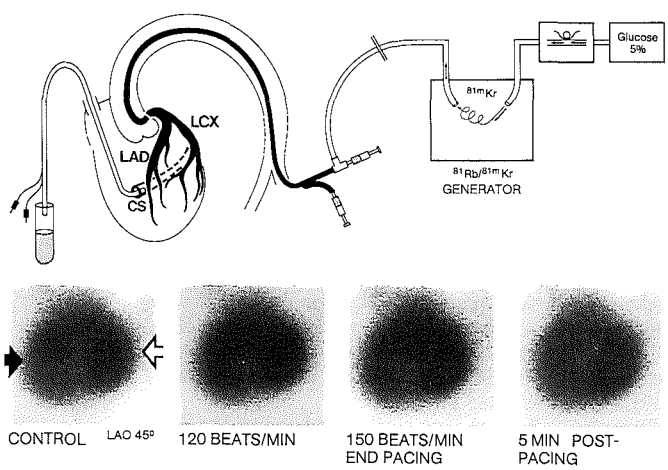


**FIGURE 1.** ***Top,*** **schematic representation of intracoronary krypton-81m perfusion.** ***Bottom,*** **sequential imaging during incremental atrial pacing in a patient with normal coronary arteries. Studies were performed in the 45° left anterior oblique (LAO) position. CS = coronary sinus; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery.**

**TABLE I Clinical and Angiographic Data**

| | | | Angiographic Data | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Coronary % Diameter Reduction | | | | | |
| Pt | AP | Previous MI | LAD | LC | RCA | Collat | LVEF (%) | Wall Motion |
| 1 | + | 0 | 70-90 | 70-90 | 0 | 0 | 70 | N |
| 2 | + | 0 | >90 | <70 | 0 | 0 | 62 | N |
| 3 | + | 0 | >90 | 0 | 0 | +LAD | 49 | Ant hk |
| 4 | + | Ant | >90 | >90 | >90 | +LC | 42 | Ant hk/inf ak |
| 5 | + | Post | >90 | >90 | 0 | 0 | 66 | N |
| 6 | + | 0 | 0 | 100 | 0 | +LC | 64 | Post hk |
| 7 | + | 0 | 70-90 | 70-90 | 0 | 0 | 68 | N |
| 8 | + | Ant | 70-90 | 0 | <70 | 0 | 54 | Ant hk |
| 9 | + | Ant | >90 | <70 | 0 | 0 | 61 | Ant hk |
| 10 | + | 0 | 0 | 70-90 | 0 | 0 | 60 | N |
| 11 | + | 0 | >90 | >90 | >90 | 0 | 59 | Ant hk |
| 12 | 0 | Ant | 100 | 0 | 0 | +LAD | 47 | Ant dk |
| 13 | + | Post | >90 | 100 | 0 | +LC | 45 | Post ak/ant hk |
| 14 | + | Post | <70 | 100 | 0 | 0 | 45 | Post hk |
| 15 | + | 0 | 70-90 | 0 | 0 | 0 | 57 | N |
| 16 | + | 0 | >90 | 0 | 0 | 0 | 50 | Ant hk |
| 17 | + | Ant | 100 | 70-90 | 0 | +LAD | 62 | Ant hk |

ak = akinesia; ant = anterior; AP = preexisting angina pectoris or angina-like symptoms; collat = collateral filling from left coronary artery; dk = dyskinesia; EF = ejection fraction; hk = hypokinesia; inf = inferior; LAD = left anterior descending artery; LC = left circumflex artery; LV = left ventricular; MI = myocardial infarction; n = normal; post = posterior; RCA = right coronary artery; + = present; 0 = absent.

ry is 0.012 mmol/liter. Hypoxanthine was assayed by dual-column high-pressure liquid chromatography (first column: $\mu$-Bondapak-C-18 [Waters Assoc.], reversed phase, and second column: Nucleosil-10 SA [Chrompack]), using a Valco CV-6-UH Pa-N-60 valve as described by van Gennip et al[20] and 0.0025 *M* $KH_2PO4$ (pH 2.9) as mobile phase. Hypoxanthine was determined by UV detection ($\lambda$ = 254 nm). The standard deviation of the determination is 0.2 $\mu M$/liter.

**Electrocardiographic measurements:** Leads I, II and $V_5$ were monitored throughout the study. ST-segment changes were measured 0.08 second after the J point in 5 consecutive beats at a paper speed of 100 mm/s.

**Study protocol:** A stabilization period of 15 minutes was allowed after instrumentation. Thus, at least 30 minutes was allowed between the last coronary angiogram and baseline measurements. Next, krypton-81m perfusion was monitored during one 10-minute observation period. The procedures to detect and correct for streaming artifacts are described in detail elsewhere.[4] Only patients in whom the baseline krypton-81m distribution pattern was stable entered the study. Multiple control hemodynamic, metabolic and scintigraphic determinations were then made. Subsequently, atrial pacing was carried out with increments in heart rate of 10 beats every 2 minutes until limiting angina or atrioventricular block occurred or a maximal heart rate of 170 beats/min was reached. All measurements were repeated during pacing at prefixed heart rates (100, 120, 140, 160 and 170 beats/min), during angina or block, followed by determinations at 15 seconds and 1, 2 and 5 minutes after pacing.

Statistical analysis was performed using the Student *t* test for paired values. All values are expressed as mean ± standard error of the mean.

## Results

Patient data and angiographic findings are listed in Table I. Fifteen patients had exercise-induced angina pectoris and 7 had electrocardiographic signs of an old myocardial infarction, all corresponding to areas of angiographically visible regional left ventricular wall motion disturbances. In another 5 areas hypokinesia or akinesia was present distal to a high-grade coronary artery lesion without electrocardiographic signs of infarction. All patients had more than 70% diameter reductions of 1 or more major branches of the left coronary artery, considered to be within the sampling area of the coronary sinus catheter; 14 areas with more than 90% and 9 with 70 to 90% diameter reductions. In 3 patients right CAD was present as well, without collateral supply from the left coronary artery.

**Control period:** During the control period no clinical signs of myocardial ischemia were found. Lactate metabolism was normal in all patients. However, stable, persistent reductions in krypton-81m distribution were found in 4 areas, all in regions with a left ventricular wall motion abnormality, 3 of which corresponded with an old myocardial infarct. Two patients with diminished krypton-81m distribution at rest produced hypoxanthine during the control period, without other signs of ischemia or lactate production (Fig. 2).

**Pacing period:** During pacing, 15 patients had angina and 14 significant (more than 0.1 mV) ST-segment changes. Angina started at 131 ± 6 beats/min and ST-segment changes (0.12 ± 0.04 mV, $p < 0.05$ vs control) (Fig. 3) at 120 beats/min.

**Krypton-81m perfusion during pacing:** A progressive decrease in krypton-81m perfusion occurred in 22 of the 23 regions with more than 70% stenosis. In areas

**TABLE II Changes in Regional Coronary Flow and Myocardial Metabolism During and After Atrial Pacing**

| | | Pacing Periods | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 100 beats/min | 120 beats/min | AP | 15 Sec P-P | 1 Min P-P | 5 Min P-P |
| 81m Kr distribution (%) | | | | | | | |
| >90% areas (n = 14) | 100 | 83 ± 4.5* | 77 ± 6* | 68 ± 7* | 78 ± 6* | 81 ± 6* | 78 ± 5* |
| 70-90% areas (n = 9) | 100 | 96 ± 2 | 83 ± 3.5* | 80 ± 4* | 79 ± 3.5* | 83 ± 4* | 87 ± 3 |
| Lactate (mmol/liter) | | | | | | | |
| A | 0.68 ± 0.04 | 0.72 ± 0.06 | 0.69 ± 0.06 | 0.69 ± 0.06 | 0.73 ± 0.06 | 0.71 ± 0.06 | 0.75 ± 0.07 |
| V | 0.60 ± 0.04 | 0.65 ± 0.07 | 0.70 ± 0.08 | 0.74 ± 0.08* | 0.85 ± 0.10* | 0.72 ± 0.08* | 0.65 ± 0.11 |
| Extraction (%) [(A-V/A) × 100%] | 16 ± 2 | 8 ± 4 | −1 ± 5 | −6 ± 5* | −15 ± 7* | −3 ± 7* | 12 ± 5 |
| Hypoxanthine (μmol/liter) | | | | | | | |
| A | 1.69 ± 0.1 | 1.58 ± 0.16 | 1.58 ± 0.15 | 1.67 ± 0.11 | 1.84 ± 0.18 | 1.86 ± 0.16 | 1.54 ± 0.2 |
| V | 1.64 ± 0.16 | 1.73 ± 0.2 | 2.62 ± 0.5* | 3.73 ± 0.76* | 5.52 ± 1.58* | 5.96 ± 1.53* | 2.96 ± 0.57* |
| A-V difference | 0.05 ± 0.17 | 0.45 ± 0.42 | 1.26 ± 0.42* | 1.49 ± 0.41* | 2.19 ± 0.5* | 2.64 ± 0.77* | 1.41 ± 0.57* |

*p <0.05 vs control; all values mean ± standard error of the mean.
A = arterial; AP = maximal paced heart rates; P-P = postpacing; V = venous.

with more than 90% stenosis, krypton-81m perfusion diminished early to 83 ± 4.5% of control (p <0.05), at 100 beats/min, before angina or ST-segment changes, whereas in areas with 70 to 90% stenosis a significant reduction to 83 ± 3.5% of control was observed later, at 120 beats/min, together with ST-segment changes but before the onset of angina (Fig. 3). During maximal heart rates (137 ± 5 beats/min) krypton-81m perfusion had further decreased to 68 ± 7% of control in areas with more than 90% CAD and to 80 ± 4% of control in 70 to 90% regions (both p <0.05 vs control) (Table II).

**Metabolic changes during pacing:** Arterial hypoxanthine and lactate levels remained unchanged throughout the study (Table II). Coronary sinus hypoxanthine and lactate values progressively increased during pacing. Both were significantly elevated at 120 beats/min, which resulted in simultaneous lactate production and hypoxanthine release at this period (lactate extraction −1 ± 5% vs 16 ± 2% (control) and difference in arteriovenous hypoxanthine −1.26 ± 0.42 μM/liter vs 0.08 ± 0.17 μM (control) (both p <0.05). Thus, changes in lactate and nucleoside metabolism were only preceded by krypton-81m distribution changes in areas of severe (more than 90%) narrowing (Fig. 3). During maximal pacing rates, lactate production increased to −6 ± 5% and the difference in arte-

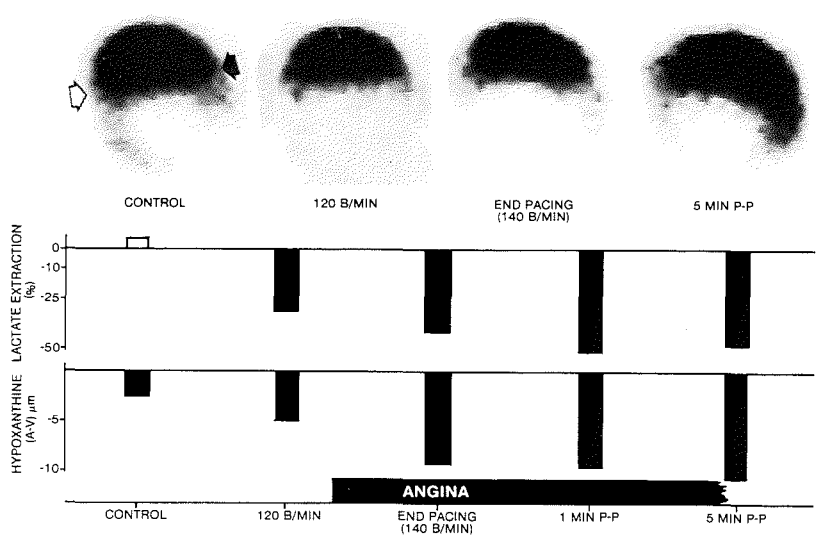


**FIGURE 2. Krypton-81m perfusion and metabolic changes in a patient with a 99% proximal stenosis in the left anterior descending coronary artery (LAD) (*open arrow*) and a 100% occlusion of the left circumflex artery (LC) (*closed arrow*) with anterograde collaterals and electrocardiographic signs of an old lateral infarction. At rest, there is decreased krypton-81m perfusion in the LAD and LC areas and significant hypoxanthine release, without other signs of ischemia. During pacing, krypton-81m perfusion decreases even more in both areas, accompanied by progressive metabolic changes, which persist 5 minutes after pacing (P-P), together with reduced krypton-81m perfusion to the LAD region, but despite an increase in (collateral) supply to the LC area.**

riovenous hypoxanthine levels to −1,49 ± 0,41 $\mu M$/liter.

**Postpacing period:** Immediately after pacing pulmonary capillary wedge pressures increased to 22 ± 3 mm Hg ($p < 0.05$ vs 13 ± 1 mm Hg at control), but only in 9 patients. Pressures had normalized already 1 minute after pacing. Angina lasted for 2.2 ± 0.5 minutes after pacing and abnormal ST-segment changes for 2.3 ± 0.3 minutes.

**Krypton-81m distribution after pacing:** In areas with more than 90% stenosis, krypton-81m perfusion increased slightly but significantly between the period of maximal pacing heart rates and after 1 minute postpacing, whereas no changes in occurred areas with 70 to 90% stenosis during this period. At 5 minutes after pacing, krypton-81m distribution was still significantly reduced (78 ± 5%) in areas with more than 90% stenosis ($p < 0.05$ vs control) but no longer in areas with 70 to 90% stenosis (87 ± 3%, difference not significant vs control).

**Metabolic changes after pacing:** Maximal release of both metabolites occurred after pacing. Lactate production values were highest 15 seconds after pacing (lactate extraction −15 ± 7%, $p < 0.05$ vs control) and maximal hypoxanthine release occurred 1 minute after pacing (Δ arteriovenous −2.64 ± 0.8 $\mu M$/liter, $p < 0.01$ vs control). Lactate production occurred in 15 patients, whereas significant hypoxanthine release (Δ arteriovenous more than −0.4 $\mu M$/liter) was observed in all 17 patients. Changes in lactate metabolism were brief and had disappeared 2 minutes after pacing. In contrast, significant hypoxanthine release was still present 5 minutes after pacing (Δ arteriovenous −1.4 ± 0.6 $\mu M$/liter, $p < 0.05$ vs control). An example of persis-

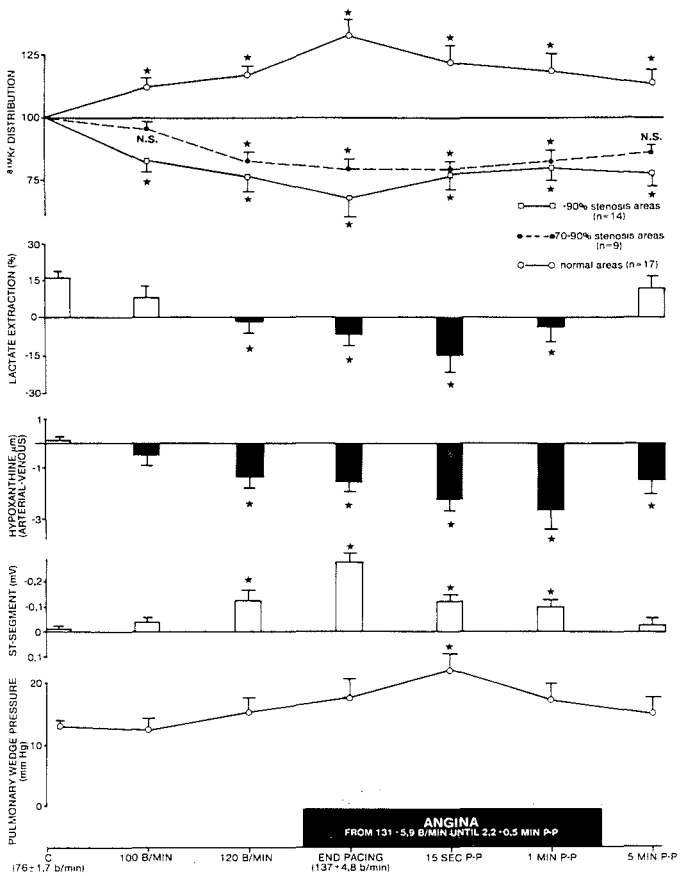


**FIGURE 3. Temporal relation of krypton-81m distribution changes, myocardial lactate production and hypoxanthine release and general indexes of ischemia during and after incremental atrial pacing (P-P). Although lactate and hypoxanthine are produced early and simultaneously, only preceded by krypton-81m perfusion changes in areas with more than 90% stenosis, maximal lactate production and hypoxanthine release occur at 15 seconds and 1 minute after pacing, respectively, irrespective of krypton-81m distribution changes. After pacing, both a reduction in krypton-81m perfusion (in areas with more than 90% stenosis) and hypoxanthine release persist, indicating ongoing flow reduction and ischemia. N.S. = not significant.**

tent reduction in poststenotic krypton-81m perfusion and ongoing hypoxanthine release is shown in Figure 4.

## Discussion

**Changes in myocardial metabolism and coronary flow during pacing:** In the present study a close temporal relation was found between changes in poststenotic regional coronary flow and myocardial metabolism during pacing-induced ischemia. This, to our knowledge, has not been investigated in humans, perhaps because in humans repetitive measurements of regional coronary flow changes at short intervals are difficult to carry out. We recently described a technique using intracoronary krypton-81m that permits continuous determination of changes in regional coronary flow pattern.[3,4] In the previous studies,[3,4] a progressive decrease in krypton-81m distribution was seen in areas with more than 70% CAD. These changes occurred well before the onset of angina, suggesting that angina is a late phenomenon in ischemia. Although angina is usually considered the standard for ischemia, changes in lactate metabolism, particularly when measured 15 seconds after pacing-induced stress, are more sensitive as a marker of myocardial ischemia.[7] Whether this also implies that lactate production in humans is an early event after coronary flow reduction and onset of ischemia is unknown. Changes in myocardial lactate and nucleoside metabolism during ischemia and the temporal relation with coronary flow reductions have been studied extensively in animal experiments. Although myocardial adenine nucleoside accumulation and release from the heart occurs soon after the onset of coronary flow reduction, the results of some studies indicate that myocardial lactate production is an earlier event during ischemia than adenine nucleotide breakdown and that lactate efflux from the heart precedes myocardial nucleoside release.[11,18] In a previous study in humans, we reported that during pacing-induced ischemia lactate production also occurs early, before hypoxanthine release.[14] In the latter study significant hypoxanthine changes were only found after angina had developed, whereas in the present investigation hypoxanthine release occurred early, simultaneously with lactate production, and before the onset of angina. This apparent discrepancy may be explained by the fact that in the early study an enzymatic method was used, which did not differentiate between xanthine and hypoxanthine. Small but significant early elevations in hypoxanthine may have therefore remained unnoticed in the (hypoxanthine) xanthine pool. With the present method the hypoxanthine and xanthine peaks are measured separately. The value of this method has already been reported,[16,20] and although several adaptations were incorporated in our own assay technique, it is comparable to the method of Harmsen et al.[16] Of the different nucleosides, only hypoxanthine could be measured in adequate amounts. Because of high activity of adenosine deaminase and nucleoside phosphorylase and subsequent fast turnover of adenosine and inosine to hypoxanthine in humans, these intermediate metabolites of nucleoside metabolism could only be detected in amounts too small to make them useful for diagnostic purposes (unpublished data). This is in accordance with the results of our previous study[14] and the work of other investigators.[16]

**Validity of hypoxanthine and lactate as markers of ischemia in humans:** The present investigation suggests that hypoxanthine is a more sensitive indicator of ischemia than lactate, for a variety of reasons. First, although changes in coronary flow occurred in all pa-

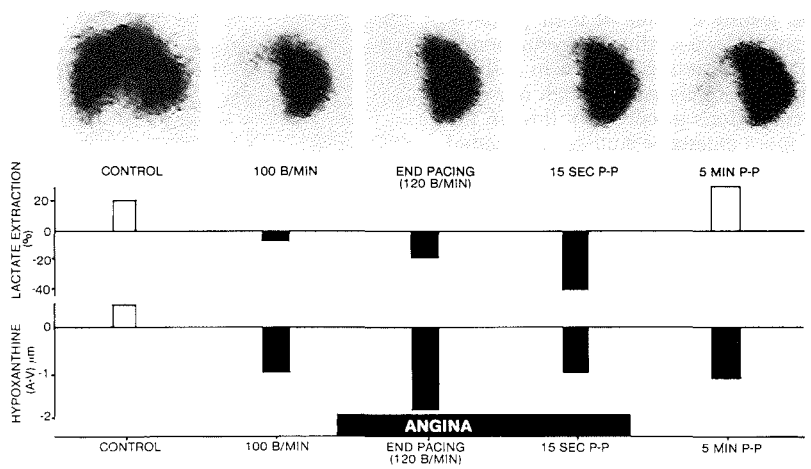


**FIGURE 4. Typical changes in krypton-81m distribution, lactate and hypoxanthine metabolism in a patient with a proximal 90% stenosis of the left anterior descending artery (LAD). Early during pacing, krypton-81m distribution decreases over the LAD area, together with lactate and hypoxanthine release, but before angina. After pacing (P-P), both the krypton-81m perfusion defect and hypoxanthine release persist, indicating ongoing (silent) ischemia despite normalization of lactate metabolism.**

tients, myocardial lactate production was only observed in 15. In contrast, significant hypoxanthine release occurred in all 17 patients. Second, whereas the arterial levels of both metabolites did not change during the study, the percent increase in coronary venous hypoxanthine levels greatly exceeded the concomitant alterations in coronary venous lactate levels, both during and after pacing. Finally, in 2 of the 4 patients with diminished krypton-81m perfusion during control conditions, and thus, presumably, a reduction in regional coronary flow, hypoxanthine release was the only indication of ischemia at rest.

**Changes in myocardial metabolism and coronary flow after pacing:** At least 5 minutes after pacing, krypton-81m perfusion was still reduced in areas with more than 90% stenosis. We previously reported this observation.[3,4] At that time after pacing, all general indexes of ischemia, such as angina and electrocardiographic and hemodynamic changes, were no longer present for several minutes at least.

Likewise, lactate metabolism had normalized between 1 and 2 minutes after pacing. In contrast, significant amounts of hypoxanthine were still released 5 minutes after pacing, suggesting ongoing (silent) myocardial ischemia, despite normalization of myocardial oxygen demand after pacing. This indicates that at this stage other factors are involved, which maintain ischemia after pacing-induced stress. The ongoing hypoxanthine release after pacing supports the hypothesis that persistent changes in coronary flow after pacing may be caused by continuing adenosine production in the poststenotic area.[4] Prolonged ischemia or reductions in regional coronary flow after stress testing were reported by Selwyn and et al,[21] who observed abnormal rubidium-82 uptake persisting for as long as 20 minutes after stress testing in some of their patients. Whether this was caused by a continuous reduction in regional flow or by a persistent reduction in cellular uptake of rubidium-82 is unclear. Although our data suggest persistent reductions in coronary flow in the postpacing period, more extensive studies are needed to evaluate the duration of coronary flow reductions after stress testing.

**Clinical implications:** Our data suggest that hypoxanthine is more sensitive as a marker of ischemia than either lactate, angina or ST-segment changes. In contrast to lactate, it is still released during longer periods of ischemia. Hypoxanthine, therefore, should be a valuable indicator of longer lasting periods of ischemia in humans and may be useful to validate interventions during the early phase of infarction. The observation in this study of persistent perfusion abnormalities and ischemia after pacing emphasizes the necessity of sufficiently long intervals between repetitive atrial pacing stress tests to allow for reproducibility of pacing-induced ischemia.[22]

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# Chapter VIII:

# ASSESSMENT OF THE ACUTE ANTIISCHEMIC PROPERTIES OF VASOACTIVE COMPOUNDS IN MAN FOCUSING ON METABOLISM.

# VIII.1.

## Acute Hemodynamic and Antiischemic Effects of Intravenous Amiodarone.

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# Acute Hemodynamic and Antiischemic Effects of Intravenous Amiodarone

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**The acute hemodynamic and antiischemic properties of amiodarone were investigated in 16 patients with more than 70% diameter reduction of a left coronary artery. Two successive atrial pacing stress tests (APST I and II) were performed, with an interval of 40 minutes in between, and amiodarone, 5 mg/kg/5 min, was infused 30 minutes after APST I. Hemodynamic changes during amiodarone administration consisted of a 20% decrease in left ventricular (LV) systolic pressure, a 13% decrease in systemic vascular resistance and an 18% decrease in stroke work. Coronary vascular resistance was reduced 19% and coronary sinus flow increased 23%. Despite a secondary 14% increase in heart rate, contractility decreased 21%, accompanied by a 45% increase in LV end-diastolic pressure, which persisted until APST II. Although most hemodynamic changes were observed only during the infusion, contractility and LV systolic pressure were still diminished at the beginning of APST II and remained so during pacing, resulting in a reduction in myocardial oxygen demand compared to APST I. Although overall myocardial oxygen consumption and coronary flow were equal during both pacing tests, amiodarone significantly reduced pacing-induced myocardial ischemia. Lactate metabolism remained normal during APST II (lactate extraction 12 ± 3% vs −28 ± 8% (APST I) at maximal pacing rates [$p < 0.05$]), while ST-segment depression, LV end-diastolic pressure postpacing and angina were also significantly reduced during APST II. Thus, in humans, intravenous amiodarone reduces vascular resistance and contractility and inhibits pacing-induced myocardial ischemia, presumably by reducing myocardial oxygen demand.**

**(Am J Cardiol 1985;55:639–644)**

Amiodarone, widely used as an antiarrhythmic agent, was originally introduced as an antianginal compound,[1] primarily because of its hemodynamic properties, which indicate that it has the potential to reduce myocardial ischemia. In the intact animal it reduces vascular resistance and afterload, and increases coronary flow as a result of its smooth-muscle relaxing properties,[2–5] while, in addition to its negative chronotropic activity, it reduces contractility as well at the higher dose levels.[6] Thus, the myocardial oxygen supply/demand ratio is favorably altered and myocardial oxygen consumption reduced. Although the results of the various animal studies are similar, the hemodynamic changes by the same intravenous dose in humans varies from study to study.[7–9] In addition, the effect of these hemodynamic changes on myocardial ischemia in humans has not been investigated. In this study, the instantaneous cardiovascular actions of amiodarone in patients with coronary artery disease, as well as the effects on pacing-induced myocardial ischemia, were investigated.

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## Methods

**Patients:** We studied 16 patients (15 men and 1 woman), aged 42 to 67 years (mean 53) with stable, exercise-induced angina pectoris and objective signs of ischemia during stress testing. All cardiac medication except short-lasting nitroglycerin was withheld 48 to 72 hours before the study. Catheterization was performed without premedication for the patient and after an overnight fast. To participate, a stenosis of more than 70% in the left anterior descending artery, the left circumflex artery or proximal marginal branches had to be present, thus ensuring that venous blood from the stenotic area was collected by the coronary sinus catheter. Three patients had 1-vessel, 9 had 2-vessel and 4 had 3-vessel disease. Left ventricular (LV) ejection fraction was slightly decreased (47 ± 4%) and LV end-diastolic volume index was normal (81 ± 8 ml/m$^2$) (Table I).

**TABLE I Patient Characteristics**

| Pt | Age (yr) & Sex | Previous MI | LVEDV (ml/m²) | LVEF (%) | % Diameter Reduction | | |
|---|---|---|---|---|---|---|---|
| | | | | | LAD | LC | RCA |
| 1 | 57M | 0 | 94 | 61 | 80 | 90 | 0 |
| 2 | 58F | 0 | 61 | 63 | 80 | 90 + 100 | 0 |
| 3 | 54M | Inf | 97 | 22 | 70 | 0 | 100 |
| 4 | 53M | Inf | 37 | 70 | 90 | 0 | 100 |
| 5 | 59M | Inf | 150 | 38 | 70 | 80 | 90 |
| 6 | 67M | Ant | 76 | 35 | 100 | 100 | 0 |
| 7 | 51M | Inf | 74 | 34 | 70 + 90 | 0 | 100 |
| 8 | 42M | 0 | 46 | 51 | 80 | 0 | 100 |
| 9 | 56M | 0 | 89 | 25 | 99 | 90 | 100 |
| 10 | 55M | 0 | 42 | 50 | 80 + 90 | 90 | 60 |
| 11 | 50M | Inf | 89 | 44 | 0 | 80 | 90 |
| 12 | 47M | 0 | 80 | 60 | 100 | 0 | 70 |
| 13 | 50M | Ant | 48 | 49 | 80 | 90 | 99 |
| 14 | 53M | Ant | 115 | 23 | 100 | 0 | 0 |
| 15 | 50M | Ant | 67 | 67 | 100 | 70 + 90 | 0 |
| 16 | 57M | 0 | 81 | 51 | 90 | 0 | 0 |
| Mean ± SEM | 53 ± 2 | | 78 ± 7 | 46 ± 4 | | | |

Ant = anterior; Inf = inferior; LAD = left anterior descending coronary artery; LC = left circumflex artery; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; RCA = right coronary artery; SEM = standard error of the mean.

**Catheterization procedures:** After routine left and right coronary arteriography was carried out using the Seldinger technique, a No. 7Fr Millar micromanometer catheter was positioned in the left ventricle using a Desilet system in the femoral artery. The side arm of this Desilet system was used to record aortic pressures. A No. 7Fr Swan Ganz catheter was advanced through a femoral vein with its tip in a pulmonary artery and its proximal lumen in the right atrium. Then, a thermodilution pacing catheter (Wilton Webster Laboratory) was placed with its tip in the midportion of the coronary sinus, such that its position was stable and blood could be drawn from the coronary sinus quickly enough to allow a rapid sequence of blood samples to be collected.

**Measurements:** After positioning, the catheters were calibrated using a 0 reference level set at midchest. The micromanometer pressures were balanced to 0 and calibrated with the fluid-filled system. Recordings of pressures and LV dP/dt were made on paper in the appropriate pressure ranges, using a CGR 1000 Cath Lab system. Cardiac output was measured by the thermodilution technique. All pressures and pressure-derived contractility indexes (LV peak dP/dt pos, dP/dt/P at 40 mm Hg (VCE40) and Vmax total pressure) as well as cardiac output were determined online by a Mennen-Cathlab computer system. Calculations were made from 12 consecutive beats. Coronary sinus blood flow was determined with the continuous infusion thermodilution method as originally described by Ganz et al.[10] Thirty to 35 ml of glucose 5% at room temperature was infused over 30 seconds into the coronary sinus. During the infusion both the mean and pulsatile flow were recorded simultaneously and measurements were made during midrespiration after the mean flow curve had stabilized. The external thermistor was always well inside the coronary sinus ostium to prevent atrial reflux interfering with the flow measurements. This was confirmed by a bolus injection of saline solution into the right atrium. A coronary venous angiogram using 3 ml of Renografin 76® was recorded on a video disk to allow rechecking of the position of the catheter tip during the investigation.

**Metabolic and electrocardiographic measurements:** Simultaneous sampling of 2 ml of blood for lactate essay was performed from the coronary sinus and left ventricle which was then directly transferred into tubes containing 2 ml of ice-cold 8% $HClO_4$, thoroughly mixed and kept on ice. After centrifugion, lactate was determined as described by Guttman and Wahlefeld.[11] Venous and arterial $O_2$ saturation values were measured using an American Optical oximeter.

Throughout the studies leads I, II and $V_5$ were continuously monitored, and ST-segment changes, 0.08 second after the J point, measured in 5 consecutive beats at a paper speed of 50 mm/s.

**Calculations:** From the measured values, the following calculations were made: coronary vascular resistance (mean arterial pressure/coronary sinus blood flow); myocardial $O_2$ consumption or uptake (difference in arterial and coronary sinus $O_2$ content × coronary sinus blood flow); modified tension-time index or double product, an index of myocardial $O_2$ demand (heart rate × LV systolic pressure). Systemic vascular resistance, derived as 80 (arterial mean pressure − atrial mean pressure)/cardiac output; pulmonic vascular resistance, derived as 80 (pulmonary mean pressure − LV mean diastolic pressure)/cardiac output; stroke work index was expressed as stroke index (mean aortic pressure − LV end-diastolic pressure) × 0.0136. Myocardial lactate extraction (%), derived as 100 (arteriocoronary sinus/arterial lactate concentration).

**Study protocol:** After positioning of the catheters, a stabilization period of at least 15 minutes was allowed to reach a minimum interval of 30 minutes between coronary angiography and the study. Multiple control determinations of all variables were then made to ensure a stable baseline. Then, the first atrial pacing stress test (APST I) was carried out with increments in heart rate of 10 beats/2 minutes until angina, atrioventricular block or a maximal heart rate of 170 beats/min. All variables were again determined at prefixed heart rates during pacing (e.g., at 100, 120, 140 and 160 beats/min), during angina or block, followed by measurements 15 seconds, 1, 2 and 5 minutes after pacing. After a 25-minute stabilization period after APST I, control measurements were carried out and at 30 minutes after APST I, amiodarone (5 mg/kg body weight) was infused over 5 minutes. All determinations were repeated at 1, 3 and 5 minutes after the onset of amiodarone administration, followed by measurements 5 minutes after the infusion. A second atrial pacing stress test (APST II) was carried out in an identical fashion as APST I. To ensure identical pacing heart rates, the patients received 0.5 mg atropine before each APST.

**Statistical analysis:** Our data-analytic approach was 2-fold. First, the measurements during and after amiodarone

**TABLE II Systemic Hemodynamic Changes During Amiodarone Administration**

| | Control | During Amiodarone Infusion | | | 5 min After Infusion |
|---|---|---|---|---|---|
| | | 1 min | 3 min | 5 min | |
| HR (beats/min) | 78 ± 4 | 84 ± 4.5* | 89 ± 4* | 87 ± 4* | 77 ± 3 |
| LVSP (mm Hg) | 141 ± 6 | 123 ± 6* | 113 ± 4* | 118 ± 5* | 128 ± 5* |
| MAP (mm Hg) | 103 ± 4 | 96 ± 3* | 87 ± 3* | 92 ± 3* | 99 ± 3 |
| CO (liters/min) | 4.8 ± 0.3 | ... | ... | 4.9 ± 0.2 | ... |
| SVR (dynes s $cm^{-5}$) | 1,698 ± 76 | ... | ... | 1,475 ± 84* | ... |
| SI (ml/beat/$m^2$) | 31 ± 2 | ... | ... | 30 ± 2 | ... |
| SWI (g/m/$m^2$) | 39 ± 3 | ... | ... | 32 ± 3* | ... |
| LV peak dP/dt pos (mm Hg $s^{-1}$) | 1,789 ± 61 | 1,609 ± 109 | 1,543 ± 120* | 1,419 ± 100* | 1,355 ± 77* |
| Vmax ($s^{-1}$) | 49 ± 3 | 46 ± 2 | 49 ± 3 | 44 ± 2* | 40 ± 3* |
| VCE 40 ($s^{-1}$) | 34 ± 2 | 32 ± 2 | 32 ± 3 | 28 ± 2* | 27 ± 2* |
| LVEDP (mm Hg) | 11 ± 1 | 10 ± 1 | 12 ± 1 | 15 ± 2* | 16 ± 2* |
| PAS (mm Hg) | 25 ± 1 | 26 ± 2 | 31 ± 2 | 32 ± 2* | 32 ± 1* |
| PAD (mm Hg) | 13 ± 1 | 14 ± 1 | 17 ± 1* | 17 ± 1* | 17 ± 1* |
| PVR (dynes s $cm^{-5}$) | 202 ± 16 | ... | ... | 220 ± 14 | ... |

* $p < 0.05$ vs control.
CO = cardiac output; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean aortic pressure; PAD = diastolic pulmonary artery pressure; PAS = systolic pulmonary artery pressure; PVR = pulmonary vascular resistance; SI = stroke index; SVR = systemic vascular resistance; SWI = stroke work index.

**TABLE III Coronary Hemodynamic Changes During Amiodarone Administration**

| | Control | During Amiodarone Infusion | | | 5 min After Infusion |
|---|---|---|---|---|---|
| | | 1 min | 3 min | 5 min | |
| CSBF (ml/min) | 127 ± 11 | 146 ± 13* | 153 ± 13* | 152 ± 15* | 139 ± 13 |
| CVR (mm Hg/ml/min) | 0.9 ± 0.1 | 0.7 ± 0.05* | 0.6 ± 0.05* | 0.7 ± 0.05* | 0.8 ± 0.1 |
| $\Delta$A-CS $O_2$ content (vol%) | 7.2 ± 0.3 | 6.5 ± 0.4* | 5.4 ± 0.4* | 5.8 ± 0.5* | 7.2 ± 0.4 |
| $MVO_2$ (ml/min) | 9.1 ± 0.9 | 9.3 ± 0.9 | 8.1 ± 1 | 8.3 ± 0.8 | 9.5 ± 1 |
| TTI (HR × LVSP × $10^{-3}$) | 10.4 ± 0.5 | 10.1 ± 0.4 | 9.9 ± 0.5 | 10.1 ± 0.5 | 102 ± 0.6 |

* $p < 0.05$ vs. control.
A = arterial; CS = coronary sinus; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; HR = heart rate; LVSP = left ventricular systolic pressure; $MVO_2$ = myocardial oxygen consumption; TTI = modified tension-time index or double product.

administration were compared with baseline (control) value. A *t* test for paired observations with 11 degrees of freedom was used, as the objective of this analysis was to test differences with the baseline observation. A 2-tailed p value <0.05 was considered significant.

Second, comparisons of measurements during atrial pacing before and after amiodarone administration were made, again using a *t* test for paired observations, with a 2-tailed p value <0.05 indicating a significant difference from 0. All values are expressed as mean ± standard error of the mean.

## Results

**Systemic hemodynamic changes during amiodarone infusion:** The systemic hemodynamic effects of amiodarone are given in Table II. During the infusion an immediate decrease in LV systolic and aortic pressures was observed with a maximal decrease of 20% and 16% during the third minute of amiodarone administration. LV systolic pressure was still reduced 5 minutes after the infusion. At the end of the infusion systemic vascular resistance and stroke work index had decreased 13% and 18% from their control values ($p < 0.05$), while cardiac output and stroke index remained unchanged. Heart rate increased immediately, with a 14% increase at the third minute of infusion ($p < 0.05$ vs control). These changes lasted a short time and had returned to baseline levels 5 minutes after amiodarone administration. In contrast to the early and short-lasting increase in heart rate, a significant decrease in contractility occurred later during the infusion, which was longer lasting with a maximal reduction 5 minutes after amiodarone (Vmax 18% and VCE40 21% vs control). Concomitant with these changes in contractility, LV end-diastolic pressure increased significantly, from 11 ± 1 mm Hg (control) to 15 ± 2 mm Hg at the end of the infusion and to 16 ± 2 mm Hg 5 minutes after amiodarone administration. Systolic and diastolic pulmonary pressure increased from 25 ± 1 and 13 ± 1 mm Hg before amiodarone to 32 ± 1 and 17 ± 1 mm Hg 5 minutes after amiodarone administration ($p < 0.05$). Pulmonary vascular resistance did not change.

**Coronary hemodynamic changes during amiodarone (Table III):** Coronary vascular resistance decreased immediately during amiodarone administration, with a maximal change of 19% ($p < 0.05$). Coronary sinus blood flow increased significantly with a maximal 23% increase during the third minute. These changes were short-lasting and only found during amiodarone administration. Although during the same period the difference in arterial and coronary sinus $O_2$ content diminished by 27% ($p < 0.05$ vs control), myocardial oxygen consumption remained unchanged, as did myocardial oxygen demand.

**Effects of amiodarone on hemodynamics during atrial pacing stress tests (Table IV):** During both

**TABLE IV Effects of Amiodarone on Hemodynamics During Atrial Pacing Stress Test**

| | | Control | 100 beats/min | 120 beats/min | AP | 1 min P-P | 2 min P-P | 5 min P-P |
|---|---|---|---|---|---|---|---|---|
| LVSP (mm Hg) | I | 145 ± 5 | 142 ± 5 | 139 ± 7 | 146 ± 8 | 149 ± 7 | 149 ± 8 | 137 ± 6 |
| | II | 128 ± 5* | 131 ± 5* | 126 ± 5* | 129 ± 6* | 137 ± 6* | 136 ± 6* | 129 ± 6* |
| TTI ($\times 10^{-3}$) | I | 11.6 ± 0.6 | 14.3 ± 0.5 | 17 ± 0.8 | 20.7 ± 1.0 | 11.4 ± 0.7 | 11.6 ± 0.8 | 10 ± 0.4 |
| | II | 10.2 ± 0.6* | 13.2 ± 0.5* | 15 ± 0.6* | 17.5 ± 0.9* | 10.6 ± 0.6 | 10.4 ± 0.6* | 10.2 ± 0.6 |
| LV dP/dt pos (mm Hg $s^{-1}$) | I | 1,788 ± 93 | 1,904 ± 91 | 2,066 ± 165 | 2,099 ± 132 | 1,828 ± 121 | 1,821 ± 117 | 1,594 ± 94 |
| | II | 1,355 ± 77* | 1,323 ± 52* | 1,547 ± 72* | 1,675 ± 124* | 1,605 ± 126* | 1,423 ± 68* | 1,478 ± 70 |
| $VCE_{40}$ ($s^{-1}$) | I | 35 ± 2 | 38 ± 2 | 39 ± 2 | 42 ± 2 | 35 ± 2 | 33 ± 2 | 31 ± 1.5 |
| | II | 26 ± 2* | 26 ± 1* | 32 ± 2* | 35 ± 3* | 31 ± 2.5* | 29 ± 2* | 29 ± 1.5 |
| CSBF (ml/min) | I | 137 ± 11 | 152 ± 15 | 159 ± 14 | 178 ± 15 | 152 ± 12 | 135 ± 13 | 131 ± 10 |
| | II | 139 ± 13 | 144 ± 13 | 158 ± 16 | 164 ± 13 | 146 ± 15 | 135 ± 14 | 144 ± 16 |
| CVR (mm Hg/ml/min) | I | 0.83 ± 0.06 | 0.71 ± 0.08 | 0.72 ± 0.05 | 0.68 ± 0.07 | 0.7 ± 0.04 | 0.81 ± 0.08 | 0.8 ± 0.05 |
| | II | 0.78 ± 0.06 | 0.77 ± 0.07 | 0.73 ± 0.08 | 0.68 ± 0.05 | 0.81 ± 0.07 | 0.79 ± 0.07 | 0.83 ± 0.08 |
| $MVO_2$ (ml/min) | I | 9.8 ± 0.9 | 10.7 ± 1.1 | 11.5 ± 1.1 | 12.2 ± 1.2 | — | 9.1 ± 0.9 | 9 ± 0.9 |
| | II | 9.5 ± 1 | 9.9 ± 1 | 10.9 ± 1.1 | 11.7 ± 0.8 | — | 9.2 ± 1.4 | 9.4 ± 1 |

* $p < 0.05$ APST I (before amiodarone) vs APST II (after amiodarone).
AP = end of pacing due to angina or block; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; LVSP = left ventricular systolic pressure; P-P = post-pacing; TTI = tension-time index; I = first atrial pacing stress test; II = second APST.

APSTs maximal heart rates were comparable: 144 ± 6 beats/min during APST I and 141 ± 6 beats/min during APST II. The tension-time index, however, was significantly diminished throughout APST II because of a reduction in LV systolic pressure. Contractility, already diminished after amiodarone administration, remained significantly decreased during APST II. The reduction in tension-time index and contractility and, thus, in myocardial oxygen demand, did not result in significant changes in coronary sinus blood flow, coronary vascular resistance or overall myocardial oxygen consumption.

**Effects of amiodarone on pacing-induced ischemia:** Amiodarone clearly inhibited the development and extent of myocardial ischemia. During APST I myocardial lactate production was already evident at 120 beats/min before angina, increasing to −28 ± 8% at the end of pacing and to a maximal value of −41 ± 18% 15 seconds after pacing (Fig. 1). In contrast, during APST II after amiodarone administration mean lactate extraction values remained positive, although in a few patients some lactate production was found immediately after pacing. Individual lactate production values were, however, always less during APST II than during APST I. In addition, ST-segment changes were significantly less during APST II (0.17 ± 0.04 vs 0.26 ± 0.04 mV during APST I) (Fig. 2). Also, there was less elevation in LV end-diastolic pressure immediately after pacing during APST II (17 ± 2 vs 22 ± 1.8 mm Hg during APST I, $p < 0.05$) (Fig. 3).

Finally, 14 patients complained of angina during APST I; during APST II, symptoms were absent in 8 patients, less severe in 4 and similar in 4.

**Adverse effects:** Amiodarone was generally well tolerated. Some patients experienced a warm feeling and facial flushing. In 2 patients (nos. 6 and 13), lactate production occurred during the last minute of infusion and 5 minutes after infusion, accompanied by ST-segment changes and a short-lasting period of angina in patient 6.

## Discussion

Amiodarone, a benzofuran derivate, was shown in this study to have important hemodynamic and antiischemic properties in humans when given intravenously. These acute hemodynamic changes consist of an immediate reduction in systemic and coronary vascular resistance, a decrease in LV systolic and aortic pressures and in cardiac work, an increase in coronary blood flow and a reduction in the difference in arterial and coronary sinus $O_2$ content. Although a short-lasting increase in heart rate is observed, a later, but longer-lasting reduction in contractility occurs with an elevation of LV filling pressure.

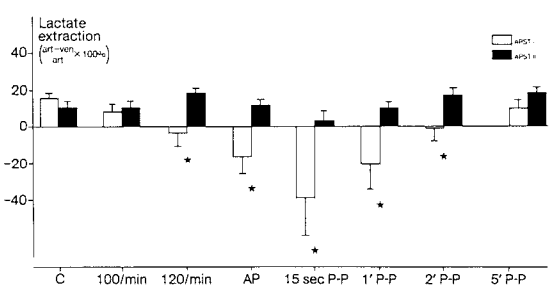


**FIGURE 1.** Lactate extraction pattern during atrial pacing stress test (APST) before and after amiodarone administration. During the first stress test (APST I), before amiodarone, lactate production is present at 120 beats/min and increases to a peak value of −41 ± 18.4% 15 seconds after pacing. During the second test (APST II), after amiodarone, there is a significant improvement in metabolism with lactate extraction values throughout the atrial pacing stress test. AP = maximal pacing rate; P-P = postpacing. *$p < 0.05$.

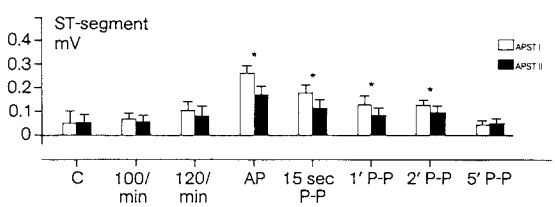


**FIGURE 2.** ST-segment depression values during pacing before and after amiodarone administration. There is significantly less ST-segment depression during the second atrial pacing stress test (APST II), after amiodarone. AP = maximal pacing rate; P-P = postpacing. * $p < 0.05$.

These cardiovascular effects compare well with hemodynamic changes in the intact animal.[3-6] Some differences exist, however. In the animal studies, the vasodilating effect is more prominent in the coronary than in the systemic vasculature, with major changes in coronary resistance and flow and only a moderate decrease in systemic resistance and afterload.[4,5,12] In the present study, the magnitude of changes in afterload and coronary blood flow were comparable.

Also, as amiodarone has negative chronotropic actions, heart rate has been reported to decrease in animal studies, in contrast to our study results, in which an increase was found, presumably secondary to the decrease in afterload. This also suggests that in humans, the vasodilating effect on the systemic vasculature is more important than in the intact animal model. However, one must be cautious with this deduction because the hemodynamic changes in man are a consequence of the combined effects of amiodarone and its solvent, Tween 80, whereas in the animal studies pure amiodarone is used.

The acute hemodynamic effects of intravenous amiodarone in humans have been studied by other investigators using an identical dose.[7-9] The rate of administration, however, varied from 1 to 4 minutes. Also, various types of patients, as well as normal persons, were studied. Although this may explain the dissimilarity in the results of some of these studies, in 2 of these investigations the same rate of infusion was used in the same type of patients. Still, opposing hemodynamic effects were reported.[7,9]

This apparent dissimilarity was one of the reasons for conducting the present investigation. Only patients with coronary artery disease were studied and dose and rate of administration chosen on purpose, as this regimen, followed by a 1,500-mg infusion over 24 hours, was effective in patients with ventricular tachycardias (unpublished observation).

Patients with a diminished LV ejection fraction were not excluded from the study, because in clinical practice patients receiving amiodarone for acute ischemia are likely to have a diminished ventricular function as well. Theoretically, in these patients the reduction in contractility and increase in LV filling pressure during the amiodarone bolus infusion may induce a deterioration of LV function and an aggrevation instead of an improvement of myocardial ischemia. In this study this was only observed in 1 out of 6 patients with a LV ejection fraction less than 40%, indicating that amiodarone may be given safely even to patients with diminished ventricular function. Whether this still holds true under less stable circumstances, i.e., ischemia or infarction, must be further investigated.

The hemodynamic changes observed in this study could be partially caused by the solvent Tween 80. Sicart et al[8] compared the hemodynamic effects of amiodarone with solvent and pure Tween 80. They concluded that the early vasodilation and reduction in afterload observed during amiodarone administration were primarily caused by Tween 80 and the later effects, i.e., changes in contractility and LV end-diastolic pressure, by amiodarone itself. The hemodynamic changes induced by Tween 80 only last a few minutes and almost certainly will have disappeared at the onset of APST II in this study.

**The antiischemic properties of amiodarone in man:** Amiodarone was originally introduced as an antianginal agent and was clinically effective in patients with angina pectoris.[13-16] It was reported recently to limit infarct size in the dog.[17] In patients with variant angina pectoris, amiodarone had beneficial effects.[18] Apart from a preliminary report from our laboratory, its antiischemic properties during acute episodes of pacing-induced myocardial ischemia have not been reported, nor have the hemodynamic actions involved.[19] In this study, amiodarone clearly inhibited the occurrence and extent of pacing-induced (regional) myocardial ischemia, although it did not affect overall myocardial $O_2$ consumption during atrial pacing. These antiischemic properties were demonstrated in particular by normalization of myocardial lactate metabolism after amiodarone treatment. Myocardial lactate production, in our experience, is a sensitive indicator of ischemia when assessed immediately (15 seconds) after pacing. Although the classic indexes of myocardial ischemia (i.e., angina, ST-changes and LV end-diastolic pressure after pacing) are reproducible at shorter pacing intervals, an interval of at least 30 minutes between successive pacing tests is needed for a reproducible lactate production pattern.[20] A 40-minute period between the 2 pacing stress tests was therefore chosen in this study and no placebo-controlled patients were used. The antiischemic effects of amiodarone were, apart from the reduction of anaerobic glycolysis, emphasized as well by a significant reduction in angina, ST-segment depression and abnormal LV end-diastolic pressure elevation during APST II. Our study results indicate that a reduction of tension-time index and contractility, and thus myocardial $O_2$ demand, is a major factor in this antiischemic action. During pacing, after amiodarone, the reduction of coronary vascular resistance had already disappeared and overall coronary flow was similar during both pacing stress tests. This makes an increase in regional flow in the ischemic area less likely. However, as we did not measure regional flow in this study, this must remain an assumption.

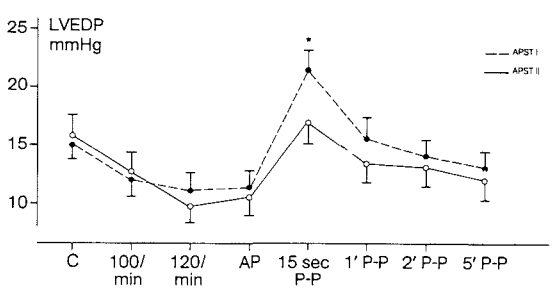


**FIGURE 3.** Left ventricular end-diastolic pressure (LVEDP) values during and after atrial pacing stress test (APST), before and after amiodarone administration. LVEDP immediately after pacing (15 sec P-P) is significantly less elevated after amiodarone. AP = maximal pacing rate; P-P = postpacing. * $p < 0.05$.

When an infusion of amiodarone is added to the bolus injection, a longer-lasting increase in coronary blood flow may result, yielding an increase in regional oxygen delivery. Combined with the reduction in myocardial $O_2$ demand, this will further diminish ischemia, provided LV filling pressure remains within the normal range. It may be anticipated that such an approach will benefit the acutely ischemic patient. Especially in the patient with infarction who has ventricular dysrhythmias as well, intravenous amiodarone may be considered the treatment of choice.

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VIII.2.

# Dose Related Coronary and Systemic Hemodynamic Effects of Intravenous Bepridil in Patients with Coronary Artery Disease.

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# Dose related coronary and systemic haemodynamic effects of intravenous bepridil in patients with coronary artery disease

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KEY WORDS: Bepridil, calcium-blocking drug, coronary artery disease.

*The acute coronary and systemic haemodynamic effects of intravenous bepridil were investigated in 27 patients with coronary artery disease; 13 (group 1) received 2 mg kg$^{-1}$ and 14 (group 2) 4 mg kg$^{-1}$ over 5 min. An immediate systemic and coronary vasodilation occurred in both groups during and immediately after the infusion. Changes were dose-related with a maximal decrease in left ventricular (LV) systolic pressure of 11% (group 1) and 18% (group 2), in mean aortic pressure of 11% (group 1) and 19% (group 2), and in coronary resistance of 23% (group 1) and 41% (group 2). Coronary flow increased by 17% (group 1) and 47% (group 2) (all changes significantly different from control (C) values and between groups). Cardiac output, measured immediately after bepridil, was unaltered, although in group 2 stroke volume index increased (14%) and systemic resistance decreased (16%), both $P<0.05$ vs C. In group 2, heart rate (HR) and contractility initially increased (8% and 10%, respectively, $P<0.05$ vs C), secondary to the greater fall in afterload, followed by a significant reduction at 5 and 10 min after bepridil (9% and 10%, respectively), accompanied by a 36% increase in LV enddiastolic pressure ($P<0.05$ vs C). No such changes were observed in group 1, apart from a simultaneous decrease in HR (9%, $P<0.05$ vs C). Thus, in humans, a dose-related, biphasic haemodynamic pattern is observed with intravenous bepridil, consisting of an acute, short-lasting vasodilation, followed by late negative chronotropic and inotropic effects, which, with longterm bepridil administration, may be beneficial during myocardial ischaemia.*

## Introduction

Bepridil hydrochloride is a new, long-acting compound with both antiischaemic and antiarrhythmic properties[1–10]. Although it is primarily regarded as a calcium-channel blocking agent[11], it also affects the fast sodium channel[12]. A competitive inhibition of calcium-dependent calmodulin has been described as well[13]. In tissue preparations and in the perfused heart model, it induces systemic and coronary arterial vasodilation[14] and has negative inotropic and chronotropic properties[11]. In the intact cardiovascular system, however, these direct effects of the drug may be attenuated or even counteracted by reflex mechanisms, the resulting haemodynamic profile predominantly depending on dose and rate of administration. Until recently only minor systemic and coronary haemodynamic effects of bepridil have been reported in humans, in a dose which caused significant electrophysiologic changes[15]. However, as measurements were performed only after bepridil administration, some of its direct haemodynamic properties may have remained unnoticed. In another 2 studies the systemic haemodynamic changes during intravenous administration of low dosages of bepridil in humans have been investigated[16,17]. The acute coronary and systemic cardiovascular properties of bepridil in dose regimens that result in antiischemic effects in humans have not been reported yet, apart from some preliminary communications[1,2,18]. The present study, therefore, was performed to compare

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the direct and secondary coronary and systemic haemodynamic effects of some of these dose regimens in patients with coronary artery disease in order to define the optimal dose in terms of safety and of haemodynamic changes, which may be relevant to its antiischaemic properties.

## Methods

### PATIENT POPULATION

Twenty-seven patients were studied, 23 men and 4 women, aged 32 to 62 years (mean 49), with stable exercise-induced angina pectoris and objective signs of myocardial ischaemia during stress testing. Only patients with >50% coronary diameter reductions, without any other form of heart disease, participated in the study. Patients with a recent infarction (less than 3 months old), hypertension, evidence of congestive heart failure (New York Heart Association functional class III and IV) or conduction disturbances were excluded. Beta-blocking agents, calcium blocking drugs and long-acting nitrates were withheld 48–72 hours prior to the study. Short-acting nitroglycerin was allowed until 12 hours before the investigation. None of the patients studied received other vasodilating drugs, digitalis or diuretics. After the nature of the investigation and of the compound and the extra time needed for the study were explained, all patients gave their consent on the day previous to the investigation.

*Catheterization procedures*

Catheterizations were carried out without premedication after an overnight fast. The study protocol was preceded by left and right coronary arteriography via the Seldinger technique and a routine atrial pacing stress test. Next, a no. 7 F thermodilution pacing catheter (Wilton Webster Laboratory) was advanced into the midportion of the coronary sinus via a branchial vein and a 7 F Millar micromanometer catheter positioned in the left ventricle via a Desilet introducer system in the femoral artery. The side-arm of this Desilet system was used to record aortic pressures. Finally, a no. 7 F Swan Ganz thermodilution catheter was advanced with its tip in a pulmonary artery and its proximal lumen in the right atrium via a femoral vein.

### MEASUREMENTS

After positioning, the catheters were calibrated using a zero reference level set at mid-chest. The micromanometer pressure was balanced to zero and calibrated with the fluid-filled system. Recordings of all pressures and of the first derivative of left ventricular (LV) pressure (LV dp/dt), were made on paper in the appropriate pressure ranges, using a CGR 1000 Cath Lab System. Cardiac output (CO) was measured in triplicate by the thermodilution technique. All pressures and pressure-derived contractility and relaxation indices (LV peak dp/dt positive and negative, dp/dt/P at 40 mmHg (VCE40) and Vmax total pressure) as well as cardiac output were determined on-line by a Mennen Cath Lab Computer System. Calculations were made from 12 consecutive beats. Coronary sinus blood flow (CSBF) was determined with the continuous infusion thermodilution method. Approximately 30–35 ml of glucose 5% at room temperature was infused over 30 seconds into the coronary sinus. During the infusion, both the mean and pulsatile flow were recorded simultaneously and measurements were made during mid-respiration after the mean flow curve had stabilized. The external thermistor was always well inside the coronary sinus ostium to prevent atrial reflux interfering with the flow measurements. This was confirmed by a bolus injection of saline in the right atrium. A coronary venous angiogram, using 3 ml of Renografin 76®, was recorded on a video disc to allow re-checking of the position of the catheter tip during the investigation.

### METABOLIC AND ELECTROCARDIOGRAPHIC MEASUREMENTS

Coronary venous and arterial $O_2$ saturation values were measured using an American Optical Oxymeter. Throughout the study, leads I, II and $V_5$ were continuously monitored for determination of heart rate and the PQ, QRS and QT interval. Measurements were performed at a paper speed of 100 mm $s^{-1}$. The $QT_c$ interval was determined according to Bazett's formula.

### CALCULATIONS

From the measured values, the following calculations were made: coronary vascular resistance by dividing mean arterial pressure by coronary sinus blood flow; myocardial $O_2$ consumption by multiplying the difference in arterial and coronary sinus $O_2$ content by coronary sinus blood flow; modified tension time index or double product, an index of myocardial $O_2$ demand, by multiplying heart rate by LV systolic pressure. Systemic vascular resistance was derived as 80 × (arterial mean pressure − atrial mean pressure)/cardiac output and pulmonary vascular resistance as 80 × (pulmonary mean

pressure − LV mean diastolic pressure)/cardiac output; stroke work index was expressed as stroke index (mean aortic pressure − LV end diastolic pressure) × 0.0136.

STUDY PROTOCOL

After instrumentation, a period of 30 min was allowed for stabilization. The studies were therefore initiated at least 60 min after angiography. Multiple control determinations of all variables were then made over a period of 20 min to ensure stable baseline values for all parameters. Next, 2 mg $kg^{-1}$ (group 1, 13 patients) or 4 mg $kg^{-1}$ (group 2, 14 patients) bepridil was administered over a period of 5 min. All variables were again determined at 2, 4, 6, 10, 15 and 30 min after the onset of the infusion, with the exception of cardiac output, which could not be measured during bepridil administration, since the proximal lumen of the Swan Ganz catheter was used for drug infusion. This route of administration was chosen as the long-term, infusion of bepridil in a peripheral vein may induce thrombophlebitis (unpublished observation).

DATA-ANALYSIS

Our approach to data analysis was twofold. Firstly, electrocardiographic and haemodynamic changes during and after bepridil infusion were expressed as percentages of baseline values.

A *t*-test for paired observations was used, as the objective of this analysis was to test differences from the baseline observations. A two-tailed *P*-value $< 0{\cdot}05$ was considered significant.

Secondly, baseline values and mean percent changes were compared between the two patient groups using a *t*-test for unpaired observations. All values are expressed as means ± 1 standard error of the mean (SEM).

## Results

General patient data and baseline angiographic findings are listed in Table 1. Although in the high-dose group, multivessel disease was more frequent than in group 1, baseline left ventricular angiographic data were not different.

ELECTROCARDIOGRAPHIC CHANGES DURING AND AFTER BEPRIDIL ADMINISTRATION

The baseline values of the QT and $QT_c$ interval were different between both groups. [397 ± 11 ms and 447 ± 10 ms, respectively (group 1) and 365 ± 10 ms and 408 ± 8 ms, respectively (group 2) ($P < 0{\cdot}05$, group 1 *vs* group 2)]. Both the values for the PQ and QRS interval were comparable. Immediately after the onset of bepridil administration a significant lengthening of the QT and $QT_c$ interval occurred in both groups compared with control, which lasted until the end of the investigation (Table 2). Changes were dose-related with a maximal lengthening of the $QT_c$ interval of 10% (group 1) and of 18% (group 2), 4 min after onset of bepridil administration. The percent changes were, however, only significantly different between both groups, at a later stage, at 5 and 10 min after the infusion.

*Table 1 General patient data and angiographic findings*

| | Group 1 Bepridil 2 mg $kg^{-1}$ $(5\ min)^{-1}$ | Group 2 Bepridil 4 mg $kg^{-1}$ $(5\ min)^{-1}$ |
|---|---|---|
| Patients (*N*) | 13 | 14 |
| Sex (M/F) | 9/4 | 14/0 |
| Age (yrs) mean | 47 ± 2·3 | 52 ± 2·4 |
| range | 36 ± 62 | 32 − 60 |
| Old Myocardial infarct | 5 | 6 |
| CAD: 1-vessel | 8 | 5 |
| 2-vessel | 1 | 5 |
| 3-vessel | 4 | 4 |
| LVEF (%) | 52 ± 4 | 57 ± 3 |
| LVEDV (ml $m^{-2}$) | 75 ± 7 | 69 ± 4 |
| LV wall motion abnormalities (*N*) | 12 | 13 |

CAD — coronary artery disease (> 50% diameter reduction)
LVEDV — left ventricular end diastolic volume index
LVEF — left ventricular ejection fraction

SYSTEMIC HAEMODYNAMIC CHANGES DURING AND AFTER BEPRIDIL

The systemic haemodynamic changes during and after bepridil administration are given in Table 3 and in Figs 1 and 2. All baseline values were comparable. In both groups, an immediate, but brief decrease in aortic pressures (Fig. 1) and in LV systolic pressure occurred, only observed during and 1 min after bepridil administration. These changes were dose-dependent and significantly different between the 2 groups. At the end of the infusion period a maximal reduction in LV systolic pressure of 11% (group 1) and of 18% (group 2) and in mean aortic pressure of 11% (group 1) and of 19% (group 2) were present (all values $P < 0{\cdot}05$ *vs* control). Systemic vascular resistance decreased only in the high-dose group from 1423 ± 116 dynes s

*Table 2 Electrocardiographic changes during and after bepridil administration*

| Variable | | Control | Minutes after onset of bepridil administration | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | During bepridil infusion | | After bepridil infusion | | | |
| | | | 2 min | 4 min | 6 min | 10 min | 15 min | 30 min |
| PQ-interval (ms) | Group 1 | 156±8 | 160±7 | 160±6 | 163±7 | 162±7 | 160±6 | 162±6 |
| | Group 2 | 164±6 | 167±6 | 171±6 | 171±6 | 174±6 | 170±6 | 169±7 |
| QRS-interval (ms) | Group 1 | 90±6 | 93±6 | 96±7 | 93±6 | 90±6 | 90±6 | 90±6 |
| | Group 2 | 76±5 | 72±7 | 74±4 | 74±4 | 79±3 | 76±7 | 73±7 |
| QT-interval (ms) | Group 1 | 397±11 | 399±11 | 418±15† | 429±11† | 435±11† | 436±11† | 422±14† |
| | Group 2 | 365±10‡ | 408±10† | 422±10† | 434±10† | 441±12*† | 436±11*† | 423±12† |
| $QT_c$-interval (ms) | Group 1 | 447±10 | 473±8† | 490±10† | 484±9† | 476±8† | 473±10† | 474±10† |
| | Group 2 | 408±8‡ | 477±6† | 483±7† | 478±8† | 470±10*† | 468±9*† | 462±8† |

*$P<0.05$ group 1 *vs* group 2 (percent change from baseline values); † $P<0.05$ *vs* control; ‡$P<0.05$ group 1 *vs* group 2 (baseline values).

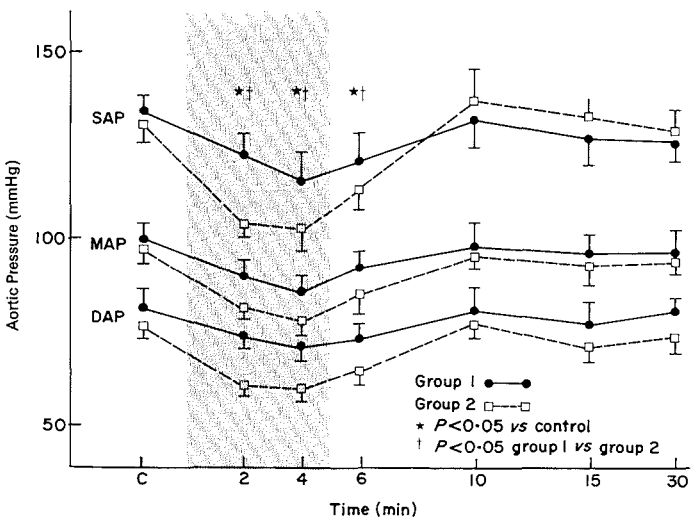


*Figure 1* Effect of bepridil, 2 mg $kg^{-1}$ (5 min)$^{-1}$ (13 patients, group 1) and 4 mg $kg^{-1}$ (5 min)$^{-1}$ (14 patients, group 2), on systolic, diastolic and mean aortic pressure (SAP, DAP and MAP, resp.). Values are mean±SEM.

$cm^{-5}$ (control) to 1196±103 dynes s $cm^{-5}$, 1 min after bepridil ($P<0.05$). No changes were observed in the low-dose group. Heart rate increased by 8% in the 4 mg $kg^{-1}$ group at the beginning of the infusion ($P<0.05$ *vs* control), however, it decreased gradually thereafter, with a significant 9% reduction 10 and 15 min after the onset of bepridil admiminstration. In the 2 mg $kg^{-1}$ group heart rate did not change during the infusion, but decreased by a similar percentage as in the high dose group, also at 10 and 15 min after onset of the infusion. No contractility changes occurred in the low dose group. In contrast, in the high-dose patients, a significant initial increase in contractility (10%) was observed, together with the increase in heart rate. This was followed by a longer lasting significant 10%

*Table 3 Systemic haemodynamic changes during and after bepridil administration*

| Variable | | Control | Minutes after onset of bepridil administration | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | During bepridil infusion | | After bepridil infusion | | | |
| | | | 2 min | 4 min | 6 min | 10 min | 15 min | 30 min |
| HR (beats $min^{-1}$) | Group 1 | 78±3·5 | 84±4 | 82±4 | 77±3 | 71±3* | 72±3* | 75±3 |
| | Group 2 | 76±4 | 83±3* | 79±3 | 74±3 | 70±3* | 70±3* | 73±2·5 |
| LVSP (mmHg) | Group 1 | 131±6 | 118±5* | 117±5·5* | 120±6* | 129±7 | 126±5·5 | 124±6 |
| | Group 2 | 132±4 | 112±3*† | 108±2·5*† | 116±3*† | 134±4 | 134±4 | 132±4 |
| LVEDP (mmHg) | Group 1 | 13±2 | 12±1·5 | 13±1·5 | 14±2 | 14±2 | 14±2 | 12±2 |
| | Group 2 | 11±1 | 11±1 | 14±1·5* | 15±2* | 15±2* | 14±2* | 11±2 |
| LV dp/dt pos (mmHg $s^{-1}$) | Group 1 | 1577±101 | 1709±172 | 1569±130 | 1543±123 | 1527±113 | 1477±97 | 1506±104 |
| | Group 2 | 1686±100 | 1654±92 | 1569±130 | 1529±106*† | 1529±95*† | 1563±104* | 1634±96 |
| VCE40 ($s^{-1}$) | Group 1 | 34±2·5 | 36±2·5 | 33±2 | 32±2 | 32±2 | 31±3 | 34±3 |
| | Group 2 | 34±1 | 36±1* | 33±1·5 | 31±2 | 30±1·5* | 31±1·5* | 32±1 |
| Vmax TP ($s^{-1}$) | Group 1 | 48±3 | 52±3 | 50±3 | 46±5 | 47±3 | 45±3 | 49±3·5 |
| | Group 2 | 50±1 | 55±2·5* | 51±2·5 | 48±2·5 | 45±2* | 46±2* | 48±2 |
| LV dp/dt neg (mgHg $s^{-1}$) | Group 1 | 1701±123 | 1746±150 | 1605±137 | 1617±131 | 1706±140 | 1653±129 | 1737±139 |
| | Group 2 | 1861±159 | 1589±163*† | 1508±148*† | 1483±107*† | 1685±150 | 1847±184 | 1782±165 |
| CO (l $min^{-1}$) | Group 1 | 6·1±0·5 | — | — | 5·8±0·4 | 5·5±0·3 | 5·7±0·35 | 5·9±0·5 |
| | Group 2 | 5·5±0·3 | — | — | 6·0±0·4 | 5·6±0·45 | 5·5±0·4 | 5·7±0·3 |
| SVI (ml $beat^{-1}$ $m^{-2}$) | Group 1 | 41±3 | — | — | 39±2 | 41±2 | 42±2·5 | 41±2 |
| | Group 2 | 39±2 | — | — | 43±2*† | 42±3 | 40±3 | 41±2 |
| SWI (g m $m^{-2}$) | Group 1 | 50±4 | — | — | 41±4 | 43±5 | 47±4 | 44±6 |
| | Group 2 | 46±3 | — | — | 41±4 | 54±6 | 50±6 | 47±3 |
| SVR (dynes s $cm^{-5}$) | Group 1 | 1281±108 | — | — | 1190±99 | 1395±149 | 1326±114 | 1315±112 |
| | Group 2 | 1423±116 | — | — | 1196±103*† | 1471±178 | 1444±168 | 1417±140 |
| PAS (mmHg) | Group 1 | 27±3 | 25±2 | 28±2·5 | 28±2 | 27±3 | 27±3 | 27±4 |
| | Group 2 | 24±2 | 27±2 | 28±2 | 29±2·5 | 29±3 | 27±2 | 26±1·5 |
| PAD (mmHg) | Group 1 | 12±2 | 12±1 | 13±1·5 | 13±1 | 13±1·5 | 13±1 | 13±2 |
| | Group 2 | 10±1 | 11±1 | 12±1 | 12±1 | 12±1 | 11±1 | 10±1 |
| PAM (mmHg) | Group 1 | 17±2 | 16±1 | 18±2 | 18±1·5 | 17±2 | 17±1 | 18±1 |
| | Group 2 | 15±1 | 17±1 | 18±1 | 19±1·5 | 17±2 | 18±1 | 17±1 |
| PVR (dynes s $cm^{-5}$) | Group 1 | 238±32 | — | — | 259±35 | 259±32 | 254±28 | 242±38 |
| | Group 2 | 216±15 | — | — | 257±22 | 262±35 | 260±26 | 242±14 |

CO — cardiac output; LVEDP — left ventricular end diastolic pressure; LVSP — left ventricular systolic pressure; HR — heart rate; PAD — pulmonary artery diastolic pressure; PAM — pulmonary artery mean pressure; PAS — pulmonary artery systolic pressure; PVR — pulmonary vascular resistance; SVI — stroke volume index; SVR — systemic vascular resistance; SWI — stroke work index. *$P<0·05$ *vs* control, †$P<0·05$ Group 1 *vs* Group 2 (percent change from baseline values).

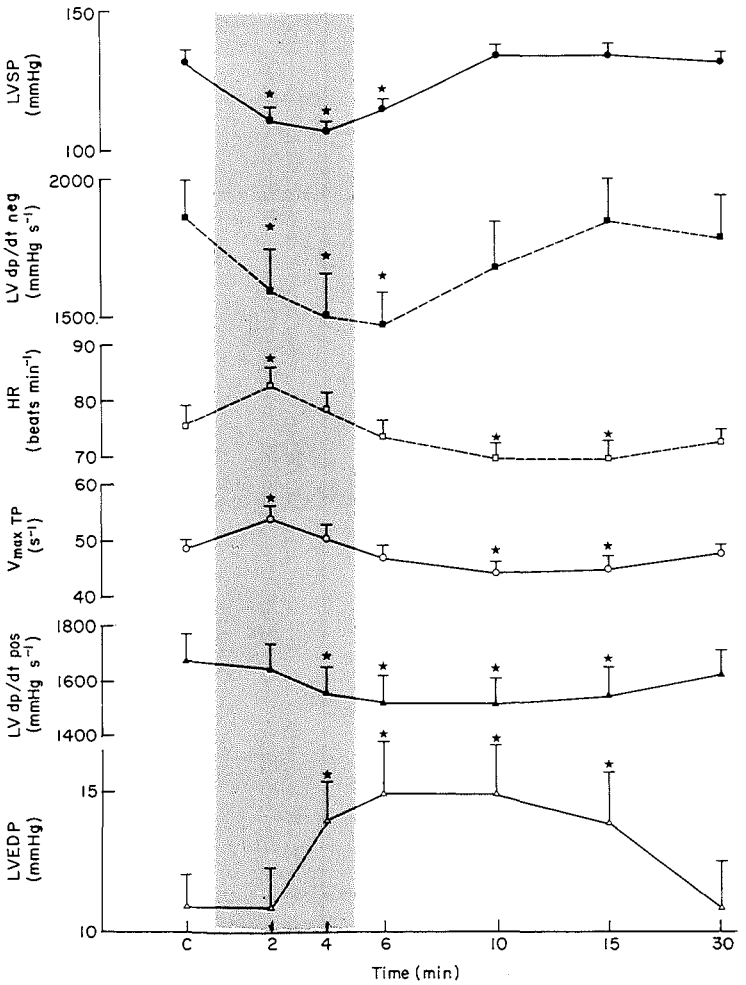


*Figure 2* Temporal relation between changes in heart rate (HR), contractility (LV dp/dt, $V_{max}$, LV P dp/dt negative and left ventricular end diastolic pressure (LVEDP) in the 4 mg kg$^{-1}$ group (see text). Values are mean ± SEM. ★$P<0.05$ *vs* control.

decrease after bepridil administration, whereas positive LV dp/dt was already reduced toward the end of the infusion period, from 1861 ± 159 mmHg s$^{-1}$ (control) to 1508 ± 148 mmHg s$^{-1}$, 4 min after onset of bepridil ($P<0.05$) (Fig. 2). At the same time, the peak negative value of LV dp/dt diminished by 20% in group 2 ($P<0.05$), but did not change in group 1. Concomitant with these reductions in contractility and, possibly, in relaxation, LV end diastolic pressure increased significantly by 36%, from 11 ± 1 mmHg (control) to 15 ± 2 mmHg (1 min after bepridil infusion), a rise in LV filling pressure, which lasted for 10 min after the infusion. In the 2 mg kg$^{-1}$ group, LV filling pressure did not change. Although cardiac output, which was first measured 1 min after the infusion, remained unaltered in both groups, stroke volume index increased by 14% in group 2, immediately after the infusion ($P<0.05$). Stroke work index, however, did not alter. Pulmonary artery pressures and pulmonary vascular resistance remained unchanged in both groups.

### CORONARY HAEMODYNAMIC CHANGES DURING AND AFTER BEPRIDIL ADMINISTRATION (TABLE IV)

Baseline coronary haemodynamic variables were comparable in both groups, except for the values of myocardial $O_2$ consumption and of the difference in

*Table 4 Coronary haemodynamic changes during and after bepridil administration*

| Variable | | Control | Minutes after onset of bepridil administration | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | During bepridil infusion | | After bepridil infusion | | | |
| | | | 2 min | 4 min | 6 min | 10 min | 15 min | 30 min |
| CSBF (ml $min^{-1}$) | Group 1 | 121±7 | 135±9 | 142±10† | 136±9 | 117±8 | 121±8 | 118±9 |
| | Group 2 | 113±8 | 164±15*† | 166±14*† | 137±14 | 133±15 | 115±14 | 121±14 |
| CVR (mmHg $ml^{-1}$ $min^{-1}$) | Group 1 | 0·83±0·07 | 0·69±0·05† | 0·65±0·06† | 0·76±0·07 | 0·91±0·10 | 0·83±0·08 | 0·90±0·09 |
| | Group 2 | 0·90±0·08 | 0·59±0·11*† | 0·53±0·07*† | 0·70±0·07*† | 0·89±0·12 | 0·98±0·11 | 0·91±0·12 |
| A-CS $O_2$ content (vol %) | Group 1 | 6·8±0·3 | 6·3±0·3 | 6·4±0·3 | 7·1±0·3 | 7·5±0·4 | 7·4±0·3 | 7·0±0·3 |
| | Group 2 | 9·1±0·7‡ | 6·9±0·93*† | 6·7±0·7*† | 8·2±0·9 | 9·2±0·6 | 9·2±0·7 | 9·6±0·8 |
| $MVO_2$ (ml $min^{-1}$) | Group 1 | 8·1±0·5 | 8·7±0·7 | 9·3±0·7 | 9·5±0·7 | 9·1±0·7 | 9·1±0·7 | 8·5±0·7 |
| | Group 2 | 10·2±0·8‡ | 10·5±1·1 | 10·4±1·2 | 10·5±1·2 | 11·7±1·4 | 10·4±1·2 | 11·0±1·3 |
| TTI | Group 1 | 10·2±0·7 | 9·9±0·7 | 9·7±0·8 | 9·2±0·6 | 9·2±0·8 | 9·1±0·6 | 9·3±0·6 |
| (HR × LVSP × $10^{-3}$) | Group 2 | 10·1±0·7 | 9·4±0·6 | 8·6±0·5† | 8·6±0·5† | 9·4±0·6 | 9·7±0·6 | 9·4±0·5 |

A — arterial; CS — coronary sinus; CSBF — coronary sinus blood flow; CVR — coronary vascular resistance; MVO2 = myocardial oxygen consumption; TTI — tension time index or double product: *$P<0·05$ group 1 *vs* group 2 (percent change from baseline values; †$P<0·05$ *vs* control; ‡$P<0·05$ group I *vs* group II (baseline values).

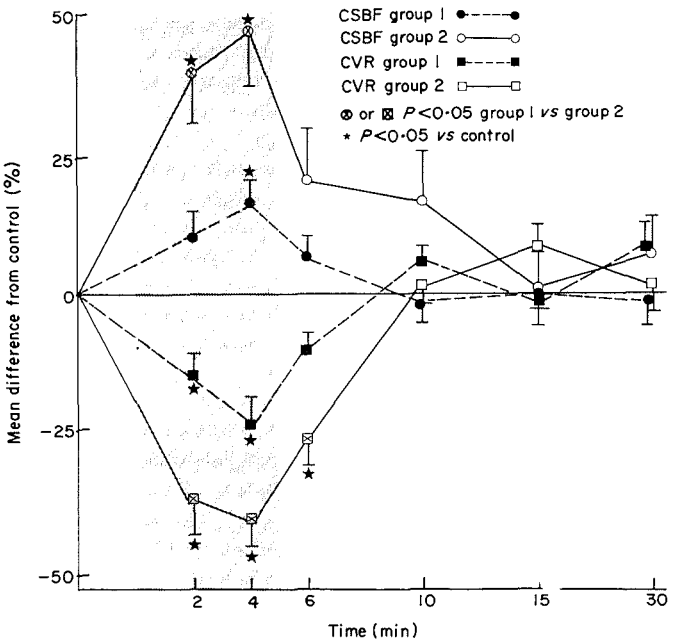


*Figure 3* Percent changes in coronary sinus flow (CSBF) and coronary vascular resistance (CVR) from baseline values in both groups during and after bepridil administration. Values are mean ± SEM.

arterial and coronary sinus $O_2$ content, which were higher in group 2 (26% and 34%, resp., $P<0{\cdot}05$ *vs* group 1). Bepridil induced coronary vasodilation, simultaneously with the systemic vascular changes. A dose-related reduction in coronary vascular resistance immediately followed the onset of bepridil administration with a maximal decrease of 23% in group 1 and of 41% in group 2 (both $P<0{\cdot}05$ *vs* control). These changes were brief. In the low-dose group they were only observed during the infusion-period, whereas in group 2 a significant reduction in coronary resistance was still present 1 min after bepridil administration. Changes in coronary flow were dose-related as well. A maximal increase of 47% occurred in the high-dose group, from $113 \pm 8$ ml min$^{-1}$ (control) to $166 \pm 14$ ml min$^{-1}$ 4 min after onset of bepridil ($P<0{\cdot}05$). In contrast, at the same time coronary flow only rose by 17% in group 1, from $121 \pm 7$ ml ml$^{-1}$ to $142 \pm 10$ ml min$^{-1}$; an increase which was just significantly different from the control value. The percent changes in coronary resistance and flow from their respective control values were significantly different between the 2 groups as well (Fig. 3). Although during the infusion period, the difference in arterial and coronary sinus $O_2$ content diminished by 26% in the 4 mg kg$^{-1}$ group ($P<0{\cdot}05$ *vs* control), myocardial oxygen consumption remained unchanged, despite a significant 15% reduction in tension time index, an index of myocardial oxygen demand. In the low-dose group no changes were observed in coronary sinus $O_2$ content, neither in myocardial $O_2$ consumption nor demand.

### ADVERSE EFFECTS

Bepridil was well tolerated. None of the patients developed symptomatic hypotension, bradycardia or clinical signs of heart failure. The only side-effect, which was observed in a few patients in groups 2, was a short period of a generalised feeling of warmth and of facial flushing during the infusion period.

## Discussion

The acute anti-ischaemic and antiarrhythmic properties of intravenous bepridil in humans have been reported by various investigators[1,2,6,8–10]. In these studies, bepridil was always administered as a bolus infusion of 2 or 3 mg kg$^{-1}$ over a few minutes. At a comparable dose and rate of administration, the compound has marked coronary and systemic

haemodynamic effects in animal experiments[19–21]. In humans, the immediate cardiovascular actions of these dose regimens have not been reported yet, apart from some preliminary communications[1,2,18]. In one early publication, the haemodynamic effects of bepridil were compared with other calcium-blocking agents[22]. However, as in this study all patients received concomitant beta-blocking therapy, the haemodynamic changes observed do not necessarily reflect the cardiovascular profile of bepridil. This investigation therefore is the first to examine the haemodynamic effects of intravenous bepridil in humans in dosages that should be relevant to its acute antiischemic properties.

### DOSE-RELATIONSHIP OF HAEMODYNAMIC AND ELECTROCARDIOGRAPHIC CHANGES

The hemodynamic changes during bepridil administration were clearly dose-related, both in magnitude and direction. During the low-dose administration only a moderate reduction in systemic and coronary resistance occurred with a small decrease in LV systolic and aortic pressures and a moderate increase in coronary flow. In contrast, when 4 mg $kg^{-1}$ was administered, a 47% increase in coronary flow and an equivalent reduction in coronary resistance was observed, despite a greater reduction in aortic diastolic pressure.

The inotropic changes during and after bepridil were clearly dose-related as well, whereas the chronotropic effects were not. Although an initial rise in heart rate occurred in the high-dose group, this, presumably, was a reflex mechanism secondary to the greater decrease in afterload and not a direct effect of bepridil. The concomitant initial increase in contractility also can be explained by a temporary increase in sympathetic tone. Although the reduction in afterload was maximal at the end of the infusion, both heart rate and contractility decreased already during the later stages of bepridil administration, indicating that the intrinsic myocardial effects of the drug were in operation at the time of the infusion. Judging by the reduction in the velocity parameters alone, a significant decrease in contractility occurred in the high-dose group, however, only at a later stage, 5 min after termination of bepridil administration, at a time that afterload had already normalized. When on the other hand the early decrease in positive LV dp/dt is taken into account as well, it may be argued that, besides changes in this parameter, caused by the decrease in afterload, a reduction in contractility was already present at the end of the infusion period, which then also explains the significant increase in LV filling pressure at the end of bepridil administration. This increase in LV filling pressure might be explained as well, though, by the reduction in negative LV dp/dt at this time, which indicates a decrease in relaxation. Changes in relaxation have been reported with nifedipine in humans[23]. However, as in our study the decrease in negative LV dp/dt coincided with the fall in LV systolic pressure (Fig. 2), it may also have been a pressure-dependent phenomenon, and not necessarily an indication of a decrease in relaxation alone.

The reductions in heart rate observed after bepridil administration were similar in both groups at a time that the peripheral vasodilating effects of bepridil were no longer present. The other electrophysiologic changes, however, e.g. the lengthening of the QT and $QT_c$ interval, were clearly dose-dependent. They also persisted throughout the entire investigation in both groups, in contrast with the short-term vascular and the intermediate inotropic effects of bepridil. These differences in duration of action between electrophysiological and haemodynamic effects reflect the complex cellular activity of bepridil. In the high-dose group, coronary venous $O_2$ content increased significantly during bepridil administration, whereas no changes occurred in the low-dose group. In animal experiments, both an increase in coronary venous $O_2$ content and a reduction in myocardial $O_2$ consumption has been described[20,21]. In our study, myocardial $O_2$ consumption did not decrease, however, despite a reduction in the double product, whereas contractility was unchanged at this point of the infusion. An explanation for the fact that bepridil did not reduce myocardial $O_2$ consumption may be the increase in LV enddiastolic pressure and therefore presumably, in diastolic wallstress.

### BIPHASIC HAEMODYNAMIC PATTERN OF BEPRIDIL

The haemodynamic actions of bepridil in this investigation can be divided into 2 phases: an immediate and short-lasting coronary and systemic vasodilation followed by late negative inotropic and chronotropic effects. This dual mode of action has been observed in animal experiments as well[19,20]. In these studies an immediate, shortlasting reduction in vascular resistance and a persistant bradycardia were reported, both with high and low doses of bepridil. Negative inotropic effects, however, only occurred at the higher dose level[19], which is in

agreement with our results. In the study by Marshall and Muir[20], a negative inotropic effect was not observed, though, despite the administration of 5 mg $kg^{-1}$ of bepridil. However, as in their study haemodynamic changes were only measured immediately and 20 min after bepridil administration, intermediate alterations in contractility may have remained unnoticed.

CLINICAL IMPLICATIONS

The minor haemodynamic effects observed with the 2 mg $kg^{-1}$ dose, indicate that with this dose regimen a substantial anti-ischemic effect is not to be expected. On the other hand, the negative inotropic changes and, particularly, the elevation of LV filling pressure, which occur with the 4 mg $kg^{-1}$ dose, may be hazardous when administered to patients with an already jeopardized myocardial function. It follows, that a 3 mg $kg^{-1}$ dose, preferably infused over 5 min, may be more suitable, provided that the bolus infusion is followed by long-term administration of bepridil, in order to maintain the vascular effects of the drug. Although the haemodynamic effects of such a dose regimen should be tested first before making definite statements about its clinical efficacy, it may be anticipated, that patients with unstable angina or an acute myocardial infarction will benefit from the antiischemic properties of bepridil during a similar dose regimen.

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## VIII.3.

# Acute Hemodynamic and Antiischemic Properties of Intravenous Bepridil in Coronary Artery Disease.

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# Acute Hemodynamic and Antiischemic Properties of Intravenous Bepridil in Coronary Artery Disease

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**The acute hemodynamic and antiischemic effects of intravenous bepridil (3 mg/kg/5 minutes followed by 1 mg/kg/hour) were studied in 19 patients with coronary artery disease under basal conditions and during 2 identical pacing stress tests 30 minutes before (pace test I) and 15 minutes after (pace test II) onset of infusion. Bepridil immediately decreased coronary and systemic vascular resistance (26 and 17%, respectively). This resulted in a 19 and 21% reduction in left ventricular systolic and mean aortic pressures and a 15% increase in coronary flow and stroke index ($p < 0.05$ vs control for each). These vasodilating effects were short lasting, persisting for 5 minutes after the bolus infusion, followed by significant reductions in heart rate (15%) and contractility (10%) and a temporary 46% increase in left ventricular filling pressure. During both pace tests heart rate, contractility, coronary flow and myocardial $O_2$ consumption were comparable. In contrast, bepridil prevented the significant increase in systemic resistance and mean aortic pressure observed during pace test I (11 and 15%, respectively). Subsequently, myocardial $O_2$ demand was significantly less during pacing after bepridil, due to an 11% reduction in left ventricular systolic pressure ($p < 0.05$ vs control and pacing test II vs I). This resulted in marked antiischemic effects: normalization of lactate extraction and reduction in ST-segment depression ($-14 \pm 7$ vs $3 \pm 6$% and $0.2 \pm 0.02$ vs $0.13 \pm 0.02$ mV, respectively, pace test I vs II, $p < 0.05$), and in less or no angina in 18 patients. Thus, intravenous bepridil significantly reduces acute myocardial ischemia during pacing-induced stress, predominantly through its systemic vasodilating effects and subsequent reduction in myocardial $O_2$ demand.**

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Bepridil hydrochloride is a novel compound with a complex pharmacologic profile, consisting of slow calcium- and fast sodium-channel blocking properties,[1-3] and a dose-related inhibitory effect on calcium-dependent vascular calmodulin.[4] These cellular properties provide for systemic and coronary vasodilatation together with negative inotropic and chronotropic activity in vitro,[1,5] which may indicate a potential toward antiischemic effects in vivo. In humans, bepridil given orally reduces exercise-induced ischemia[6-8] and compares favorably to other antianginal compounds.[9,10] Its antiischemic efficacy during intravenous administration has not yet been described. We investigated the potential antiischemic properties and underlying hemodynamic effects of bepridil administered in dosages, which, in a previous study, were identified as optimal in terms of hemodynamic activity and safety.[11]

## METHODS

**Patients:** After approval of the protocol by the Institutional Ethical Review Board and informed consent, 19 patients with exercise-induced angina or an old myocardial infarct and objective signs of ischemia during stress testing participated. There were 18 men and 1 woman, aged 38 to 68 years (mean 57). Patients with unstable angina, valvular heart disease, hypertension or heart failure were excluded. Cardiovascular therapy was withheld 48 to 72 hours before the investigation, but short-acting nitroglycerin was allowed until 6 hours before the study. To participate, a diameter reduction of ≥70% in at least 1 proximal branch of the left coronary artery had to be present. Catheterization was performed without premedication after an overnight fast. Individual clinical and catheterization data are listed in Table I.

**Catheterization procedures:** After routine coronary angiography, a 7Fr thermodilution pacing catheter (Webster Laboratories) was advanced from a brachial vein into the midportion of the coronary sinus, such that its position was stable, no interference by atrial reflux occurred and blood could be drawn quickly enough to allow for fast repetitive sampling. The catheter's position was identified radiographically by injecting 3-ml iopamidol and the data were stored on video disc to allow rechecking during the study. Next, a 7Fr thermodilution catheter was positioned with its tip in a pulmonary artery via a femoral vein and a 7Fr Millar micromanometer catheter advanced into the left ventricle

**TABLE I** Patient Characteristics

| Pt | Age (yrs), Sex | Previous MI | LVEF (%) | LVEDV (ml/m²) | Percent Diameter Reduction of Coronary Artery | | |
|---|---|---|---|---|---|---|---|
| | | | | | LAD | LC | Right |
| 1 | 49, M | 0 | 50 | 88 | 100 | | |
| 2 | 68, M | Inferior | 41 | 78 | | 90 | 90 |
| 3 | 62, M | 0 | 53 | 68 | 99 | 80 | 90 |
| 4 | 57, M | Anterior | 52 | 40 | 99 | 70 | |
| 5 | 47, M | 0 | 42 | 73 | 100 | 90 | 100 |
| 6 | 64, M | Anterior | 38 | 88 | 70 | 70 | |
| 7 | 59, M | Inferior | 43 | 66 | 70 | | 100 |
| 8 | 54, M | Anterior | 22 | 47 | 70 | | 90 |
| 9 | 54, M | Inferior | 38 | 60 | 90 | 90 | 99 |
| 10 | 59, M | Anterior | 40 | 70 | 50 | 100 | 100 |
| 11 | 61, M | 0 | 41 | 65 | 70 | 100 | 70+100 |
| 12 | 47, M | Anterior | 60 | 82 | 100 | 99 | 80 |
| 13 | 55, M | Inferior | 52 | 49 | 100 | | 100 |
| 14 | 66, M | Anterior | 46 | 96 | 99 | 70 | 100 |
| 15 | 66, M | Inferior | 51 | 59 | 99 | 99 | |
| 16 | 63, M | Anterior | 27 | 99 | 90 | 70 | |
| 17 | 60, M | Anterior | 53 | 59 | 100 | 70 | 100 |
| 18 | 38, M | 0 | 60 | 70 | 99 | 99 | |
| 19 | 57, F | 0 | 68 | 105 | 70 | | |
| Mean ± SEM | 58 ± 1.8 | | 47 ± 2.7 | 73 ± 4.5 | | | |

LAD = left anterior descending; LC = left circumflex; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; SEM = standard error of the mean.

from the right femoral artery via a Desilet introducer system. Arterial pressures were recorded through the side arm of this system.

**Hemodynamic, metabolic and electrocardiographic measurements:** All fluid-filled catheters were calibrated using a 0 reference level set at midchest. Right atrial, femoral and pulmonary artery pressures were measured using Bentley transducers. The micromanometer pressure was balanced to 0 and superimposed on the conventional pressure tracing. All pressures and left ventricular dP/dt were recorded at different paper speeds, e.g., 10, 25 and 100 mm/s, using a CGR 1000 Cathlab system. Cardiac output was measured in triplicate by the thermodilution technique. Throughout the study, pressures, pressure-derived contractility indexes and cardiac output were determined on-line by a Mennen Cathlab computer system. Coronary sinus flow was calculated by the method of Ganz et al.[12] Both pulsatile and mean coronary flow were measured during a continuous infusion of 30 to 35 ml of 5% glucose at room temperature over 30 seconds by motor-driven infusion pump. Electrocardiographic leads I, II and $V_5$ were continuously monitored for heart rate. ST-segment changes were measured in 5 consecutive beats, 0.06 second after the J point, at a paper speed of 100 mm/s using a calibrated magnifying lense. $O_2$ saturation levels were measured with an American Optical oximeter. The technique used for collecting blood samples for lactate and the sensitivity of its determination have been reported.[13]

**Calculated variables:** Derived hemodynamic variables (stroke index, stroke work, systemic, pulmonary and coronary resistances, myocardial $O_2$ consumption and the double product, an index of myocardial $O_2$ demand) were calculated using standard formulas.[14] Myocardial lactate extraction (%) was calculated as 100 × (arterial − coronary sinus/arterial lactate concentration).

**Study protocol:** Multiple control determinations of all variables were performed 20 and 40 minutes after instrumentation and coronary angiography, respectively, to ensure stable baseline values without residual effects of the contrast material. A first atrial pacing stress test (pace I) was then carried out with 10 beats/2 min increase in heart rate until moderate to severe angina, atrioventricular block or a maximum rate of 170 beats/min occurred. At fixed intervals (100, 120, 140 and 160 beats/min), during maximal pacing rates and at 15 seconds, 1, 2 and 5 minutes after pacing all variables were again determined. Thirty minutes were allowed for stabilization after pace I, followed by multiple control measurements. Next, bepridil, 3 mg/kg over 5 minutes, was administered, followed by a continuous infusion at a rate of 1 mg/kg/hour until the end of the study. Variables were reexamined 1, 3, 5, 10 and 15 minutes after onset of bepridil administration, followed immediately by a second pacing test (pace II), identical to pace I. Cardiac output was measured before and 5 minutes after each pacing test and before and 5 and 15 minutes after onset of bepridil administration. Patients received 0.5 mg atropin before each pace test to ensure reproducibility of pacing. Bepridil plasma levels were determined at 1, 3, 5, 10, 15, 25, 35 and 45 minutes after onset of drug infusion and during maximal pacing rates.

**Statistical analysis:** Values are expressed as mean ± 1 standard error of the mean. Each patient served as his own control. Measurements during bepridil infusion before pacing were compared with control values, using a *t* test for paired observations with 19 degrees of free-

**TABLE II** Systemic Hemodynamic Changes During Bepridil Administration

| | Control | During Bepridil Infusion Before Pacing | | | | |
|---|---|---|---|---|---|---|
| | | 1 minute | 3 minutes | 5 minutes | 10 minutes | 15 minutes |
| HR (beats/min) | 81 ± 2 | 86 ± 2* | 84 ± 3 | 79 ± 2 | 69 ± 2* | 72 ± 2* |
| LVSP (mm Hg) | 143 ± 6 | 128 ± 5* | 116 ± 3* | 119 ± 4* | 140 ± 5 | 143 ± 6 |
| MAP (mm Hg) | 108 ± 5 | 93 ± 4* | 85 ± 4* | 87 ± 4* | 102 ± 4* | 105 ± 6 |
| CO (liters/min) | 5.5 ± 0.3 | — | — | 5.8 ± 0.2 | — | 5.4 ± 0.3 |
| SVR (dynes · s · $cm^{-5}$) | 1,554 ± 140 | — | — | 1,284 ± 90* | — | 1,444 ± 112 |
| SI (ml/beats/$m^2$) | 35 ± 2 | — | — | 39 ± 2 | — | 41 ± 2* |
| SWI (g/m/$m^2$) | 46 ± 1.4 | — | — | 43 ± 2.1 | — | 46 ± 1.8 |
| LV peak dP/dt pos (mm Hg · $s^{-1}$) | 1,628 ± 71 | 1,571 ± 81 | 1,525 ± 59 | 1,470 ± 55* | 1,440 ± 57* | 1,477 ± 52* |
| Vmax ($s^{-1}$) | 48 ± 2 | 50 ± 3 | 51 ± 2 | 48 ± 2 | 43 ± 2* | 45 ± 2 |
| VCE 40 ($s^{-1}$) | 35 ± 2 | 35.5 ± 2 | 35 ± 2 | 32.5 ± 2 | 31 ± 2* | 31 ± 1* |
| LV peak dP/dt neg (mm Hg · $s^{-1}$) | 1,762 ± 69 | 1,674 ± 83 | 1,464 ± 67* | 1,511 ± 70* | 1,639 ± 65* | 1,668 ± 71 |
| LVEDP (mm Hg) | 10 ± 1 | 10 ± 1 | 13 ± 1* | 15 ± 2* | 14 ± 1* | 12 ± 1 |
| PAS (mm Hg) | 25 ± 2 | 25 ± 2 | 27 ± 2* | 29 ± 1.5* | 26 ± 1 | 28 ± 2 |
| PAD (mm Hg) | 11 ± 1 | 11 ± 1 | 12 ± 1 | 12 ± 1 | 11 ± 3 | 11 ± 5 |
| PVR (dynes · s · $cm^{-5}$) | 185 ± 76 | — | — | 146 ± 102 | — | 156 ± 82 |

* p <0.05 vs control.
CO = cardiac output; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean aortic pressure; PAD = diastolic pulmonary artery pressure; PAS = systolic pulmonary artery pressure; PVR = pulmonary vascular resistance; SI = stroke index; SVR = systemic vascular resistance; SWI = stroke work index.

**TABLE III** Coronary Hemodynamic Changes During Bepridil Administration

| | Control | During Bepridil Infusion Before Pacing | | | | |
|---|---|---|---|---|---|---|
| | | 1 minute | 3 minutes | 5 minutes | 10 minutes | 15 minutes |
| CSBF (ml/min) | 151 ± 8 | 165 ± 9 | 169 ± 11 | 173 ± 10* | 155 ± 9 | 153 ± 10.5 |
| CVR (mm Hg/ml/min) | 0.75 ± 0.06 | 0.6 ± 0.05* | 0.5 ± 0.04* | 0.5 ± 0.04* | 0.7 ± 0.07 | 0.7 ± 0.06 |
| Δ A-CS $O_2$ content (vol %) | 6.8 ± 0.25 | 5.7 ± 0.3* | 5.5 ± 0.3* | 5.7 ± 0.3* | 6.7 ± 0.3 | 7.1 ± 0.3 |
| $MVO_2$ (ml/min) | 10.4 ± 0.8 | 9.5 ± 0.9 | 9.55 ± 0.6 | 9.9 ± 1.0 | 10.45 ± 0.65 | 10.3 ± 0.8 |
| TTI (HR × LVSP × $10^{-3}$) | 11.5 ± 0.55 | 10.8 ± 0.5 | 9.8 ± 0.4* | 9.4 ± 1.0* | 9.8 ± 0.4* | 10.2 ± 0.5* |

* p <0.05 vs control.
A = arterial; CS = coronary sinus; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; HR = heart rate; LVSP = left ventricular systolic pressure; $MVO_2$ = myocardial oxygen consumption; TTI = modified tension-time index or double product.

**TABLE IV** Effects of Bepridil on Hemodynamics During Atrial Pacing

| | | Control | 100 beats/min | 120 beats/min | MAX | 1 min P-P | 2 min P-P | 5 min P-P |
|---|---|---|---|---|---|---|---|---|
| LVSP (mm Hg) | I | 148 ± 6 | 148 ± 6 | 143 ± 6 | 148 ± 6 | 158 ± 8 | 155 ± 6 | 153 ± 6 |
| | II | 143 ± 6 | 135 ± 5* | 129 ± 3* | 131 ± 6* | 138 ± 6* | 136 ± 4.5* | 140 ± 6* |
| TTI (× $10^{-3}$) | I | 10.2 ± 0.5 | 14.8 ± 0.6 | 17.4 ± 0.7 | 21.5 ± 0.7 | 12.9 ± 0.7 | 12.35 ± 0.7 | 12.6 ± 0.7 |
| | II | 10.2 ± 0.5 | 13.1 ± 0.4* | 14.3 ± 0.4* | 19.0 ± 0.7* | 12.5 ± 0.5 | 11.9 ± 0.5 | 12.2 ± 0.5 |
| Vmax ($s^{-1}$) | I | 45 ± 2 | 55 ± 2.2 | 59 ± 2.8 | 58 ± 3.3 | 49 ± 2.6 | 53 ± 2.5 | 51 ± 3.1 |
| | II | 45 ± 1.9 | 50 ± 2.2 | 53 ± 2.4 | 58 ± 2.5 | 52 ± 2.8 | 52 ± 2.3 | 51 ± 2.8 |
| CSBF (ml/min) | I | 144 ± 11 | 188 ± 17 | 210 ± 20 | 229 ± 16 | — | 193 ± 21 | 185 ± 16 |
| | II | 153 ± 10.5 | 166 ± 11 | 175 ± 10.5 | 220 ± 18 | — | 162 ± 12 | 161 ± 11 |
| CVR (mm Hg/ml/min) | I | 0.76 ± 0.05 | 0.65 ± 0.22 | 0.59 ± 0.23 | 0.56 ± 0.05 | — | 0.62 ± 0.22 | 0.66 ± 0.05 |
| | II | 0.71 ± 0.06 | 0.69 ± 0.25 | 0.65 ± 0.22 | 0.55 ± 0.05 | — | 0.69 ± 0.23 | 0.675 ± 0.05 |
| $MVO_2$ (ml/min) | I | 9.8 ± 1.0 | 12.7 ± 2.05 | 14.75 ± 1.8 | 15.7 ± 1.5 | — | 12.1 ± 1.4 | 12.8 ± 1.6 |
| | II | 10.3 ± 0.8 | 12.0 ± 1.2 | 13.3 ± 2.1 | 15.0 ± 1.5 | — | 10.3 ± 1.0 | 11.1 ± 0.9 |

* p <0.05, I (before bepridil) vs II (after bepridil).
CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; LVSP = left ventricular systolic pressure; I = first atrial pacing stress test; II = second atrial pacing stress test; MAX = maximal pacing rates; $MVO_2$ = myocardial oxygen consumption; P-P = after pacing; TTI = tension-time index.

dom, as the object of this analysis was to test differences with the baseline observation. A *t* test for paired observations was also used for measurements during both pacing tests before and during bepridil. A 2-tailed p value <0.05 was considered significant.

## RESULTS

**Systemic hemodynamic changes during bepridil administration before pacing:** Bepridil immediately reduced left ventricular systolic and mean aortic pressures (19 and 21% respectively, at 3 minutes of bepridil administration) (Table II). These changes were short lasting; they were only observed during the first 5 to 10 minutes of the infusion. Likewise, systemic vascular resistance also decreased (17% vs control, p <0.05), but only at 5 minutes of bepridil. Heart rate initially increased significantly but thereafter gradually decreased by 15% at 10 minutes of bepridil administration (p

<0.05 vs control). The velocity parameters decreased simultaneously with a significant 10% reduction at 10 and 15 minutes after onset of bepridil. In contrast, left ventricular dP/dt was already diminished at 5 minutes of bepridil administration, closely following the reduction in arterial pressures. This early change in contractility was accompanied by a significant 46% increase in left ventricular end-diastolic pressure. Cardiac output remained unaltered, but stroke index steadily improved with a significant 15% increase at 15 minutes.

**Coronary hemodynamic changes during bepridil administration before pacing:** Coronary blood flow increased immediately with a maximum increase of 15% after 5 minutes of bepridil, accompanied by a 26% reduction in coronary resistance (both $p < 0.05$ vs control) (Table III). These changes were short lasting, and only observed during the bolus infusion. Myocardial $O_2$ consumption did not change, despite a significant 19% reduction in the difference in arterial and coronary venous $O_2$ content during the bolus infusion and a persistent 15% reduction in the double product ($p < 0.05$ vs control).

**Effects of bepridil on hemodynamics during and after pacing:** Maximal heart rates were similar during both pacing tests (145 ± 4 and 146 ± 4 beats/min [pace I and II, respectively]) (Table IV). In contrast, throughout pace II, left ventricular systolic pressure and the double product were significantly less (11 and 12%, respectively) compared with pace I. Mean arterial pressure also was significantly less during pace II (106 ± 4 vs 120 ± 6 mm Hg [pace I] during maximal rates). Moreover, whereas systemic resistance increased during pace I from 1,394 ± 129 (control) to 1,553 ± 150 dynes · s · $cm^{-5}$ (5 minutes after pacing, $p < 0.05$), it tended to decrease during pacing with bepridil (1,444 ± 112 [control] vs 1,399 ± 111 dynes · s · $cm^{-5}$ [5 minutes after pacing]). Changes in contractility and in coronary flow and resistance were comparable during both pacing tests. Myocardial $O_2$ consumption was also similar despite the significant reduction in myocardial $O_2$ demand during pace II.

**Effects of bepridil on pacing-induced myocardial ischemia:** Bepridil clearly reduced myocardial ischemia, evidenced by normalization of lactate metabolism during pace II, compared with pace I (Figure 1). Furthermore, at 15 seconds after pacing, lactate extraction was −57 ± 16% during pace test I, compared with only −9 ± 9% during pacing with bepridil ($p < 0.05$). Moreover, during pace II there was less ST-segment depression than during pace I (−0.13 ± 0.02 vs −0.20 ± 0.02 mV, respectively, $p < 0.05$, Figure 2). Left ventricular end-diastolic pressure was significantly reduced (23 ± 2 [pace I] vs 15 ± 1 mm Hg [pace II], 15 seconds after pacing, Figure 2). Finally, whereas all patients had severe angina during pace I, 10 had no angina, 8 had less

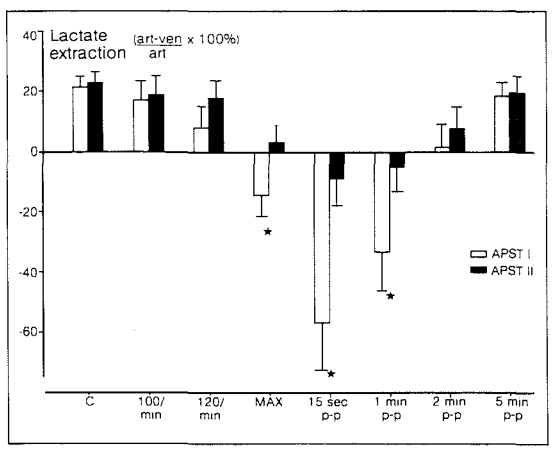


**FIGURE 1. Changes in lactate extraction during pacing before (APST I) and during bepridil administration (APST II). Lactate production, present during APST I at maximal pacing rates (MAX) and during the first minute after pacing (p-p), is significantly less during APST II. Art = arterial; ven = venous.**

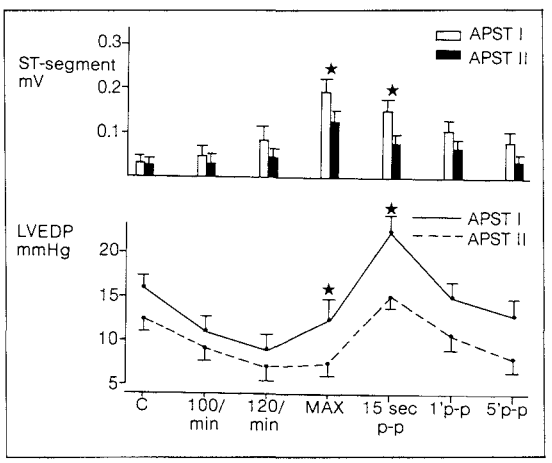


**FIGURE 2. Effect of pacing without and with bepridil (APST I and APST II, respectively) on ST-segment changes and left ventricular end-diastolic pressure (LVEDP). Both ST-segment depression and LVEDP are significantly reduced during maximal pacing rates (MAX) and 15 seconds after pacing (p-p). C = control.**

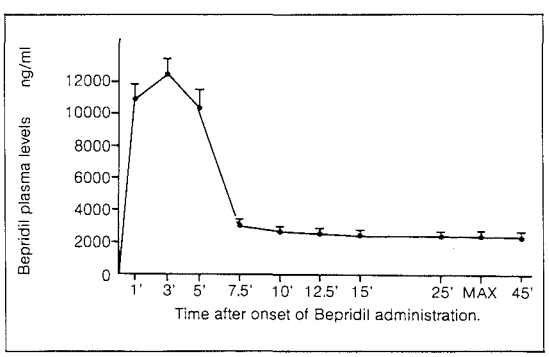


**FIGURE 3. Bepridil plasma levels during the bolus infusion, 3 mg/kg, given over the first 5 minutes and during the following maintenance infusion of 1 mg/kg/hour. MAX = maximal pacing rates; ' = minute.**

angina and only 1 patient had similar angina during pacing with bepridil.

**Plasma bepridil levels:** High plasma levels were observed immediately after onset of administration (Figure 3), with maximal values at 3 minutes (12,456 ± 920 ng/ml). Following the bolus infusion they decreased abruptly and subsequently stabilized at 7.5 minutes of bepridil, thereafter fluctuating around the 2,500-ng/ml level. As the average duration of pacing during both tests was 15.3 ± 0.6 minutes, bepridil plasma levels had been stable for approximately 20 to 23 minutes at the end of pace II.

**Adverse effects:** Bepridil was well tolerated. No side effects were reported. However, in patient no. 13, left ventricular end-diastolic pressure increased from 15 (control) to 38 mm Hg (5 minutes of bepridil) and in patient no. 10 lactate production was observed immediately after the bolus administration. Both instances were asymptomatic.

## DISCUSSION

Bepridil, a compound with predominant calcium-antagonistic properties, is known to reduce exercise-induced ischemia and to alleviate angina after oral administration.[6-10] The present study is the first to demonstrate that it also has important antiischemic properties during intravenous administration, predominantly due to a marked reduction in myocardial oxygen demand during pacing-induced stress. This antiischemic effect was achieved at dosages that were well tolerated and did not result in proarrhythmic or untoward hemodynamic effects, despite a reduced left ventricular function at baseline in most patients.

**Hemodynamic effects of bepridil before pacing:** Bepridil induced early but short lasting vasodilatation, which led to a reduction in systemic and coronary resistance, a decrease in left ventricular systolic and aortic pressures and, despite the simultaneous reduction in coronary perfusion pressure and myocardial $O_2$ demand, a significant increase in coronary flow.

We had previously observed that 5-minute bolus infusions of 2 and 4 mg/kg bepridil result in dose-related coronary and systemic arterial vasodilating effects, which only occur during the infusion period.[11] The present dosage scheme—a maintenance infusion in addition to the bolus administration—was designed to achieve sustained systemic and, particularly, coronary vasodilatation. This was obviously not successful, because in the present study vasodilatation only persisted for 10 minutes after onset of infusion. This early but short lasting vasodilatation was followed by negative inotropic and chronotropic effects, comparable to our previous results with a 4 mg/kg bolus infusion.[11] Certain differences do, however, exist. In the present study, the negative inotropic effects of bepridil were shorter lasting and the improvement in left ventricular pump function was more sustained. This indicates a more optimal balance between negative inotropic and vasodilator properties of bepridil with the present dosage scheme. This scheme also compares favorably with the dosages used in other studies with intravenous bepridil in humans.[15-17] In most of these studies, which all used a relatively slow infusion rate of 2 to 4 mg/kg over 15 minutes, a reduction in contractility, an increase in left ventricular filling pressure and either an unchanged or diminished left ventricular pump function was observed. In only 1 study did cardiac output increase transiently after the administration of 3 mg/kg over 15 minutes.[17]

**Hemodynamic and antiischemic effects during pacing:** Bepridil significantly reduced myocardial ischemia, indicated in particular by the reduction in myocardial lactate production during and immediately after pacing. Moreover, the significant reduction in angina, ST-segment changes and increase in left ventricular filling pressure during pace II also underline its antiischemic properties.

This antiischemic effect of bepridil is most likely due to a reduction in myocardial $O_2$ demand during pacing, comparable to the effects of other calcium antagonists in similar investigations.[18-20] Although bepridil initially increased coronary flow during the bolus infusion before pacing, these changes were no longer apparent during the subsequent pacing test. An increase in myocardial $O_2$ supply therefore appears less likely as a mechanism to explain its antiischemic effects. However, as the coronary sinus thermodilution technique only measures overall left ventricular function and not regional perfusion, this assumption cannot be verified. Theoretically, because bepridil reduces left ventricular diastolic pressure during pacing, this may have resulted in improved perfusion of the poststenotic subendocardial area.

During both pacing tests, heart rates were identical by design and contractility changes were similar. Thus, bepridil's predominant mode of action during pacing appears to be a decrease in myocardial oxygen demand, due to a reduction in left ventricular systolic pressure. Moreover, it prevented the increase in arterial pressure and systemic resistance observed during the first pace test. We recently reported systemic neurohumoral activation and subsequent vasoconstriction during pacing-induced ischemia.[21] It may be postulated that the latter is prevented by arterial vasodilators such as bepridil. Thus, intravenous bepridil significantly reduces ischemia and is well tolerated. Its potential usefulness in unstable angina and infarction should be further investigated.

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# VIII.4.

# Acute Antiischemic Properties of High Dosages of Intravenous Diltiazem in Humans in Relation to Its Coronary and Systemic Hemodynamic Effects.

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*European Heart Journal* (1987) **8**, 965–974

# Acute antiischaemic properties of high dosages of intravenous diltiazem in humans in relation to its coronary and systemic haemodynamic effects

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KEY WORDS: Intravenous diltiazem, haemodynamics, pacing-induced ischaemia.

*The antiischaemic properties of intravenous diltiazem in recommended therapeutic doses are disputed. In 17 patients with coronary artery disease the systemic and coronary haemodynamic effects of diltiazem were assessed during a high-dose infusion (0·4 mg kg$^{-1}$ per 5 min, followed by 0·4 mg kg$^{-1}$ per 10 min). In addition, its potential antiischaemic properties were investigated during identical pacing stress tests, 30 minutes before ($P_1$) and immediately after diltiazem administration ($P_2$). Diltiazem reduced left ventricular systolic pressure from 133±5 to 116±5 mmHg ($P<0·005$, $\bar{x}\pm SEM$), persisting until after $P_2$. It decreased systemic and coronary resistance by 32% ($P<0·001$) and 29% ($P<0·005$), respectively, with a sustained increase in cardiac output from 5·9±0·4 to 7·3±0·6 l min$^{-1}$ ($P<0·01$), but a brief 20% rise in coronary flow ($P<0·05$), after the bolus infusion only. Heart rate, contractility, left ventricular filling pressure and myocardial $O_2$ consumption remained unchanged. Despite high plasma levels (673±81 µg l$^{-1}$) diltiazem was well tolerated. During identical maximal pacing rates diltiazem considerably reduced myocardial $O_2$ demand (double product: 16·3±0·8 ($P_2$) vs 21·1±1·1 ($P_1$), $P<0·005$), due to an 18% decrease in left ventricular systolic pressure, resulting in diminished coronary flow and myocardial $O_2$ consumption during $P_2$ (14% and 15%, respectively, $P<0·05$ vs $P_1$). Diltiazem also significantly reduced pacing-induced ischaemia, indicated by normalization of myocardial lactate extraction (1±8% ($P_2$) vs −41±12% ($P_1$), $P<0·05$), and left ventricular filling pressure (13±2 ($P_2$) vs 27±3 mmHg ($P_1$), $P<0·01$), less ST-segment depression (0·12±0·01 ($P_2$) vs 0·24±0·02 mV ($P_1$), $P<0·01$) and improved contractility ($V_{max}$ 59±5 ($P_2$) vs 48±3 s$^{-1}$ ($P_1$), $P<0·05$). Angina was absent or less in 15 patients during pacing after diltiazem. Thus, diltiazem, in high dosages, induces continuing systemic but short lasting coronary vasodilation, improves pump function without negative chronotropic and inotropic effects and has pronounced antiischaemic properties, predominantly due to diminished myocardial $O_2$ demand.*

## Introduction

The antianginal efficacy of orally administered diltiazem has been reported in a multitude of studies[1–8]. In contrast, the potential antiischaemic effects of diltiazem, when given intravenously, are less well established. Moreover, the results of the few studies reported, in which the effect of intravenous diltiazem on either pacing- or exercise-induced stress was investigated, are conflicting with regard to antiischaemic efficacy and effect on coronary haemodynamics[9–12]. This may be explained by the difference in dosages used in these investigations, which ranged from 18 to 35 µg kg$^{-1}$ min$^{-1}$. In this dose-range coronary flow is not affected by diltiazem[9,11–16]. As the systemic, and particularly, the coronary effects of diltiazem are dose-dependent[13] and as, in humans, a dose-dependent increase in coronary flow has been demonstrated with other calcium-entry blocking agents as well[17,18], it may be anticipated that with higher dosages the antiischaemic properties of diltiazem become more manifest,

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predominantly because of improved myocardial oxygen supply. The present study therefore investigated the acute and systemic haemodynamic effects of relatively high dosages of intravenous diltiazem in patients with coronary artery disease, the safety of such a drug regimen, and the subsequent anti-ischaemic properties of the compound during pacing induced stress.

## Methods

### PATIENTS

The protocol was approved by the human studies committee of the Zuiderziekenhuis. Seventeen patients (16 men and 1 woman) were studied after informed consent was obtained. All patients had stable, exercise-induced angina pectoris and/or a documented old myocardial infarction, whereas 11 patients had objective signs of ischaemia during stress testing (Table 1). The mean age was 54 years (range 39 to 67). Thirteen patients had documented myocardial infarctions (9 anterior and 8 inferior). All cardiac medication was tailed off 48–72 hours before the study, apart from shortacting nitroglycerin which was allowed until 8 hours before the investigation. Only patients with a $\geqslant 70\%$ diameter reduction in one or more branches of the left coronary artery within the sampling area of the coronary sinus catheter participated. Nine patients had 1-vessel, three had 2-vessel and five had 3-vessel coronary artery disease. Overall left ventricular ejection fraction was slightly decreased ($47 \pm 4\%$), but left ventricular end diastolic volume index was normal ($79 \pm 8$ ml m$^{-2}$).

### CATHETERIZATION PROCEDURES

Catheterization was performed without premedication after an overnight fast using the Seldinger technique. After routine left and right coronary arteriography with non-ionic contrast medium (Iopamidol), a 7 Fr Wilton–Webster thermodilution pacing catheter was advanced from a brachial vein into the mid portion of the coronary sinus and positioned such that blood could be drawn quickly enough to allow a rapid sequence of blood samples to be collected. The stability of the catheter tip

*Table 1 Clinical and angiographic data*

| Patient | Age (years) Sex | Previous MI | Ergo | AP | LVEDV (ml m$^{-2}$) | LVEF (%) | Coronary angiogram (% luminal narrowing) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | LAD | LCX | RCA |
| 1 | 46 M | ant | 0 | + | 107 | 38 | 100 | 50 | 0 |
| 2 | 39 M | 0 | + | + | 46 | 51 | 80 | 90 | 0 |
| 3 | 60 F | 0 | + | + | 62 | 67 | 99 | 0 | 0 |
| 4 | 64 M | ant/inf | + | + | 147 | 34 | 100 | 100 | 80 |
| 5 | 58 M | 0 | + | + | 36 | 56 | 70 | 0 | 0 |
| 6 | 40 M | ant | 0 | 0 | 71 | 23 | 99 | 0 | 0 |
| 7 | 51 M | inf | 0 | + | 86 | 42 | 0 | 99 | 0 |
| 8 | 52 M | inf | + | + | — | — | 0 | 90 | 0 |
| 9 | 55 M | ant/inf | + | + | 125 | 25 | 100 | 99 | 99 |
| 10 | 63 M | inf | 0 | + | 61 | 51 | 70 | 70 | 70+100 |
| 11 | 55 M | inf | + | + | 80 | 47 | 90 | 0 | 100 |
| 12 | 62 M | ant/inf | + | + | 134 | 22 | 100 | 90 | 100 |
| 13 | 61 M | ant | + | + | 52 | 64 | 100 | 0 | 0 |
| 14 | 62 M | 0 | + | + | 62 | 50 | 100 | 100 | 0 |
| 15 | 47 M | ant | 0 | 0 | 70 | 54 | 100 | 0 | 0 |
| 16 | 67 M | ant | + | + | 72 | 68 | 90 | 0 | 50 |
| 17 | 44 M | ant/inf | 0 | + | 56 | 61 | 99 | 90 | 70 |
| Mean ± SEM | 54 ± 2·1 | | | | 79 ± 8·1 | 47 ± 3·8 | | | |

AP = exercise induced angina, ant = anterior, ergo = positive bicycle ergometry test; inf = inferior; LAD = left anterior descending artery; LCX = left circumflex artery; LVEDV = left ventricular end diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; RCA = right coronary artery; SEM = standard error of the mean; + = present; O = absent; — = no data.

was checked and the possibility of atrial reflux investigated by a bolus injection of saline solution into the right atrium. A coronary venous angiogram, using 4 ml of iopamidol was recorded on video disk to allow rechecking of the position of the catheter tip during the study. Next, a 7 Fr Millar micromanometer catheter or an 8 Fr Honeywell pigtail angiographic micromanometer catheter was positioned in the left ventricle via a Desilet system in the right femoral artery. The side-arm of this Desilet system was used to record arterial pressures. Finally, a 7 Fr Swan Ganz thermodilution catheter was advanced from a femoral vein with its tip in a pulmonary artery and its proximal lumen in the right atrium.

MEASUREMENTS

All fluid filled catheters were calibrated using a zero reference level set at midchest. The micromanometer pressures were balanced to zero and superimposed on the conventional pressure tracings. All pressures and the first derivative of left ventricular pressure (LV dp/dt) were recorded on paper in the appropriate pressure ranges at different paper speeds, e.g. 25, 100 and 250 mm $s^{-1}$, using a CGR 1000 Cath Lab system. Cardiac output was measured with the thermodilution method and recorded on paper at a speed of 10 mm $s^{-1}$. All pressures, pressure-derived indices of contractility (LV peak dp/dt positive, dp/dt/P at 40 mmHg (VCE 40), $V_{max}$ total pressure) as well as cardiac output were determined on-line by a Mennen Cath Lab Computer system and calculations made from 12 consecutive beats. The system allows for interaction with the computer before final calculations are made by showing representative tracings and measuring points before each determination. Coronary sinus blood flow was determined with the thermodilution techique during a continuous 30 second infusion of 30–35 ml of glucose 5% at room temperature into the coronary sinus[19]. Both the mean and pulsatile flow were recorded continuously. Calculations were made during mid-respiration after the mean flow curve had stabilized, towards the end of the infusion period. At the end of the investigation, the pressure curves from the femoral artery were compared with a simultaneous recording from the aortic root by the micromanometer catheter. Throughout the study, leads I, II and $V_5$ were continuously monitored and recorded at a paper speed of 100 mm $s^{-1}$ for the determination of heart rate and/or ST-segment changes. The ST-segment was measured in 5 consecutive beats, 0·08 s after the J point, using a calibrated magnifying lens.

METABOLIC MEASUREMENTS

One ml of blood was collected simultaneously from the left ventricle and coronary sinus for lactate assay, transferred directly into precooled tubes containing 1 ml of icecold 8% $HCLO_1$, mixed thoroughly and kept on ice during the study. After the investigation, lactate was determined in triplicate using an enzymatic method, described by Guttman and Whalefeld[20]. Standard deviation of the determination in our laboratory is 0·012 mmol $l^{-1}$. Venous and arterial $O_2$ saturation values are measured using an American Optical Oxymeter.

CALCULATIONS

Derived haemodynamic values (stroke volume index; stroke work index; systemic, pulmonary and coronary vascular resistance; myocardial $O_2$ consumption and the double product (DP), an index of myocardial $0_2$ demand) were calculated using standard formulas[21].

Myocardial lactate extraction (%) was calculated as

$$\frac{L_A - L_{CS}}{L_A} \times 100\%,$$

where $L_A$ and $L_{CS}$ are the arterial and coronary sinus lactate levels, respectively.

STUDY PROTOCOL

A stabilization period of 20 min was allowed after positioning of the catheters in order to reach a minimum interval of 40 min between the last coronary angiogram and the study. Multiple control determinations of all variables, except cardiac output, were then made to ensure stable baseline values, which were then followed by the first atrial pacing stress test (APST I). During pacing, heart rate was elevated with increments of 10 beats per 2 min until either angina pectoris was present to a similar degree as would discontinue physical exercise in normal life, atrioventricular block occurred, or a maximal pacing heart rate of 170 beats $min^{-1}$ was reached. All variables were re-evaluated at prefixed intervals during pacing, e.g. at 100, 120, 140, 160 beats $min^{-1}$, during maximal pacing heart rates, followed by measurements 15 s, 1, 2 and 5 min after pacing. The degree of angina during pacing was assessed using a scoring system ranging from 0

(no angina) to 10 (severe angina). After a 30 minute stabilization period after pacing, multiple control measurements were again determined. Diltiazem was thereupon administered intravenously by a motor driven infusion pump in a dose of 0·4 mg $kg^{-1}$ over 5 min, followed by 0·4 mg $kg^{-1}$ over 10 min. Repeat measurements of all variables were carried out 1, 3, 5, 10 and 15 min after the onset of the infusion. Cardiac output measurements were made at baseline before the infusion, followed by repeat determinations at 5 and 15 min after onset of diltiazem administration. Immediately after the infusion a second atrial pacing stress test (APST II) was carried out, identical to APST I. To ensure similar pacing heart rates during both pacing stress tests, all patients received 0·5 mg atropine before each APST. Blood sampling for diltiazem assay was carried out at control before diltiazem infusion, at 5 and 15 minutes after onset of diltiazem administration and in some patients at maximal pacing heart rates during APST II.

STATISTICAL ANALYSIS

Measurements during diltiazem administration were compared with baseline (control) values. A *t* test for paired observations with 16 degrees of freedom was used, as the object of this analysis was to test differences with the baseline observation. In a separate analysis a linear regression model was used to study the effect measures in relation to the plasma levels of diltiazem. A 2-tailed *P* value $<0·05$ was considered significant. The comparisons of measurements during atrial pacing before and after diltiazem were also made using a *t* test for paired observations, with a 2-tailed *P* value $<0·05$ indicating significant differences from zero. Data are presented as mean values $\pm 1$ standard error of the mean.

## Results

SYSTEMIC HAEMODYNAMIC CHANGES DURING DILTIAZEM INFUSION (TABLE 2)

Initially, no changes occurred. However, 3 min after the onset of diltiazem administration both left ventricular systolic pressure and aortic pressures decreased significantly. A reduction, which persisted throughout the infusion period with a maximal decrease in left ventricular systolic pressure of 13% at the end of the infusion period, from $133 \pm 5·5$ mmHg (control) to $116 \pm 5·5$ mmHg (15 min diltiazem infusion, $P<0·005$) and a similar maximal reduction in mean aortic pressure, 5 and 10 min after the onset of the diltiazem infusion

*Table 2 Systemic haemodynamic changes during diltiazem administration*

| Haemodynamic variables | baseline | Time after onset of diltiazem infusion (0·4 mg $kg^{-1}$ per 5 min, followed by 0·4 mg $kg^{-1}$ per 10 min) | | | | |
|---|---|---|---|---|---|---|
| | | 1 min | 3 min | 5 min | 10 min | 15 min |
| HR (beats/min) | 84±4 | 85±4 | 87±4 | 86±3 | 81±3 | 79±3 |
| LVSP (mmHg) | 133±5 | 130±6 | 121±6*† | 117±5** | 117±5** | 116±5** |
| LVEDP (mmHg) | 13±2 | 13±2 | 13±2 | 14±2 | 14,5±2 | 14±2 |
| MAP (mmHg) | 102±3 | 99±3 | 92±3·5** | 88±3** | 88±4** | 89±3** |
| CO (l/min) | 5·9±0·4 | — | — | 7·3±0·6*† | — | 6·8±0·4† |
| SVR (dynes s $cm^{-5}$) | 1343±104 | — | — | 944±85†† | — | 908±91†† |
| SI (ml/beat/$m^2$) | 37±3 | — | — | 45±3*† | — | 43±3† |
| SWI (g m $m^2$) | 45±4 | — | — | 44±4 | — | 44±4 |
| LV peak dp/dt pos (mmHg.$s^{-1}$) | 1487±86 | 1504±92 | 1523±107 | 1493±101 | 1489±106 | 1434±102 |
| $V_{max}(s^{-1})$ | 45±3 | 47±3 | 48±3 | 49±3 | 48±3 | 46±2 |
| PAS (mmHg) | 28±3 | 25±2 | 28±3 | 26±2 | 27±2 | 31±4 |
| PAD (mmHg) | 13±2 | 12±1 | 14±2 | 13±1 | 13±1 | 16±2 |
| PAM (mmHg) | 18±2 | 16±2 | 19±2 | 17±1 | 17±1 | 21±2·5 |
| PVR (dynes s $cm^{-5}$) | 166±32 | — | — | 149±31 | — | 148±28 |

*$P<0·05$; †$P<0·025$; *†$P<0·01$; **$P<0·005$; ††$P<0·001$ *vs* baseline. Values are mean ± SEM.
CO = cardiac output; HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean aortic pressure; min = minute; PAD = pulmonary artery diastolic pressure; PAM = pulmonary artery mean pressure; PAS = pulmonary artery systolic pressure; PVR = pulmonary vascular resistance; s = seconds; SI = stroke index; SVR = systemic vascular resistance; SWI = stroke work index.

from 102±3 mmHg (control) to 88±3 mmHg (5 min diltiazem, $P<0.005$). Systemic vascular resistance decreased from 1343±104 dynes s $cm^{-5}$ (control) to 944±85 dynes s $cm^{-5}$ at 5 min ($P<0.001$), and to 908±91 dynes s $cm^{-5}$ at 15 minutes after the onset of diltiazem infusion ($P<0.001$), a reduction of 32%. Cardiac output concomitantly increased by 24% from 5·9±0·41 $min^{-1}$ (control) to 7·3±0·61 $min^{-1}$ (5 min diltiazem, $P<0.001$) and was still elevated by 15% at the end of the infusion. Heart rate, contractility indices and left ventricular end diastolic pressure remained unaltered. Pulmonary artery pressures and pulmonary vascular resistance did not change either.

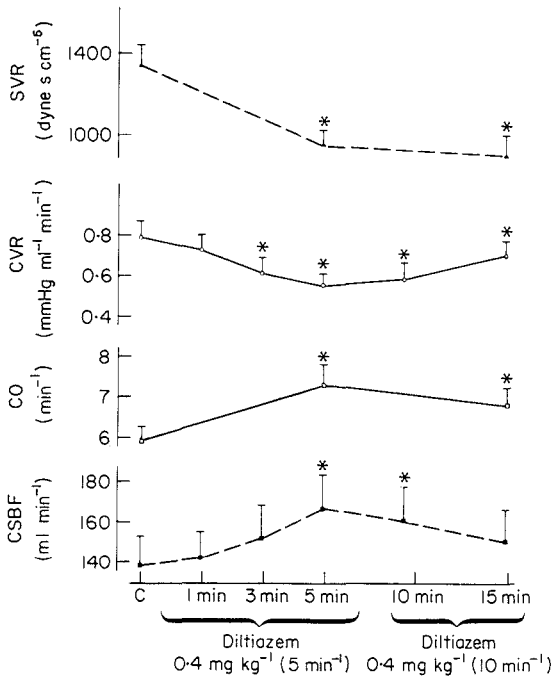


*Figure 1* Temporal relation between systemic and coronary vascular effects of diltiazem. At the end of the infusion the decrease in systemic vascular resistance persists, whereas changes in coronary vascular resistance have already returned towards baseline values. At this time cardiac output is still increased, in contrast to coronary flow. (C = control; CO = cardiac output; CSBF = coronary sinus flow; CVR = coronary vascular resistance; min = minute; SVR = systemic vascular resistance; *$P<0.05$ *vs* C).

CORONARY HAEMODYNAMIC CHANGES DURING DILTIAZEM ADMINISTRATION (TABLE 3)

A significant reduction in coronary vascular resistance was first observed 3 min after the beginning of the infusion with a maximal decrease of 29% at 5 min of diltiazem (0·56±0·05 mmHg $ml^{-1}$ $min^{-1}$ *vs* 0·79±0·08 mmHg $ml^{-1}$ $min^{-1}$ (control), $P<0.0025$). Although, in contrast to the persisting changes in systemic vascular resistance, coronary resistance increased thereafter (Fig. 1), it was still significantly reduced at the end of the infusion. Despite the reduction in aortic pressures, coronary sinus flow increased by 20%, from 138±15 ml $min^{-1}$ (control) to 166±17 ml $min^{-1}$ (5 min diltiazem), $P<0.05$. This increase in flow occurred late, however, and was short-lasting, being observed for only 5 and 10 min after the onset of diltiazem administration (Fig. 2). Myocardial oxygen extraction decreased significantly between 3 and 15 min of the infusion period with a maximal reduction of 21% at 5 min of diltiazem infusion [difference between arterial and coronary sinus $O_2$

*Table 3 Coronary haemodynamic changes during diltiazem administration*

| Haemodynamic variables | Baseline | Time after onset of diltiazem infusion (0·4 mg $kg^{-1}$ per 5 min, followed by 0·4 mg $kg^{-1}$ 10 min) | | | | |
|---|---|---|---|---|---|---|
| | | 1 min | 3 min | 5 min | 10 min | 15 min |
| CSBF (ml $min^{-1}$) | 138±15 | 142±3 | 152±16 | 166±17* | 160±17* | 151±15 |
| CVR (mmHg $ml^{-1}$ $min^{-1}$) | 0·79±0·08 | 0·73±0·07 | 0·62±0·07* | 0·56±0·05*† | 0·50±0·08** | 0·71±0·07* |
| △A−CS $O_2$ content (vol %) | 7·0±0·4 | 6·9±0·3 | 6·3±0·4** | 5·6±0·4** | 5·8±0·4** | 5·9±0·4** |
| $MVO_2$ (ml $min^{-1}$) | 9·8±1·1 | 9·8±1·0 | 9·7±1·1 | 8·8±0·9 | 9·2±0·8 | 8·9±0·9 |
| DP (HR × LVSP × $10^{-3}$) | 10·9±0·5 | 11±0·6 | 10·4±0·5 | 9·9±0·5* | 9·4±0·5* | 9·1±0·6* |

*$P<0.05$; *†$P<0.01$; **$P<0.005$ *vs*.baseline. Values are mean±SEM.
A = arterial; CS = coronary sinus; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; $MVO_2$ = myocardial oxygen consumption; DP = modified tension–time index or double product; other abbreviations as in Table 2.

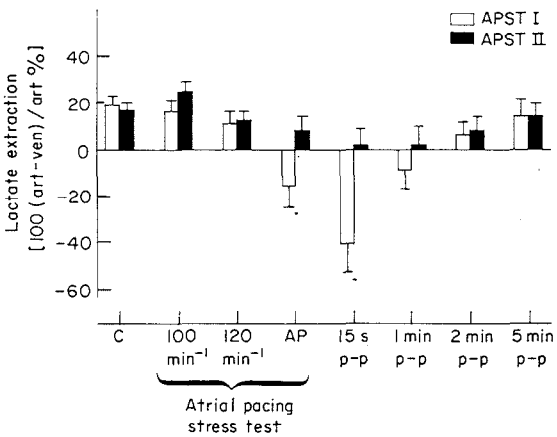


*Figure 2* Effect of diltiazem on myocardial lactate balance during pacing. During the first atrial pacing stress test (APST I) before diltiazem, lactate production is present during maximal pacing heart rates (AP) and the first minute after pacing. After diltiazem, during and after the second atrial pacing test (APST II) average lactate extraction values remain positive, indicating a significant reduction in ischaemia (art = arterial; C = control; p-p = post-pacing; ven = coronary venous $*P<0.05$ *vs* C).

content: $5.55\pm0.42$ vol % *vs* $7.0\pm0.45$ vol % (control), $P<0.005$]. Myocardial oxygen consumption did not change though, despite a 17% reduction in myocardial oxygen demand at the end of the infusion period [DP: $9.1\pm0.7$ at 15 min diltiazem *vs* $10.9\pm0.5$ (control), $P<0.05$].

EFFECTS OF DILTIAZEM ON CORONARY AND SYSTEMIC HAEMODYNAMICS DURING PACING (TABLE 4)

Maximal pacing heart rates were comparable during both pacing stress tests: $153\pm5$ beats $min^{-1}$ (APST I) and $150\pm5$ beats $min^{-1}$ (APST II). Myocardial oxygen demand (DP), however, was significantly diminished during and after APST II, because of a persisting 18% reduction in left ventricular systolic pressure during pacing after diltiazem ($P<0.01$ *vs* APST I). This resulted in a reduction in myocardial oxygen consumption ($11.1\pm1.4$ ml $min^{-1}$ (APST II) *vs* $14.7\pm1.6$ ml $min^{-1}$ (APST I), $P<0.005$) and less increase in coronary sinus flow ($187\pm16$ ml $min^{-1}$ (APST II) *vs* $217\pm19$ ml $min^{-1}$ (APST I), $P<0.05$) during maximal pacing heart rates. Contractility on the other hand, had improved during pacing after diltiazem. At the end of APST II $V_{max}$ was $59\pm4.9\ s^{-1}$ *vs* $48\pm3.2\ s^{-1}$ at the end of APST I ($P<0.05$).

EFFECTS OF DILTIAZEM ON PACING-INDUCED ISCHAEMIA

Myocardial lactate production, present during APST I, was absent during pacing after diltiazem. Whereas the arterial lactate levels did not change throughout the entire investigation and lactate extraction was normal during the control periods before both stress tests, coronary venous lactate levels rose significantly during APST I. This resulted in lactate production during maximal pacing heart rates ($-16\pm4\%$) and in the first minute after pacing ($-41\pm12\%$, 15 s post-pacing). In contrast, during APST II after diltiazem, the mean lactate extraction values remained positive (Fig. 2). Although some patients still produced lactate during the second pacing test, the individual values were significantly less than the corresponding values during the first test in all but one patient (Fig. 3). The antiischaemic properties of diltiazem

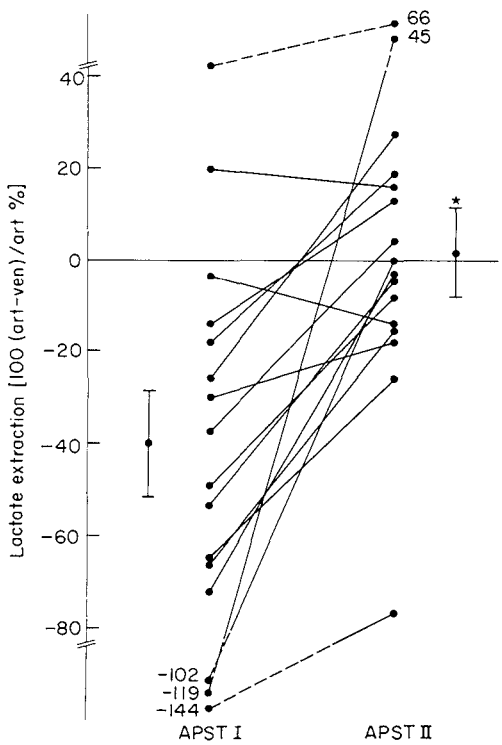


*Figure 3* Individual lactate extraction values 15 seconds after the control pacing stress test (APST I) and 15 seconds after pacing with diltiazem (APST II). There is a significant reduction of lactate production values or a return towards normal lactate extraction in all but one patient, who produced lactate during APST I (art = arterial; ven = coronary venous $*P<0.05$ APST I *vs* II).

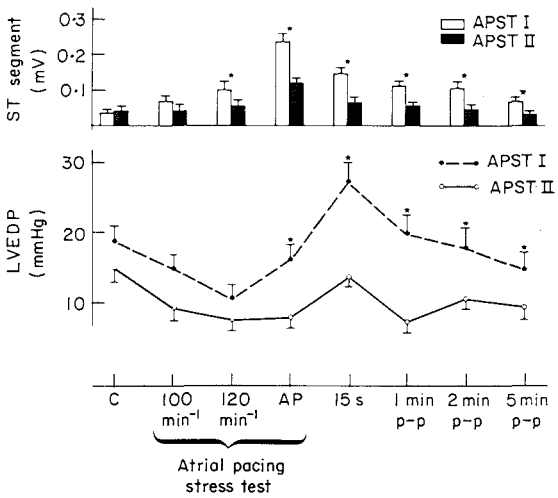


*Figure 4* Significant reduction by diltiazem of ST-segment changes and left ventricular end diastolic pressure (LVEDP) during the second pacing test (APST II) compared to the control test (APST I). These differences in ST-segment changes are already present at 120 beats $min^{-1}$ before angina and persist, as well as the difference in LVEDP, until 5 minutes post-pacing (p-p) (AP = maximal pacing heart rates; C = control; $*P<0.05$ APST I *vs* II).

were emphasized as well by a reduction in ST-segment depression during and after APST II [0·12 ± 0·01 mV *vs* 0·24 ± 0·02 mV (APST I), $P<0.05$] and by normalization of left ventricular end diastolic pressure immediately after pacing [13 ± 2 mmHg (APST II) *vs* 27 ± 2·8 mmHg (APST I), $P<0.01$ (Fig. 4)]. Moreover, whereas angina was present in all patients during APST I, it was absent in 7, less severe in 8 and similar in only 2 patients during pacing after diltiazem.

PLASMA DILTIAZEM LEVELS

Plasma diltiazem levels were high during the infusion period as would be expected. After the initial infusion rate, at 5 min the mean concentration was 673 ± 81 $\mu g\,l^{-1}$, declining to 445 ± 73 $\mu mg\,l^{-1}$ at the end of the infusion. There was no significant correlation between individual plasma levels and percent changes in haemodynamic parameters. In the last 5 patients to be studied, diltiazem plasma levels were also determined during maximal pacing heart rates during APST II, 13 ± 1·8 min after the end of the infusion period. The average diltiazem level at this time of the study had declined to 193 ± 23 $\mu g\,l^{-1}$.

*Table 4 Effects of diltiazem on coronary and systemic haemodynamics during pacing*

| Haemodynamic variables | baseline | During pacing | | | After pacing | |
|---|---|---|---|---|---|---|
| | | 100 beats $min^{-1}$ | 120 beats $min^{-1}$ | MAX | 1 min p-p | 5 min p-p |
| LVSP (mmHg) | | | | | | |
| APST I | 137 ± 6 | 132 ± 9 | 131 ± 5 | 136 ± 6 | 141 ± 6 | 136 ± 6 |
| APST II | 116 ± 5·5* | 117 ± 5* | 112 ± 4* | 111 ± 5* | 114 ± 6* | 117 ± 5* |
| DP ($\times 10^{-3}$) | | | | | | |
| APST I | 10·2 ± 0·5 | 13·0 ± 0·6 | 15·7 ± 0·5 | 21·1 ± 1·1 | 11·7 ± 0·9 | 11·6 ± 0·6 |
| APST II | 9·1 ± 0·7 | 11·6 ± 0·5*† | 13·4 ± 0·5*† | 16·3 ± 0·8** | 10·5 ± 0·6 | 10·5 ± 0·5 |
| LV dp/dt pos (mmHg $s^{-1}$) | | | | | | |
| APST I | 1466 ± 75 | 1542 ± 125 | 1693 ± 88 | 1849 ± 104 | 1602 ± 170 | 1534 ± 82 |
| APST II | 1434 ± 102 | 1540 ± 86 | 1547 ± 92 | 1704 ± 133 | 1527 ± 89 | 1508 ± 93 |
| $V_{max}$ ($s^{-1}$) | | | | | | |
| APST I | 42 ± 2 | 46 ± 2 | 51 ± 3 | 48 ± 3 | 44 ± 4 | 45 ± 3 |
| APST II | 46 ± 2·5 | 52 ± 3 | 54 ± 3 | 59 ± 5* | 52 ± 4 | 50 ± 3 |
| CSBF (ml $min^{-1}$) | | | | | | |
| APST I | 147 ± 15 | 148 ± 13 | 191 ± 24 | 217 ± 19 | 159 ± 15 | 147 ± 15 |
| APST II | 151 ± 15 | 135 ± 13 | 166 ± 17 | 187 ± 16* | 135 ± 13 | 146 ± 13 |
| CVR (mmHg $ml^{-1}$ $min^{-1}$) | | | | | | |
| APST I | 0·68 ± 0·06 | 0·70 ± 0·06 | 0·57 ± 0·06 | 0·58 ± 0·05 | 0·75 ± 0·07 | 0·73 ± 0·05 |
| APST II | 0·73 ± 0·07 | 0·72 ± 0·07 | 0·67 ± 0·07 | 0·59 ± 0·05 | 0·71 ± 0·07 | 0·66 ± 0·06 |
| $MVO_2$ (ml $min^{-1}$) | | | | | | |
| APST I | 10·9 ± 1·2 | 10·0 ± 1·6 | 12·8 ± 1·7 | 14·7 ± 1·6 | 10·3 ± 1·2 | 10·6 ± 1·3 |
| APST II | 8·9 ± 0·9 | 9·0 ± 1·3 | 11·1 ± 1·4 | 12·5 ± 1·1* | 9·2 ± 1·3 | 9·6 ± 1·1 |

$*P<0.05$; $*†P<0.01$; $**P<0.005$, APST I *vs* APST II. Values are mean + SEM.
MAX = maximal heart rates; APST = atrial pacing stress test; p-p = post-pacing; other abbreviations as in Tables 2 and 3.

ADVERSE EFFECTS

Diltiazem was well tolerated. Side-effects were not observed. In one patient (no. 9) left ventricular systolic pressure temporarily decreased from 103 mmHg to 78 mmHg halfway through the infusion, however, without signs of ischaemia.

## Discussion

ACUTE HAEMODYNAMIC EFFECTS OF DILTIAZEM

In this study, diltiazem, administered intravenously at relatively high dosages, induced pronounced systemic and coronary vasodilating effects, which resulted in a reduction in afterload, improvement of left ventricular pump function and an increase in coronary flow. Whereas with lower dosages, ranging from 18 to 35 $\mu g\,kg^{-1}\,min^{-1}$, an improvement in cardiac output is observed as well, coronary blood flow does not increase in humans[9,11]. Although coronary vascular resistance is significantly reduced with these lower dosages[11,12,16] this effect apparently is not sufficient to offset the impact of systemic vasodilation on coronary perfusion pressure in a situation where myocardial oxygen demand is reduced as well. Clearly, higher dosages of diltiazem, as used in the present study ($53\,\mu g\,kg^{-1}\,min^{-1}$) are needed to induce a significant rise in coronary flow. Apart from these quantitative differences, the effects of diltiazem on systemic versus coronary vascular beds differ in duration as well. Whereas, in the present study, coronary vascular changes already returned towards baseline values during the infusion period, the systemic vascular effects were definitely longer lasting. Systemic vascular resistance was still decreasing at the end of the infusion period, whereas the reduction in left ventricular systemic and aortic pressures persisted until at least 5 min after the second atrial pacing stress test. In contrast, the increase in coronary flow was shortlasting, only being observed halfway through the infusion period.

Despite the pronounced reduction in afterload, reflex positive chronotropic or inotropic effects, caused by baroreceptor-mediated sympathetic stimulation, did not occur. A secondary increase in heart rate is commonly found in patients with coronary artery disease during intravenous administration of calcium-entry blocking agents, particularly with the dihydropyridine derivatives nifedipine[22,23], nicardipine[24,25] and nisoldipine[26] as well as with bepridil[17,18]. In contrast, no such changes are reported with verapamil, presumably because of its pronounced negative chronotropic and inotropic effects, which offset the increase in sympathetic drive during afterload reduction[27,28]. In animal experiments the acute administration of diltiazem in dosages, which produce hypotension, does not result in changes in contractility and only leads to a modest increase in heart rate[29], whereas, in humans, diltiazem at lower dose levels has been reported to decrease heart rate[30]. The absence of a secondary increase in heart rate and contractility in our patients therefore seems best explained by the intrinsic negative chronotropic and inotropic properties of diltiazem.

EFFECTS ON HAEMODYNAMICS DURING PACING

The predominant haemodynamic effects of diltiazem during pacing consisted of a decrease in left ventricular systolic and aortic pressure, thereby reducing the double product and myocardial oxygen demand. Diltiazem did not result in an additional increase in coronary sinus flow during pacing. In contrast, coronary sinus flow was significantly reduced during pacing after diltiazem, compared with the first pacing test. Thus, it seems unlikely that the higher dosages used in the present study result in an improvement of myocardial oxygen supply to the ischaemic regions. This is an assumption that cannot be verified, however, as the thermodilution technique does not measure regional post-stenotic coronary flow. Theoretically, the reduction in coronary sinus flow, could have been the result of a reduction in oxygen demand in the normal myocardial areas alone. Moreover, even with an overall reduction in coronary flow, the decrease in left ventricular filling pressure and diastolic wall tension during pacing after diltiazem may in fact improve subendocardial post-stenotic flow. Even so, the significant reduction in myocardial oxygen demand in the present study is sufficient to explain the antiischaemic properties observed. A similar mechanism for the reduction in pacing-induced ischaemia has been reported for other calcium-entry blocking agents[31–34] and for other vasoactive drugs, such as amiodarone[35]. In these studies coronary flow did not increase during pacing after drug administration or was reported to decrease[32]. Only felodipine induced a greater increase in coronary flow during atrial pacing[36]. This suggests that, like diltiazem, these agents affect pacing-induced ischaemia predominantly by a reduction in myocardial oxygen demand, rather than an increase in oxygen supply.

EFFECT OF DILTIAZEM ON PACING-INDUCED ISCHAEMIA

The antiischaemic properties of diltiazem were clearly demonstrated by a reduction or absence of classic symptoms of ischaemia, e.g. angina, ST-segment changes and alterations in left ventricular filling pressure after pacing. More importantly, it was also demonstrated by normalization of lactate metabolism during pacing after diltiazem. Myocardial lactate production is reproducible, provided a minimum interval of 45 minutes between atrial pacing stress tests is adhered to[37]. The significant reduction in lactate production observed during the second atrial pacing stress test underlines the antiischaemic properties of diltiazem. Moreover, contractility, measured by the velocity parameters (VCE40, $V_{max}$), which are less load-dependent than conventional pressure-derived indices[38], actually increased during pacing after diltiazem. Together with the reduction in left ventricular filling pressure this suggests an improvement in left ventricular systolic function, alone or in combination with an increase in left ventricular compliance, due to the reduction of ischaemia.

PLASMA DILTIAZEM LEVELS

Despite the very high plasma diltiazem levels no untoward haemodynamic or clinical effects were observed. In one patient a drastic fall in blood pressure occurred. However, in this case diltiazem plasma levels were not unduly high. Conversely, in patients with markedly elevated plasma levels, haemodynamic changes were not different from the other patients. Thus, no correlation was present between diltiazem plasma levels and cardiovascular effects of the drug. Although in the present study a pronounced antiischaemic effect was observed, the limited number of diltiazem plasma levels during maximal pacing heart rates preclude any sensible correlation between therapeutic effects and plasma levels of diltiazem.

CLINICAL IMPLICATIONS

The present study indicates that diltiazem, known to have antianginal properties when given orally, also has pronounced antiischaemic properties during intravenous administration, provided that rather high dosages are given. As this dose regimen is well tolerated, even by patients with markedly diminished left ventricular function[39], it would appear that diltiazem in these dosages may be given safely to patients with acutely compromised ventricular function due to acute ischaemia and/or infarction. Although, at present, it is uncertain whether calcium-entry blocking agents can limit infarct size, it may be anticipated that the reduction in myocardial oxygen demand during diltiazem administration leads to a reduction of accompanying ischaemia. Moreover, diltiazem in these circumstances may improve ventricular pump function, both by a direct unloading effect on the ventricle as well as by its antiischaemic properties. The results of the present study therefore indicate that diltiazem, given intravenously, should benefit the acutely ischaemic patient and that further studies are warranted in patients with acute myocardial ischaemia and/or infarction to elucidate further its potential as an antiischaemic agent under these circumstances.

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# Chapter IX:

# PREDOMINANT EFFICACY OF ARTERIAL VASODILATORS IN VENTRICULAR DYSFUNCTION AS ASSESSED THROUGH MYOCARDIAL METABOLISM.

# IX.1.

# Hemodynamic Effects and Tolerability of Intravenous Amiodarone in Patients with Impaired Left Ventricular Function

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## SUMMARY

Acute hemodynamic effects and tolerability of intravenous amiodarone, 5 mg/kg administered over 5 minutes, were compared in patients with coronary artery disease and either a normal (left ventricular (LV) ejection fraction ≥45%, n=10, gr. N) or impaired LV function (ejection fraction <45%, n=9, gr. L).

Amiodarone acutely reduced systemic vascular resistance and LV and aortic pressures in both groups [13%, 18% and 13%, resp., (gr. N) and 15%, 17% and 15%, resp.(gr. L)]. Heart rate initially increased (18%, gr. L and 10%, gr. N), but followed by a late 6% decrease, in gr. N only, and by a progressive reduction in contractility (Vmax) together with a rise in LV end diastolic pressure [19 and 38%, resp. (N) and 17% and 58%, resp. (L)], all values $p<0.05$ vs control. Coronary flow increased significantly with 20% (gr. N) and 31% (gr. L), but only during amiodarone administration, accompanied by a 26% and 25% reduction in myocardial oxygen extraction in groups N and L resp. Stroke work decreased in both groups [20% (gr. N) and 19% (gr. L), $p<.05$ vs control]. In contrast, cardiac output only improved (10%) in patients with impaired ventricular function. Significant side effects did not occur. Thus, relatively high dosages of intravenous amiodarone are well tolerated and improve cardiac pump function in patients with an impaired, but not with a normal cardiac function. However, the tendency to increase LV end diastolic pressures cautions for careful monitoring in patients in whom preexisting LV filling pressure may be elevated.

## INTRODUCTION

Acute myocardial ischemia and/or infarction and subsequent cellular instability may result in serious ventricular dysrhythmias, requiring immediate antiarrhythmic therapy. As ventricular function frequently is decreased as a result of ischemia, contractility changes as a result of antiarrhythmic therapy[1] may be contraindicated in this situation. To achieve therapeutic drug levels quickly, relatively high dosages of the antiarrhythmic have to be administered intravenously. This may lead to a further deterioration of cardiac pump function as a result of dose-related negative inotropic properties. In this respect, it has been suggested that amiodarone, given orally, compares favourably to other antiarrhythmics, having little or no negative inotropic effects on the one hand and peripheral vasodilating properties on the other[1]. This may be different during intravenous administration. Animal studies uniformly indicate that, besides coronary and systemic effects and negative inotropic properties, intravenous amiodarone also results in a dose-dependent reduction in contractility and subsequent increase in left ventricular filling pressures[2-6]. Although the hemodynamic profile of intravenous amiodarone in humans may vary considerably, despite identical dosages[7-10], a clear reduction of contractility has been demonstrated in two studies in patients with a normal preexisting left ventricular function[10,11]. In contrast, although some studies suggest a predominant cardiodepressant effect in patients with ventricular dysfunction[12,13] the systemic and coronary hemodynamic effects of amiodarone are only incompletely evaluated in this patient group and not compared with those with a normal

ventricular function. The present study compares the hemodynamic effects and tolerability of a loading dose of intravenous amiodarone, which in earlier studies was reported effective against ventricular arrhythmias[14,15], in patients with diminished versus normal ventricular function.

## METHODS

### Patients

After informed consent, 19 patients, 18 men and 1 woman, mean age 53±1.4 yrs, range 42-67, entered into the study. To ensure a homogeneous study group, only patients with stable exercise-induced angina and objective signs of ischemia during exercise testing and/or a documented old myocardial infarction were studied. Patients with systemic hypo- or hypertension, a recent myocardial infarction (less than 3 months ago), conduction disturbances, bradycardia or signs of congestive heart failure were excluded. Cardiac therapy was tailed off 48-72 hours before the investigation with the exception of short-acting nitroglycerin, which was permitted up to 12 hours before the study. None of the patients had previously received antiarrhythmic therapy. To participate, significant coronary artery disease (≥70% diameter stenosis of one or more epicardial coronary arteries) had to be present. Based on the ventricular ejection fraction, angiographically determined, patients were separated into those with a normal left ventricular function (ejection fraction ≥45%, group N, n=10) and those with an impaired left ventricular function (ejection fraction <45%, group L, n=9). Clinical characteristics and angiographic data of patients in both groups are given in Table I. Groups were comparable with respect to sex, age and number of coronary artery lesions. By design, left ventricular ejection fraction was significantly different [57±2% (group N) versus 31±3% (group L)]. Likewise, left ventricular end diastolic volumes were different [64±6 ml/m$^2$ (group N) versus 95±9 ml/m$^2$ (group L), $p<0.05$] whereas the number of previous infarcts was greater in patients with diminished left ventricular function.

### Catheterization procedures and instrumentation

Studies were carried out during left and right heart catheterization in the morning, after an overnight fast and without premedication. First, left and right coronary angiography was performed using the Seldinger technique, to ensure eligibility for the study. Then, a nr. 7 Fr balloon-tipped triple lumen thermodilution catheter was positioned in the pulmonary artery via a Desilet system in the right femoral vein. Next a nr. 7 Fr Millar microtip manometer catheter was introduced into the left ventricle through a Desilet introducer system in the femoral artery, the side arm of which was used to record arterial pressures. Finally, a nr. 7 Fr thermodilution pacing catheter was inserted in the coronary sinus through a brachial vein positioned such that the proximal thermistor was at least 2 cm beyond the orifice of the coronary sinus and its position stable. The absence of atrial reflux was confirmed by a bolus injection of saline into the right atrium. The position of the catheters was subsequently recorded on video-disc to allow rechecking during the investigation.

### Measurements and calculations

After calibration of the fluid-filled catheters with a zero reference level set at midchest, the micromanometer pressure was balanced to zero and superimposed on the conventional pressure tracings. Pressure and flow signals and the first derivative of left ventricular pressures (LV dp/dt) were recorded on paper using a CGR 1000 cathlab system. Cardiac output was measured on-line by a Mennen cathlab computer system. Also, by way of this system, pressures and pressure-derived contractility indices were determined on-line from 12 to 15 consecutive beats. Coronary sinus blood flow was measured during a continuous 30 second infusion of 30 to 35 ml of glucose 5% at room temperature, using the method of Ganz et al[16]. Both the mean and phasic flow were recorded and calculations made from the mean flow curve. At the end of the study the pressure curves from the femoral artery were compared with a simultaneous recording from the aortic route by the micromanometer catheter, to compensate for any difference between proximal and distal arterial pressures. Arterial and coronary venous oxygen saturation values were measured using an American optical oxymeter.

### Hemodynamic measurements

Hemodynamic measurements included mean and phasic systemic arterial, pulmonary arterial and right atrial pressures (mmHg), left ventricular peak systolic and mean and end diastolic pressures (mmHg), pressure derived contractility indices [left ventricular dp/dt (mmHg.sec$^{-1}$) and Vmax total pressure (sec$^{-1}$)]. Thermodilution cardiac output was determined in triplicate. From the measured hemodynamic parameters the following

**Table I: Baseline clinical and angiographic characteristics.**

| | Gr. N (ejection fraction ≥45%) | Gr. L (ejection fraction <45%) |
|---|---|---|
| Sex | 9 male/1 female | 9 male |
| Age | 52±1.6(42-58 yrs) | 55±2.8 (43-67 yrs) |
| Previous myocardial infarction | 3 | 7 |
| Coronary angio: (≥70% diameter stenosis) | | |
| 1 - vessel | 2 | 2 |
| 2 - vessel | 5 | 5 |
| 3 - vessel | 3 | 2 |
| Left ventricular ejection fraction % | 57±2 | 31±3† |
| Left ventricular end diastolic volume (ml/$m^2$) | 64±6 | 95±9† |

† $p<0.05$ group L vs group N. Values are $\bar{x} \pm$ SEM

variables were calculated according to previously described formulas[17]: coronary vascular resistance (CVR, mmHg/ml/min), systemic vascular resistance (SVR, dynes.sec.$cm^{-5}$), stroke index (SI, ml/beat/$m^2$), stroke work index (SWI, g/m/$m^2$), myocardial oxygen extraction ($\Delta$A-$CSO_2$, vol %), myocardial oxygen consumption ($MVO_2$, ml/min).

### Studyprotocol

Approximately 30 to 45 minutes after the last coronary angiogram and 15 to 20 minutes after instrumentation, multiple control measurements of all hemodynamic variables were carried out to ensure stable baseline values. Thereafter, amiodarone was infused, 5 mg/kg over 5 minutes. Repeated measurements of all variables were performed at 1, 3, 5 and 10 minutes after onset of amiodarone administration. Due to the rapid sequence of measurements, cardiac output determinations were only carried out at 7.5 minutes after the beginning of drug infusion.

### Statistical analysis

In each group, measurements made during amiodarone administration were compared with baseline values before administration, using a t test for paired observations. A two tailed p value <.05 was considered significant. Differences in hemodynamic values between groups were compared using a t test for unpaired observations with a two tailed p value <.05 considered significant.

## RESULTS

### Baseline hemodynamic values.

At baseline, hemodynamic variables were comparable between the two groups, except for contractility parameters which were significantly lower in group L.

### Systemic hemodynamic effects of amiodarone in normal versus depressed ventricular function (Table II)

Immediately following the onset of amiodarone infusion, left ventricular systolic and mean aortic pressures decreased with 18% and 13% resp. in group N and with 17% and 15% resp. in group L. This reduction in pressures was of short duration. Already after 5 minutes following amiodarone

**Table II: Systemic hemodynamic changes during amiodarone administration.**

| | | | After onset of amiodarone infusion | | | |
|---|---|---|---|---|---|---|
| | | control | 1 min | 3 min | 5 min | 10 min |
| HR (beats/min) | group N | 83±5.8 | 89.5±6.7 | 92±5.9* | 91±5.9* | 78±5.5* |
| | group L | 78±4.1 | 82±4.3 | 92±4.4* | 87±4.8* | 81±3.0 |
| LVSP (mmHg) | group N | 139±5.1 | 125±7.0 | 114±3.2* | 119±3.7* | 127±4.2 |
| | group L | 136±1.4 | 127±9.3 | 113±8.0* | 121±9.9* | 137±9.9 |
| MAP (mmHg) | group N | 105.5±4.0 | 99.0±2.4 | 92±3.3* | 95±3.2* | 105±2.3 |
| | group L | 97±5.5 | 88±3.5*† | 83±2.9* | 88±4.8* | 91±5.2† |
| SVR ($dynes.s.cm^{-5}$) | group N | 1780±104 | | | 1551±951* | |
| | group L | 1730±108 | | | 1469±1191* | |
| CO (l/min) | group N | 4.9±0.4 | | | 5.1±0.31 | |
| | group L | 4.5±0.2 | | | 4.9±0.31* | |
| SVI ($ml/beat/m^2$) | group N | 31.3±2.6 | | | 29.7±1.41 | |
| | group L | 31.2±2.2 | | | 30.5±2.71 | |
| SWI ($g.m.m^2$) | group N | 41.1±4.0 | | | 32.6±1.61* | |
| | group L | 35.7±2.7 | | | 29.1±2.61* | |
| LV peak dP/dt pos ($mmHg.sec^{-1}$) | group N | 1867±142 | 1838±157 | 1832±211 | 1643±162* | 1500±126* |
| | group L | 1492±114 | 1450±127 | 1521±170 | 1361±111* | 1272±86* |
| Vmax ($sec^{-1}$) | group N | 56.4±4.0 | 54.5±3.7 | 58.9±6.6 | 50.4±4.1* | 45.6±4* |
| | group L | 42.1±1.6† | 42.4±1.9† | 45.4±3.4 | 40.8±2.5* | 34.7±1.1*† |
| VCE40 ($sec^{-1}$) | group N | 38.4±3.1 | 39.1±3.3 | 39.1±4.8 | 33.4±3.2* | 30.3±2.9* |
| | group L | 28.3±1.4† | 28.4±1.8† | 30.4±2.7† | 26.7±1.9*† | 23.3±0.7*† |
| LVEDP (mmHg) | group N | 10±1.7 | 10±1.6 | 11±1.7 | 12±2.1 | 13±2.3* |
| | group L | 12±1.5 | 10±1.5* | 13±2.0 | 11±2.3* | 19±2.7* |
| PAM (mmHg) | group N | 16.5±0.9 | 18±1.1 | 21±1.4* | 21±1.7* | 21±1.3* |
| | group L | 18±1.0 | 18±1.7 | 21±1.3* | 22±1.0* | 23±1.1* |
| PVR ($dynes.\ s.cm^{-5}$) | group N | 113±27 | | | 137±77 | |
| | group L | 111±35 | | | 80±46 | |

*Abbreviations: CO = cardiac output; HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean aortic pressure; min = minute(s); PAM = mean pulmonary artery pressure; PAS = systolic pulmonary artery pressure; PVR = pulmonary vascular resistance; SI = stroke index; SVI = stroke volume index; SVR = systemic vascular resistance; SWI = stroke work index. All values means ± standard error of the mean.*
** $p<.05$ vs control † $p<.05$ group L vs group N*

administration, pressures had returned to control values in both groups (Fig. 1). Amiodarone equally reduced systemic vascular resistance in both patient groups, 13% in group N and 15% in group L. Cardiac output remained unchanged in patients with a normal baseline left ventricular function. In contrast, in patients with a depressed left ventricular function, a significant 9% increase in cardiac output was observed (Fig. 1). However, stroke volume did not change, due to a significant 18% rise in heart rate, which started early after onset of drug administration and persisted until the end of the infusion (Fig. 2). In group N, heart rate also increased, but to a lesser extent (11% versus control, $p<0.05$), followed by a late reduction, 5 minutes after amiodarone. Left ventricular stroke work decreased with 21% in group N and with 18% in group L (both $p<0.05$ versus control).

Amiodarone significantly reduced contractility in both groups, which started already during the infusion, despite the concomitant increase in heart rates. Changes in left ventricular dp/dt and $V_{max}$ were progressive, with a maximal reduction of 20% and 19% in group N and of 15% and 18% in group L., 5 min after the end of amiodarone administration (Fig. 2). In group L, left ventricular end diastolic pressure initially decreased with 15%. However, subsequently it increased in both

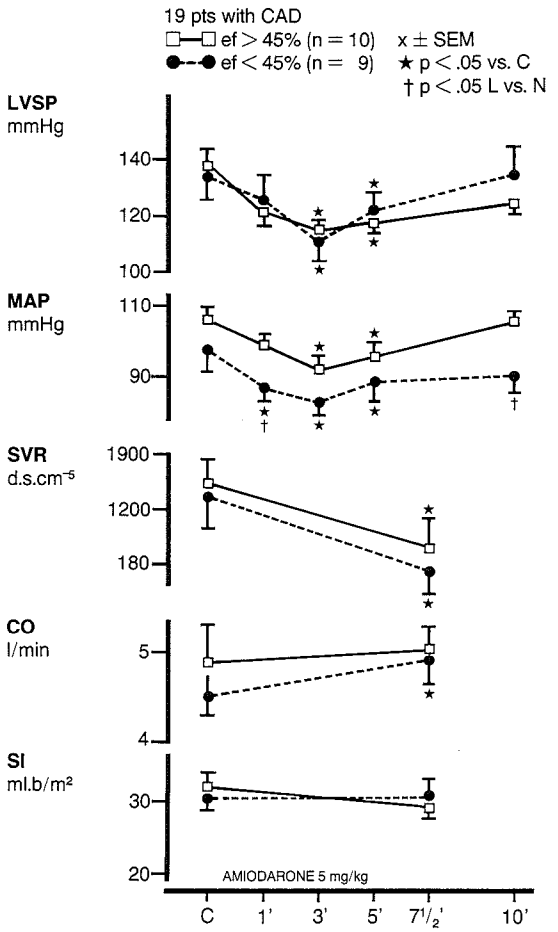


*Fig. 1: Acute, but shortlasting effects of intravenous amiodarone on systemic vascular resistance (SVR), left ventricular systolic pressure (LVSP) and mean aortic pressure (MAP), comparable in both groups.*
*Amiodarone improved cardiac output (CO) in patients with impaired ventricular function at base level (group L, closed symbols), but not in patients with a normal ventricular function (group N, open symbols).*
*SI = stroke index; ef = ejection fraction.*

groups, parallel to the changes in contractility, from 10 mmHg (control) to 13 mmHg (5 min after amiodarone) in group N and from 12 mmHg (control) to 19 mmHg (5 min after amiodarone) in group L (Fig. 2).

**Coronary hemodynamic effects of amiodarone in patients with normal versus diminished ventricular function (Table III)**

A significant but similar reduction in coronary vascular resistance was observed in both groups, again only during amiodarone infusion, with a

*Fig. 2: Effects of amiodarone on heart rate (HR), contractility indices (LV dp/dt, Vmax) and left ventricular end diastolic pressure (LVEDP). Amiodarone induced significant 11% and 18% increases in heart rate early after drug administration in groups N (open symbols) and L (closed symbols) resp., followed by a late reduction in patients with normal ventricular function at baseline. Progressive, but similar reductions in contractility are observed in both groups, accompanied by a late 30% and 55% increase in LVEDP in groups N (open symbols) and L (closed symbols) resp. ef = ejection fraction.*

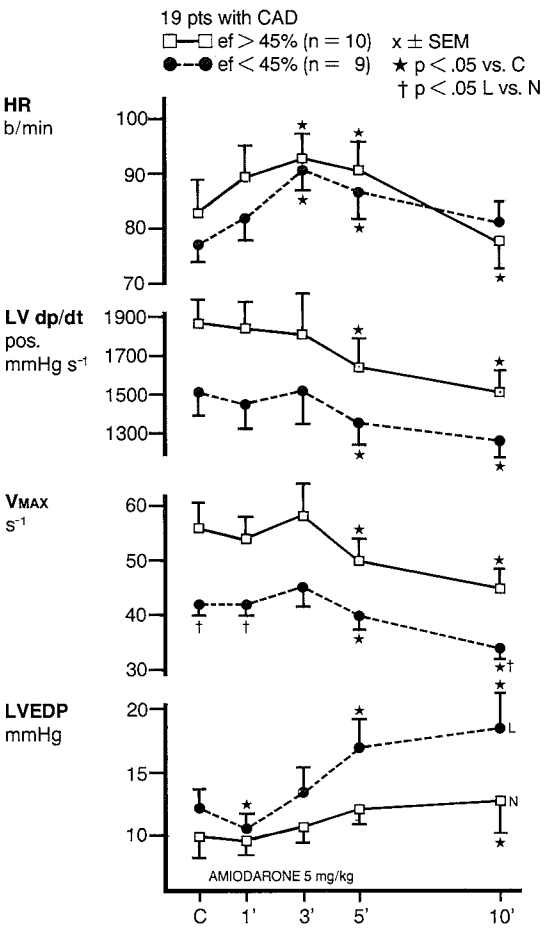

**Table III: Coronary hemodynamic changes during amiodarone administration.**

| | | | After onset of amiodarone infusion | | | |
|---|---|---|---|---|---|---|
| | | control | 1 min | 3 min | 5 min | 10 min |
| CSBF (ml/min) | group N | 135±8.7 | 151±12.6* | 162±15.0* | 149±9.3 | 136±9.9 |
| | group L | 134±19.6 | 162±21.6* | 167±21.3* | 176±27.7* | 157±15.5 |
| CVR (mmHg/ml/min) | group N | 0.79±0.06 | 0.69±0.05 | 0.60±0.05* | 0.65±0.03* | 0.79±0.05 |
| | group L | 0.83±0.10 | 0.60±0.07* | 0.58±0.09* | 0.60±0.09* | 0.67±0.08 |
| $\Delta$A-$CSO_2$ content (vol%) | group N | 12.0±0.7 | 11.0±0.7* | 8.9±0.8* | 9.1±0.8* | 11.3±0.8 |
| | group L | 11.8±0.7 | 10.3±0.7* | 8.8±0.9* | 9.9±1.1* | 11.1±1.1 |
| $MVO_2$ (ml/min) | group N | 16.9±1.7 | 16.8±2.0 | 14.8±2.5 | 13.8±1.7* | 15.5±1.6 |
| | group L | 15.6±2.1 | 15.9±1.6 | 13.6±1.1 | 15.8±1.4 | 16.6±2.2 |
| TTI (HRxLVSPx$10^{-3}$) | group N | 11.3±0.6 | 10.8±0.7 | 10.5±0.7 | 10.8±0.7 | 9.9±0.8* |
| | group L | 10.6±1.1 | 10.3±0.7 | 10.4±0.9 | 10.5±1.0 | 11.1±0.8 |

*Abbreviations: A = arterial; CS = coronary sinus; CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; HR = heart rate; LVSP = left ventricular systolic pressure; min = minute(s); MVO2 = myocardial oxygen consumption; TTI = modified tension-time index or double product. All values mean ± standard error of the mean.*
** $p<.05$ vs control*

maximum decrease of 24% (group N) and of 30% (group L). Despite the concomitant reduction in coronary perfusion pressure, coronary flow increased during this period (20% in group N and 31% in group L, both $p<0.05$ versus control).
These coronary hemodynamic changes were accompanied by a significant reduction in myocardial oxygen extraction of 26% and 25% in group N and L, resp. In contrast, myocardial $O_2$ consumption did not change appreciably in group L. In group N a moderate 18% decrease in $MVO_2$ was observed at the end of amiodarone infusion ($p<0.05$ versus control). Again, these changes in myocardial energetics were of short duration, only observed during amiodarone administration.

### Adverse effects

Amiodarone was relatively well tolerated in both groups. Shortlasting periods of facial flushing were reported in a few patients during the last minutes of amiodarone administration.

## DISCUSSION

The aim of this study was to compare the acute hemodynamic effects and tolerability of a relatively high intravenous dose of amiodarone in patients with normal versus diminished pre-existing cardiac function. Serious ventricular dysrhythmias are prone to occur during acute myocardial ischemia and/or infarction. As it is not uncommon in this situation, that myocardial function is depressed as well, subsequent aggressive antiarrhythmic therapy may further compromise the latter. The intrinsic negative inotropic effects of antiarrhythmic compounds generally are dose-related. Likewise, a progressive reduction in contractility has been reported with incremental dose-levels of intravenous amiodarone in open-chest anesthetized dogs[6]. However, as its systemic vasodilating properties are equally dose-related, progressive unloading of the left ventricle with higher dosages of amiodarone may outbalance these negative inotropic effects. Thus far, the systemic and coronary hemodynamic profile of high dosages of intravenous amiodarone, such as administered in the present study, has not been systematically investigated in patients with pre-existing ventricular dysfunction or compared with those in patients with normal ventricular function. The present dose, 5 mg/kg amiodarone, was chosen, as previous studies has suggested that this dose, administered over 5-15 minutes, alone or followed by a maintenance infusion of 1000-1500 mg/24 hours, may result in acute antiarrhythmic effects[14,15,18,19].

### Acute hemodynamic profile of intravenous amiodarone in normal versus diminished ventricular function

The present study indicates that a high dose of intravenous amiodarone, given over a relatively short period, does not further compromise cardiac pump function. If anything, it improves it in patients with pre-existing ventricular dysfunction, but not in patients with a normal cardiac function at baseline.
The hemodynamic profile of intravenous

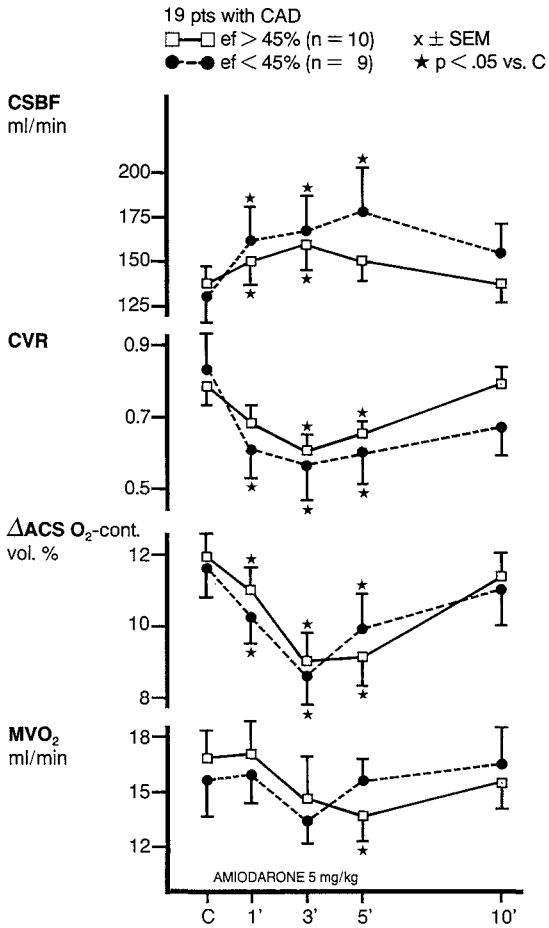


*Fig. 3: Significant similar reductions in coronary vascular resistance (CVR) in both groups, resulting in a 20% and 31% increase in coronary flow (CSBF) in groups N (open symbols) and L (closed symbols) resp.*
*Simultaneously, myocardial oxygen extraction ($\Delta$A-CS $O_2$ content) decreases in both groups, accompanied by an 18% decrease in myocardial oxygen consumption ($MVO_2$) in group N (open symbols). ef = ejection fraction*

amiodarone is biphasic. First, immediate but shortlasting systemic and coronary vasodilating effects are observed during the infusion period, accompanied by an increase in heart rate. Somewhat later, starting from the end of amiodarone administration, progressive negative inotropic and chronotropic effects become apparent. As these different hemodynamic effects did overlap at the time cardiac output measurements were performed, the progressive reduction in contractility and hence in myocardial function may still have been offset by the unloading effects of amiodarone on the left ventricle. The biphasic hemodynamic profile of amiodarone also suggests, that the improvement in cardiac output will be more pronounced during amiodarone administration when systemic vasodilatation is maximal. On the other hand, the progressive reduction in contractility may eventually result in a significant depression of cardiac output.
As in the present study cardiac output measurements were only performed before and at 7.5 minutes after amiodarone administration, it is unfortunate that these assumptions cannot be verified. The improvement of cardiac pumping performance in patients with ventricular dysfunction during amiodarone related predominantly to the pronounced increase in heart rate. Hence, stroke volume did not change significantly. There is a suggestion though, that the effect on stroke volume by amiodarone, nevertheless, may be different between both patient groups. Although the reduction in systemic vascular resistance and left ventricular systolic pressure were similar, it stands to reason that amiodarone induces a greater decrease in ventricular wall stress, in patients with enlarged hearts, e.g. in group L in our study. This, in itself, may improve systolic performance. Moreover, in these patients, with ischemic cardiomyopathy, amiodarone theoretically could result in a reduction in (silent) ischemia and in improved ventricular performance due to its effects on wall stress and, subsequently, on myocardial oxygen demand and subendocardial flow. In this respect, our data, which indicate more pronounced effects on coronary vascular resistance and coronary flow in patients with ventricular dysfunction, may support this theory. However, as none of our patients showed any sign of ischemia, this mechanism remains a very hypothetical one.

### Comparison with oral amiodarone in left ventricular dysfunction

Oral amiodarone generally is well tolerated in patients with left ventricular dysfunction[11,20,21]. As such, it compares favourably with other antiarrhythmic agents[1]. Although it has been suggested that oral amiodarone is better tolerated than the intravenous formulation[19], such a comparison is hazardous, as the clinical conditions where either form of therapy is contemplated, will be different. Following institution of oral therapy, it takes several days for antiarrhythmic efficacy to develop[23,24]. The intravenous formulation therefore is

obligatory for direct antiarrhythmic efficacy. Consequently, it is administered to patients in highly unstable conditions with arrhythmias following acute cardiovascular disorders, such as ischemia or infarction and, possibly, in addition to other antiarrhythmic agents. That hemodynamic derangements, i.e. hypotension and cardiogenic shock, have been reported[15,25] comes as no surprise. Exacerbations of heart failure have also been reported with oral amiodarone[26,27]. The propensity to develop negative effects on ventricular function following intravenous amiodarone, may depend on the rapidity of administration, however.

**Comparison with different infusion rates in ventricular dysfunction**

Theoretically, as the negative inotropic effects of amiodarone are dose-related, a slower rate of administration might prevent untoward effects on cardiac pump function. Schwartz and co-workers[12] administered 5 mg/kg amiodarone intravenously over 20 minutes to 18 patients with coronary artery disease, 9 with an ejection fraction $\geq$30%, and 9 with an ejection fraction <30%. Apparently, at this slow rate of administration, amiodarone does induce vasodilating effects. Systemic vascular resistance and arterial pressures in their study did not change. In contrast, cardiac output was clearly depressed during the first 10 minutes after infusion, particularly in patients with a low ejection fraction at baseline. Simultaneously, there was a decrease in stroke volume in these patients. These results do not indicate that a slow infusion rate of amiodarone will improve hemodynamic profile. On the other hand, the significant increase in left ventricular filling pressure, observed in our patients with pre-existing ventricular dysfunction, was not observed by Schwartz et al.[12].

**Hemodynamic effects of Tween 80**

Commercially available amiodarone is dissolved in Tween 80. The hemodynamic effects reported in this trial may, at least in part, result from cardiovascular actions of the solvent. It has been suggested, that besides vasodilating effects, Tween 80 may also result in negative inotropic properties[28]. Sicart and co-workers[8] compared the hemodynamic effects of 10 mg/kg amiodarone, dissolved in Tween 80 with an equivalent amount of the solvent alone. In their study, Tween 80 alone did not result in negative inotropic effects, but induced a moderate reduction in systemic vascular resistance. In combination with amiodarone, however, the effect on systemic vascular resistance was clearly more pronounced and resulted in a secondary increase in heart rate, not observed with Tween 80 alone. Hence, to a certain extent, Tween 80 augments the initial vasodilating effects of amiodarone, but does not further affect its cardiovascular profile.

**Side effects**

Side effects consisted of facial flushing during the period of amiodarone administration, but in accordance with the shortlasting vasodilating effects of amiodarone, were shortlived and well tolerated. Also, the reduction in blood pressure in our supine patients was well tolerated.

**Clinical implications**

The results from this study indicate that a relatively high intravenous dose of amiodarone has no negative effects on cardiac pump function and is generally well tolerated. Although, as a result of its systemic vasodilating effects, aortic pressures decrease, these changes are moderate and do not lead to clinically significant reductions in coronary perfusion pressure. In contrast, coronary flow improves in both patient groups. Moreover, as a result of systemic vasodilatation, cardiac pump function even improves, although only in patients with ventricular dysfunction.
Hence, a bolus administration of 5 mg/kg Amiodarone, administered in 5 minutes, should be considered safe in patient with a diminished ventricular function as a result of ischemia or infarction, particularly as the compound, at this dose level, has clear antiischemic effects[28]. Only in patients with elevated ventricular filling pressure before drug treatment or in patients with clinical signs of heart failure, a more cautious approach is advisable, in view of the observed increase in left ventricular end diastolic pressure in our patients with ventricular dysfunction. In the latter situation, hemodynamic monitoring is advisable to titrate the optimal rate of administration of intravenous amiodarone.

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# IX.2.

# Hemodynamic and Antiischemic Effects of High-Dose Intravenous Diltiazem in Patients with Normal versus Impaired Ventricular Function

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## Abstract.

Antiischemic efficacy, hemodynamic profile and safety of high-dose intravenous diltiazem, 0.4 mg/kg/5 min followed by 0.4 mg/kg/10 min, were compared in normotensive patients with coronary artery disease, 11 (Gr. A) with normal and 12 (Gr. B) with impaired ventricular function (ejection fraction $\leq$45%) during 2 identical pacing stress tests, 30 minutes before (APST I) and immediately following diltiazem administration (APST II). Diltiazem was well tolerated despite high peak plasma levels, 869±152 $\mu$g/l (Gr. A) and 926±169 $\mu$g/l (Gr. B). It immediately reduced systemic vascular resistance, left ventricular systolic and mean arterial pressures [27%, 15% and 13%, resp. (Gr. A) and 32%, 14% and 17%, resp. (Gr. B), $p<0.05$ vs control]. Although cardiac output improved with 19% (Gr. A) and 27% (Gr. B), the increase in stroke index was significantly greater and more prolonged in Gr. B. Also, whereas diltiazem did not affect heart rate or contractility in either group, left ventricular end diastolic pressure increased significantly by 33% in Gr. A, but not in Gr. B. Moreover, whereas diltiazem equally reduced coronary resistance in both groups and improved coronary flow by 26% (Gr. A) and by 17% (Gr. B), resp., myocardial $O_2$ extraction only decreased in Gr. B (24%, $p<0.05$ vs control). During APST II, myocardial $O_2$ demand was 26% (Gr. A) and 19% (Gr. B) less, compared to APST I ($p<0.05$). Although this resulted in less angina and ST-segment depression in both groups, antiischemic efficacy of diltiazem was more pronounced in patients with impaired ventricular function. During pacing with diltiazem, myocardial lactate metabolism normalized in Gr. B but not in Gr. A. Moreover, velocity parameters improved more during APST II in Gr. B. The rise in left ventricular pressure following pacing also was reduced to a greater extent in Gr. B than in Gr. A. Thus, high-dose intravenous diltiazem is well tolerated and improves left ventricular pump function, particularly in patients with preexisting ventricular dysfunction. Its more pronounced antiischemic effects in the latter group may suggest particular clinical usefulness when ventricular function is impaired.

## INTRODUCTION

The antianginal efficacy of oral diltiazem is, reportedly, dose-related and improves with increments in dosage[1,2]. Whereas variable antiischemic effects are observed with relatively low doses of intravenous diltiazem ranging from 18 to 35 $\mu g.kg^{-1}$, high-dose intravenous administration (i.e. 53 $\mu g.kg^{-1}$ results in clear-cut antiischemic properties[3-7]. Moreover, in humans, coronary flow improves only when such high intravenous dosages are administered[7-12]. Thus, in clinical situations where increases in coronary vascular tone and a reduction in coronary flow are likely to be present, e.g. myocardial ischemia at rest or,

possibly, the acute phase of myocardial infarction, a high, rather than a low dose administration of diltiazem may be preferable.

Several studies have shown that diltiazem may limit myocardial injury during prolonged periods of ischemia or during the acute phase of myocardial infarction, even when administered after the event has occurred[13,14]. Moreover, animal experiments indicate that during infarction and thrombolytic therapy, diltiazem further salvages myocardial tissue in addition to the effect of thrombolysis[15]. Thus, diltiazem may be useful in similar conditions in humans, providing it is given intravenously and in a sufficiently high dose to profit from its coronary vasodilating effect. However, in clinical situations such as myocardial infarction or prolonged ischemia at rest, where left ventricular function commonly is reduced, these high dosages of diltiazem may be contra-indicated because of the intrinsic negative inotropic properties of diltiazem, which, in vitro, are dose-related[16]. In patients with chronic congestive heart failure, a relatively low dose intravenous administration of diltiazem is well tolerated[17]. Conversely, in patients with a normal ventricular function, defined as a left ventricular ejection fraction $\geq$50%, high intravenous dosages do not negatively affect myocardial contractility or left ventricular pump function[7]. No data are available, however, on the acute hemodynamic effects of the latter dose regimen in patients with a diminished left ventricular function. Moreover, it has not been established as to whether the antiischemic efficacy of high-dose intravenous diltiazem is preserved in these patients. The present study compares the acute systemic and coronary hemodynamic effects of high dosages of intravenous diltiazem in patients with a normal versus impaired left ventricular function, investigates the safety of such a drug regimen, and compares the antiischemic effects of diltiazem in these two patient groups during pacing-induced stress.

## METHODS

### Patients

After approval of the study-protocol by the Human Studies Committee of the Zuiderziekenhuis on February 8, 1985 and after informed consent was obtained, 23 patients participated in this study, 21 men and 2 women (mean age 54±2 years, $\bar{x}$±SEM). All patients were catheterized for stable, exercise-induced angina with objective signs of ischemia during exercise testing and/or a documented old myocardial infarction. Patients with systemic hypertension, valvular disease, a recent myocardial infarction (less than 2 months ago), conduction disturbances or signs of congestive heart failure were not admitted to the study. Patients with unstable angina were not included, as such patients may deteriorate upon withdrawal of cardiac medication. Unstable angina was defined by the occurrence of recent episodes of angina, increased frequency and/or duration of episodes of angina during the last month or recent prolonged episodes of angina at rest (>20 minutes). Also, patients with angina, predominantly occurring at rest, and/or ECG-changes, suggestive of variant angina, were not included. All cardiac therapy was tailed off 48-72 hours before the study with the exception of short-acting nitroglycerin, which was permitted up to 6 hours before the investigation. Coumarin derivatives were withheld 72 hours pre-study and anti-platelet drugs (e.g. aspirin) were stopped at least 10 days before the investigation. To participate, it was essential that a $\geq$70% diameter reduction in either the left anterior descending artery, the proximal left circumflex artery or a proximal marginal branch of the left coronary artery was present. On the day before the investigation the left ventricular ejection fraction was determined by multigated radionuclide angiography and patients were divided into those with a normal (ejection fraction >45%, group A, n=11) and an impaired ventricular function (ejection fraction $\leq$45%, group B, n=12). Clinical characteristics and angiographic data are given in Table I. Both groups were comparable as to sex, age, exercise-inducible ischemia and number of diseased vessels. Previous infarcts, however, were more frequent in group B. In the latter group, left ventricular end diastolic volume was increased compared to group A [97±9 ml/m$^2$ (group B) versus 59±5 ml/m$^2$ (group A), $p < 0.05$] and, by design, left ventricular ejection fraction reduced [35±2% (group B) versus 58±2% (group A), $p < 0.05$].

### Catheterization procedures

Patients were catheterized in the morning after an overnight fast, without premedication. First, left and right coronary angiography was performed with non-ionic contrast material (Iopamidol) using the Seldinger technique. Next, a no 7 F thermodilution pacing catheter (Wilton Webster) was inserted in the coronary sinus through a brachial vein, such that the proximal thermistor was at least 2 cm beyond the orifice of the coronary sinus, the catheter position stable and

**Table I: Clinical characteristics and angiographic data.**

| | Gr. A left ventricular ejection fraction >45% | Gr. B left ventricular ejection fraction ≤45% |
|---|---|---|
| number of patients | 11 | 12 |
| age (yr) | 51±5 (range 39 to 67) | 51±3 (range 40 to 66) |
| male/female | 10 / 1 | 11 / 1 |
| previous infarct (n) | 4 anterior / 3 inferior | 7 anterior / 7 inferior |
| exercise-induced angina (n) | 9 | 10 |
| positive ergometry test (n) | 8 | 7 |
| coronary angiography (>70% stenosis) | | |
| 1-vessel: | 4 | 5 |
| 2-vessel: | 3 | 2 |
| 3-vessel: | 4 | 5 |
| LV ejection fraction (%) | 58±2 | 35±2* |
| LV end diastolic volume (ml/m2) | 59±5.4 | 97±8.8* |

*Abbreviations: LV = left ventricular; n = number; yr = year.*
*Values are mean ± SEM; *p<.05 Gr. A vs B.*

that blood could be drawn quickly enough to allow for a rapid sequence of blood samples. The absence of atrial reflux was confirmed by a bolus injection of saline at room temperature into the right atrium. A no 7 F balloon-tipped, triple-lumen thermodilution catheter was positioned in a pulmonary artery via a Desilet system in the right femoral vein. Care was taken, that the catheter tip was stable without baseline drift on the thermodilution signal. Finally, a no 7 or no 8 F Millar or Honeywell pigtail angiographic micromanometer catheter was introduced into the left ventricle through a Desilet introducer system in a femoral artery. The side arm of this system was used to record arterial pressures. The position of the catheters was recorded on video disc to allow rechecking of their respective positions during the study.

**Measurements**

Hemodynamic measurements included mean and phasic systemic arterial, pulmonary artery, and right atrial pressures, left ventricular (LV) peak systolic, mean and end diastolic pressures, LV pressure-derived contractility indices (LV peak dp/dt, dp/dt/P at 40 mmHg and $V_{max}$ total pressure), thermodilution cardiac output (in triplicate) and coronary sinus blood flow. All fluid filled catheters were calibrated, using Bentley transducers, with a zero reference level set at mid-chest. The micromanometer pressures were balanced to zero and superimposed on the conventional pressure tracings. After appropriate calibration, all pressure and flow signals and the first derivative of left ventricular pressure (LV dp/dt) were recorded on paper at different paperspeeds, using a CGR 1000 cath lab system. Cardiac output was measured on-line by a Mennen cath lab computer system. This system also allows for the on-line determination of all pressures and pressure-derived contractility indices, measured over 12 to 15 consecutive beats. Coronary sinus blood flow was determined during a continuous 30-second

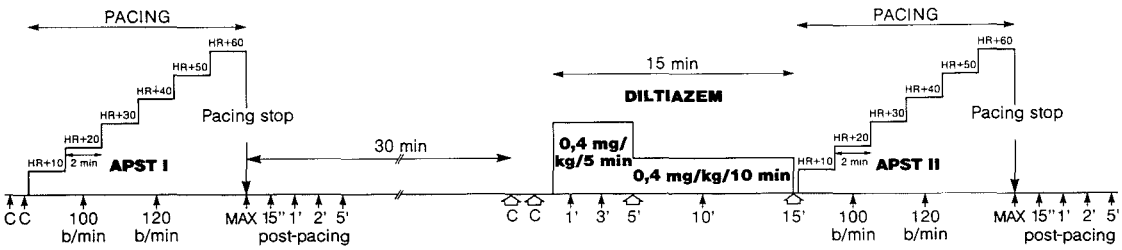


*Fig 1: Schematic representation of study protocol. Diltiazem is administered over 15 minutes, 30 minutes after and just before 2 identical atrial pacing stress tests (APST) I and II, resp. Hemodynamic and metabolic parameters are evaluated at prefixed intervals, designated by closed arrows. In addition, cardiac output is measured before and 5 and 15 minutes (') after onset of diltiazem administration (open arrows). b/min = beats/minute; C = control; HR = heart rate; MAX = maximal pacing rates.*

infusion of 30-35 ml of glucose 5% at room temperature. Both the mean and phasic flow were recorded. Calculations were made from the mean flowcurve, according to the formula: coronary sinus blood flow (ml/min) = $V_1$ x $[(T_b-T_i)/(T_b-T_{cs})-1]x1.08$, where $T_b$ is blood temperature before injection, $T_i$ is temperature of injectate, $T_{cs}$ is temperature of mixture of coronary sinus blood and injectate, and $V_1$ the rate of injectate (ml/min). At the end of the study, the pressure curves from the femoral artery were compared with a simultaneous recording from the aortic root by the micromanometer catheter, to compensate for any difference between proximal and distal arterial pressures.

### Metabolic and electrocardiographic measurements

Arterial and coronary venous blood was sampled simultaneously for the determination of oxygen and lactate. Oxygen saturation values were measured, using an American Optical Oxymeter. For the determination of lactate, 1 cc of whole blood was collected and immediately deproteinized in 1 cc ice-cold 8% $HClO_4$, vortexed and kept on ice until further assay. The procedures for lactate determination and the standard deviation of the assay in our laboratory have been previously published[18,19]. Heart rate and ST-segment changes were determined from 100 mm/sec recordings of ECG leads I, II and V5. The ST-segment was measured in 5 consecutive beats, 0.08 second after the J point, using a calibrated magnifying lens. Plasma diltiazem levels were determined by high-performance liquid chromatography analysis.

### Calculations

Coronary vascular resistance (mmHg/ml/min) was calculated as the difference between mean arterial pressure (mmHg) and LV mean diastolic pressure (mmHg), divided by coronary sinus blood flow (ml/min). Systemic vascular resistance (dynes.sec.cm$^{-5}$) was derived as [mean arterial pressure (mmHg) - mean right arterial pressure (mmHg)] x 80 / cardiac output (l/min). Stroke work index (g.m.m$^2$) was calculated as stroke index (ml/beat/m$^2$) x [mean arterial pressure (mmHg) - left ventricular end diastolic pressure (mmHg)] x 0.0136. Myocardial oxygen extraction ($mlO_2$/ml) was calculated as arterial oxygen content ($mlO_2$/ml) - coronary venous oxygen content ($mlO_2$/ml) and myocardial oxygen consumption (ml/min) as the product of myocardial oxygen extraction ($mlO_2$/ml) and coronary blood flow (ml/min). Percentage myocardial lactate extraction was calculated as 100 x [arterial lactate content (mmol/l) - coronary venous lactate content (mmol/l)]/arterial lactate content (mmol/l). Myocardial lactate uptake (mmol/min) was determined by multiplying the difference in arterial and coronary venous lactate content (mmol/ml) with coronary blood flow (ml/min).

### Study protocol

At least 40 minutes after the last coronary angiogram and 20 minutes after instrumentation, multiple control measurements of all variables, except cardiac output, were performed to ensure stable baseline values (Fig. 1). An atrial pacing stress test (APST I) then followed, during which heart rate was elevated with 10 beats/2 minutes until moderate to severe anginal pain or atrioventricular

block occurred, or a maximal heart rate of 170 beats/ minute was reached. Repeated determinations of all hemodynamic and metabolic variables were carried out at fixed intervals during pacing, e.g. halfway the 100 and 120 beats/minute period, and during maximal pacing rates (MAX), followed by measurements at 15 seconds, 1, 2, and 5 minutes after pacing. Coronary flow was measured during the last 30 seconds before pacing was interrupted. Next, coronary flow measurements were performed immediately following the 2-minute post-pacing period.

After the first pacing stress test, a 30-minute stabilization period was allowed before multiple control determinations of all hemodynamic and metabolic variables were again carried out. Next, diltiazem was administered intravenously (0.4 mg/kg during 5 minutes, followed by 0.4 mg/kg during 10 minutes) and all measurements repeated at 1, 3, 5, 10 and 15 minutes after the onset of administration. Cardiac output was determined at baseline and at 5 and 15 minutes of diltiazem administration. Immediately following diltiazem infusion, a second atrial pacing stress test (APST II) was carried out, identical to APST I. During APST II all variables were reassessed at similar time intervals as during APST I. Blood for diltiazem assay was collected just before the infusion, and at 5 and 15 minutes of infusion and, in the last 11 patients studied, also at the end of APST II, just before discontinuation of pacing. At the onset of each pacing stress test, patients received atropine (0.5 mg i.v.) to ensure identical maximal pacing heart rates, as the option of this part of the investigation was to study the antiischemic properties of the compound under identical pacing stress conditions.

### Statistical analysis

In each group, measurements made during diltiazem administration were compared with baseline values before infusion, using a t-test for paired observations. A 2-tailed p value $<0.05$ was considered significant. The same method was used to compare measurements during APST I and II within each group. Group differences in hemodynamic and metabolic mean values before and during diltiazem administration and before, during and after both atrial pacing stress tests, were compared using a t-test for unpaired observations. A 2-tailed p value $<0.05$ was considered significant. In a separate analysis, a linear regression model was applied to investigate the difference between plasma levels of diltiazem and effect measures within each group, whereas differences in diltiazem levels between both groups were compared with a t-test for unpaired observations.

## RESULTS

### Systemic hemodynamic changes during diltiazem administration in patients with normal versus impaired baseline left ventricular function (Table II)

Diltiazem equally reduced systemic vascular resistance in both groups (27%, group A and 32%, group B, both $p<0.05$ versus control), resulting in significant, similar decreases in left ventricular systolic pressure (15%, group A and 14%, group B) and in mean arterial pressure (13%, group A and 17%, group B, fig 2). Pressure changes occurred gradually, starting at 3 minutes of diltiazem administration, but persisting until the end of the study. Cardiac output improved significantly and consistently, both in group A (19%) as well as in group B (27%). In contrast, in group A, stroke index was only increased temporarily, immediately after the bolus infusion, whereas in group B it remained elevated for the full duration of diltiazem administration. Moreover, diltiazem induced a significantly greater percent increase in stroke index in group B than in group A (32±3% versus 22±7%, resp., $p<0.001$).
Neither heart rate nor the isovolumetric indices of contractility changed in either group. Although, in patients with an impaired left ventricular function, the velocity parameters were already significantly reduced at baseline, they did not further decline during diltiazem administration. Also, whereas during control, left ventricular end diastolic pressure was significantly elevated in group B compared with group A (15±2.6 mmHg versus 9±1.6 mmHg, resp., $p<0.05$), it did not further increase during diltiazem administration in patients with impaired left ventricular function. In contrast, in group A, left ventricular filling pressure rose with 33% towards the end of the infusion period ($p<0.05$ versus control). Pulmonary artery pressures and pulmonary vascular resistance did not change in either group.

### Coronary hemodynamic effects of diltiazem in normal versus impaired baseline left ventricular function (Table III)

Baseline values of coronary sinus blood flow and coronary vascular resistance were significantly different in both groups [100±11 ml/min (group A) versus 155±17 ml/min (group B) and 1.2±0.17

**Table II: Systemic hemodynamic changes during diltiazem administration in patients with normal vs impaired left ventricular function.**

| | | minutes after onset of diltiazem infusion | | | | |
|---|---|---|---|---|---|---|
| | Control | 1 | 3 | 5 | 10 | 15 |
| SVR (dynes.s.cm$^{-5}$) | | | | | | |
| Gr A | 1321±136 | | | 963±113* | | 986±107* |
| Gr B | 1267±106 | | | 865±58* | | 931±68* |
| LVSP (mmHg) | | | | | | |
| Gr A | 137±5.5 | 129±4.8 | 120±5.1* | 117±4.3* | 118±4.2* | 118±3.6* |
| Gr B | 137±7.2 | 131±7.8 | 124±7.2* | 118±6.5* | 118±6.7* | 119±7.7* |
| MAP (mmHg) | | | | | | |
| Gr A | 107±3.2 | 104±3.0 | 98±3.4* | 93±3.8* | 92±4.0* | 92±3.0* |
| Gr B | 103±4.4 | 100±5.3 | 89±4.1* | 86±4.2* | 88±4.9* | 91±4.1* |
| CO (l/min) | | | | | | |
| Gr A | 6.7±0.6 | | | 8.0±0.8* | | 7.4±0.6* |
| Gr B | 6.2±0.4 | | | 7.9±0.6* | | 7.3±0.5* |
| SI (ml/beat/m$^2$) | | | | | | |
| Gr A | 44±3.8 | | | 49.4±4.7* | | 48.6±3.6 |
| Gr B | 36±2.8 | | | 46.2±3.6* | | 43.6±3.3* |
| SWI (g.m.m$^2$) | | | | | | |
| Gr A | 58±4.9† | | | 55±4.4 | | 54±4.9 |
| Gr B | 40±3.7 | | | 45±4.9 | | 46±4.8 |
| HR (beats/min) | | | | | | |
| Gr A | 81±5 | 85±5 | 86±5 | 85±5 | 80±5 | 77±5 |
| Gr B | 89±5 | 91±5 | 94±4 | 91±4 | 86±4 | 81±4 |
| LV peak dp/dt pos (mmHg$^{-1}$) | | | | | | |
| Gr A | 1619±112 | 1674±122 | 1694±139 | 1676±127 | 1625±126 | 1576±104 |
| Gr B | 1459±81 | 1484±86 | 1526±87 | 1444±95 | 1468±89 | 1345±100 |
| $VCE_{40}$ (sec$^{-1}$) | | | | | | |
| Gr A | 33.9±2.8† | 35.2±2.9† | 35.9±2.7† | 32.7±3.7† | 34.2±2.3† | 0.7±3.7† |
| Gr B | 28.2±1.5 | 29.1±1.8 | 29.8±1.8 | 29.0±1.8 | 28.2±2.1 | 26.8±2.0 |
| Vmax (sec$^{-1}$) | | | | | | |
| Gr A | 51±3.8† | 53±4.3† | 57±3.1† | 57±2.8† | 55±2.8† | 54±2.4† |
| Gr B | 42±2.3 | 41±2.9 | 46±3.06 | 45±2.8 | 43±3.3 | 43±2.9 |
| LVEDP (mmHg) | | | | | | |
| Gr A | 9±1.6† | 8±1.2 | 10±1.4 | 11±1.7 | 12±1.4* | 12±1,4* |
| Gr B | 15±2.6 | 14±2.9 | 13±2.4 | 15±2.6 | 17±2.8 | 16±2.8 |
| MPAP (mmHg) | | | | | | |
| Gr A | 15±1.0 | 15±1.3 | 16±1.3 | 17±1.4 | 17±1.2 | 18±1.2 |
| Gr B | 18±2.9 | 16±1.9 | 19±3.2 | 16±1.7 | 19±3.5 | 19±2.3 |
| PVR (mmHg) | | | | | | |
| Gr A | 106±34 | | | 106±38 | | 110±39 |
| Gr B | 156±43 | | | 110±30 | | 115±44 |

*Abbreviations: CO = cardiac output; HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systelic pressure; min = minutes; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; sec = seconds; SI = stroke index; SVR = systemic vascular resistance; SWI = stroke work index.*

**$p<0.05$ vs control; † $p<0.05$ Gr. A vs B. Values are mean ± SEM.*

**Table III: Coronary hemodynamic changes during diltiazem infusion in patients with normal vs impaired left ventricular function.**

| | | minutes after onset of diltiazem infusion | | | | |
|---|---|---|---|---|---|---|
| | Control | 1 | 3 | 5 | 10 | 15 |
| CSBF (ml/min) | | | | | | |
| Gr. A | 100±11† | 108±12† | 116±9† | 126±11* | 122±12†* | 114±13 |
| Gr. B | 155±17 | 154±16 | 175±20 | 173±21 | 182±22* | 170±18 |
| CVR (mmHg/ml/min) | | | | | | |
| Gr. A | 1.20±0.17† | 1.10±0.14† | 0.87±0.07†* | 0.76±0.07†* | 0.82±0.12* | 0.84±0.10 |
| Gr. B | 0.74±0.07 | 0.69±0.07 | 0.58±0.06* | 0.55±0.06* | 0.57±0.07* | 0.66±0.06* |
| $\Delta$A-CS $O_2$ content (vol %) | | | | | | |
| Gr. A | 7.08±0.53 | 6.78±0.43 | 6.08±0.55 | 5.56±0.58 | 5.70±0.50 | 5.95±0.52 |
| Gr. B | 6.60±0.34 | 6.36±0.34 | 5.52±0.39* | 5.03±0.37* | 5.17±0.45* | 5.38±8.35* |
| $MVO_2$ (ml/min) | | | | | | |
| Gr. A | 7.80±0.95† | 8.19±1.15 | 7.52±0.74 | 7.50±0.83 | 7.24±0.88 | 7.99±0.97 |
| Gr. B | 10.60±1.23 | 9.88±1.30 | 9.43±1.50 | 8.73±1.10 | 8.83±1.09 | 9.20±1.28 |
| DP (HR x LVSP x $10^{-3}$) | | | | | | |
| Gr. A | 10.97±0.69 | 10.89±0.72 | 9.64±0.60 | 9.27±0.61* | 9.42±0.66* | 9.07±0.66* |
| Gr. B | 10.60±1.23 | 9.88±1.30 | 9.43±1.5 | 8.73±1.10* | 8.83±1.09* | 9.20±1.28* |

*Abbreviations: CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; $\Delta$A-CS $O_2$ content = difference in arterial and coronary sinus $O_2$ content; DP = double product; $MVO_2$ = myocardial oxygen consumption.*
**p<0.05 vs control; † p<0.05 Gr. A vs B. Values are mean ± SEM.*

mmHg/ml/min (group A) versus 0.74±0.07 mmHg/ml/min (group B), resp., p<0.05]. However, subsequent changes during diltiazem infusion were similar. Coronary sinus flow increased by 26% (group A) and by 17% (group B), albeit only for a short period, between 5 and 10 minutes of diltiazem infusion. Concomitantly, coronary vascular resistance decreased by 37% (group A) and by 26% (group B), at 5 minutes of diltiazem. The reduction in coronary vascular resistance persisted for the entire infusion period, but only in group B patients. Likewise, myocardial oxygen extraction only decreased in group B (24% compared with baseline values, p<0.05) despite similar reductions in the double product, an index of myocardial oxygen demand, and despite similar changes in coronary flow in both groups. This reduction in myocardial oxygen extraction also persisted for the entire infusion period. Myocardial oxygen consumption, although different at baseline [7.8±1.0 ml/min (group A) versus 10.6±1.2 ml/min (group B), p<0.05], did not further change during diltiazem administration in either group.

### Systemic and coronary hemodynamic effects of diltiazem on hemodynamics during pacing (Tables IV and V)

Baseline heart rates were similar during and after APST I and APST II in both groups. Maximal pacing rates during APST I were 153±6 beats/minute and 155±5 beats/minute for groups A and B, resp. By design, maximal pacing rates were also similar during APST I and II in each group. In contrast, the double products were significantly, albeit equally, reduced in both groups during APST II, compared with APST I (by 26%, group A and by 19%, group B) due to similar reductions in left ventricular systolic pressure during pacing after diltiazem in both groups. Mean arterial pres-

**Table IV: Effects of diltiazem on systemic hemodynamics during pacing.**

| | | | Control | MAX | 1 min p-p | 5 min p-p |
|---|---|---|---|---|---|---|
| HR (beats/minute) | Gr.A | APST I | 71±3 | 153±6* | 80±6 | 80±6 |
| | | APST II | 77±5 | 151±6* | 92±5 | 92±5 |
| | Gr.B | APST I | 80±4 | 155±5* | 83±6 | 84±5 |
| | | APST II | 81±4 | 155±6* | 87±5 | 88±6 |
| LVSP (mmHg) | Gr.A | APST I | 138±6 | 140±7.0 | 147±6 | 140±6.5 |
| | | APST II | 118±4# | 119±5.0# | 125±5# | 121±5# |
| | Gr.B | APST I | 143±8 | 141±7.5 | 150±8 | 143±7.5 |
| | | APST II | 119±8# | 113±7.3# | 120±6.3# | 121±6.2# |
| MAP (mmHg) | Gr.A | APST I | 102±3.5 | 116±3.9* | 107±3.5* | 102±3.8 |
| | | APST II | 95±4.3 | 103±3.5†* | 97±3 | 97±3.2 |
| | Gr. B | APST I | 102±5.9 | 113±6.7* | 111±8.3* | 108±9 |
| | | APST II | 90±3.7 | 95±4 | 93±5.3 | 93±4.7* |
| LV dp/dt pos ($mmHg.s^{-1}$) | Gr.A | APST I | 1536±98 | 2027±134* | 1828±156* | 1674±99 |
| | | APST II | 576±104 | 1927±163* | 1733±159* | 1602±136 |
| | Gr.B | APST I | 1426±66 | 1955±203* | 1811±169* | 1527±86 |
| | | APST II | 1345±100 | 1563±102* | 1537±96*# | 1445±97# |
| Vmax ($s^{-1}$) | Gr.A | APST I | 47±2 | 58±3* | 52±4* | 48±3 |
| | | APST II | 54±2 | 72±6* | 57±3.5 | 56±3 |
| | Gr.B | APST I | 39±2† | 43±3† | 43±3 | 42±3 |
| | | APST II | 43±3† | 52±3†*# | 48±3# | 47±3# |

*Abbreviations: DP = double product; HR = heart rate; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; MAX = maximal pacing rates; p-p = post-pacing;*
**p<0.05 vs control; † p<0.05 Gr. A vs B; # = p<.05 APST I vs II. Values are mean ± SEM.*

sures increased significantly during APST I by 14% (group A) and by 11% (group B) at maximal pacing rates. During APST II, arterial pressures again increased in group A (12% versus control APST II, p<0.005) but no longer in group B. Also, changes in contractility during pacing before and after diltiazem were different in both groups. In group B velocity indices ($V_{max}$) increased more during pacing after diltiazem than during the untreated, first pacing test [43±3 $sec^{-1}$ (APST I) versus 51±3 $sec^{-1}$ (APST II), p<0.05)]. In group A, on the other hand, isovolumetric indices of contractility did not further improve during APST II. Moreover, in group B, coronary flow increased less during pacing after diltiazem than during APST I [248±24 ml/min (APST I) versus 210±20 ml/min (APST II), p<0.05]. Again, in group A, changes in coronary flow were similar during both pacing tests.

Myocardial oxygen consumption was not significantly different during APST I and II in both groups, although it tended to be less during pacing after diltiazem. In contrast, myocardial $O_2$ extraction ($\Delta$A-CS $O_2$ content) was significantly greater during APST II than during APST I in group B (Table V).

**Table V: Effect of diltiazem on coronary hemodynamics during pacing.**

| | | | Control | MAX | 1 min p-p | 5 min p-p |
|---|---|---|---|---|---|---|
| CSBF (ml/min) | Gr. A | APST I | 123±10 | 181±16* | 126±10 | 116±9 |
| | | APST II | 120±13 | 160±18* | 116±9 | 116±9 |
| | Gr. B | APST I | 159±20 | 249±24*† | 188±27† | 163±21 |
| | | APST II | 174±20#† | 210±20*# | 162±26# | 162±18† |
| CVR (mmHg/ml/min) | Gr. A | APST I | 0.89±0.08 | 0.69±0.07* | 0.89±0.09 | 0.91±0.10 |
| | | APST II | 0.89±0.10 | 0.70±0.06* | 0.82±0.06 | 0.91±0.09 |
| | Gr. B | APST I | 0.70±0.07 | 0.48±0.05*† | 0.64±0.09 | 0.70±0.09 |
| | | APST II | 0.56±0.07†# | 0.48±0.05*† | 0.61±0.14 | 0.66±0.08* |
| $\Delta$A-CS $O_2$ content (vol %) | Gr. A | APST I | 12.6±0.7 | 11.4±0.6 | 10.3±0.4 | 12.3±0.8 |
| | | APST II | 10.0±1.0# | 10.8±0.8* | 8.9±0.6# | 10.4±0.6 |
| | Gr. B | APST I | 10.5±0.7† | 10.0 ±0.5 | 9.7±0.4 | 10.2±0.5 |
| | | APST II | 8.3±0.4# | 10.35±0.5* | 9.4±0.4 | 9.8±0.6 |
| $MVO_2$ (ml/min) | Gr. A | APST I | 15.9±2.2 | 20.4±2.4* | 12.4±1.4 | 14.7±1.8 |
| | | APST II | 12.4±1.1# | 17.3±1.8* | 10.6±1.7 | 12.3±1.4 |
| | Gr. B | APST I | 17.2±2.8 | 25.2±3.0* | 19.4±3.5 | 16.6±3.0 |
| | | APST II | 14.3±2.0 | 21.7±2.2* | 14.5±3.5 | 15.9±2.6 |
| DP | Gr. A | APST I | 10.1±0.6 | 20.7±1.0* | 11.8±0.8* | 11.5±0.6 |
| | | APST II | 9.2±0.7 | 17.6±0.8*† | 11.8±0.5* | 11.3±0.5* |
| | Gr. B | APST I | 11.3±0.5 | 22.2±1.3* | 13.7±1.2* | 13.0±1.0* |
| | | APST II | 9.4±0.9# | 17.2±1.3*# | 11.2±0.8* | 10.9±0.7* |

*Abbreviations: CSBF = coronary blood flow; CVR = coronary vascular resistance; $\Delta$A-CS $O_2$ content = difference in arterial and coronary sinus oxygen content; DP = double product; $MVO_2$ = myocardial oxygen consumption; MAX = maximal pacing heart rate; min = minute; p-p = post-pacing;*
** p<0.05 vs control; † p<0.05 Gr. A vs B; # = p<.05 APST I vs II. Values are mean ± SEM.*

### Antiischemic properties of diltiazem in patients with normal versus impaired left ventricular function

Diltiazem had similar antianginal effects in group A and group B. During APST II, 10 patients in each group had less or no angina when compared with the first, untreated pacing test. Also, the reduction in ST-segment depression was identical in each group during pacing before and after diltiazem (Fig. 2). In contrast, changes in myocardial lactate metabolism were different. Whereas control lactate extraction values before each APST were similar and lactate production values during and after APST I comparable in groups A and B, lactate metabolism remained normal and unchanged from baseline values during pacing after diltiazem in group B. (Fig. 3). In contrast, in group A, myocardial lactate extraction again changed to production during APST II, despite the administration of diltiazem. As a result, changes in myocardial lactate extraction were significantly

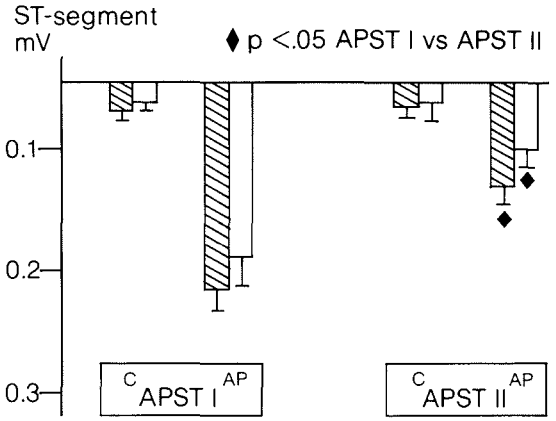


*Fig. 2: ST-segment changes during pacing before and after diltiazem in Gr. A (hatched bars) and Gr. B (open bars). Values are mean ± SEM. APST = atrial pacing stress test; C = control; MAX = maximal pacing rates.*

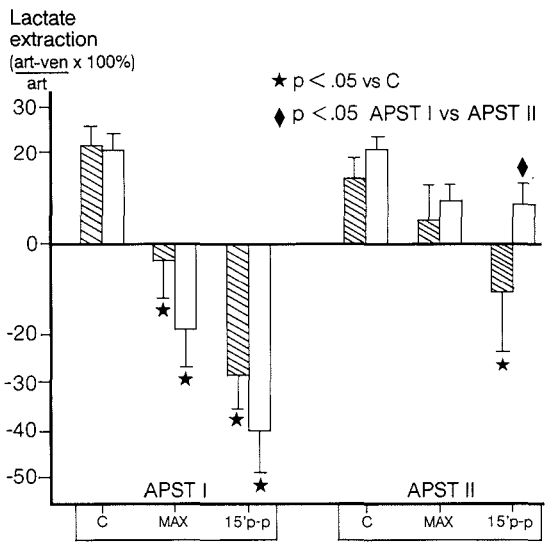


*Fig. 3: Effect of diltiazem on myocardial lactate extraction during pacing. During atrial pacing stress test (APST) I comparable lactate production is found in Gr. A (hatched bars) and Gr. B (open bars). In contrast, during APST II lactate extraction is preserved in Gr. B, significantly different from APST I, but not in Gr. A. Values are mean ± SEM.*
*C = control; MAX = maximal pacing rates; ' = minute(s); p-p = post-pacing.*

different [lactate 15 seconds post-pacing: -6±12% (group A) versus 7±5% (group B), p<0.05]. Moreover, in group B, the rise in left ventricular end diastolic pressure, measured at 15 seconds post-pacing, was 18 mmHg less during APST II than during APST I, compared with a difference of 11 mmHg in group A (Fig. 4).

### Plasma diltiazem levels

Plasma diltiazem levels were comparable in groups A and B, with values of 869±152 μg/l and 926±169 μg/l, resp., at 5 minutes diltiazem, gradually decreasing to 339±152 μg/l and 432±103 μg/l, resp., at maximum pacing rates during APST II. There was no significant relation between individual plasma levels and percentage changes in hemodynamic parameters.

### Adverse effects

No untoward clinical effects were observed. Myocardial ischemia did not occur during diltiazem administration. Only one patient complained of a short period of facial flushing.

## DISCUSSION

It has been suggested, that the usefulness of calcium-antagonists during prolonged periods of myocardial ischemia or during infarction, is restricted to their prophylactic use only[20]. However, some experimental data indicate, that intravenous diltiazem, when administered after the event, may still limit the degree of ischemia and protect the infarcting myocardium[13,14]. Moreover, when administered just before thrombolysis, it enhances salvage of reperfused ischemic myocardium[15]. Like all calcium antagonists, diltiazem could theoretically protect against ischemia-induced myocardial injury through a number of actions[20]. However, the energy-sparing properties, resulting from its intrinsic hemodynamic effects, e.g. the arterial vasodilating and negative inotropic properties of diltiazem, presumably are most important. The net result of the latter is a reduction in myocardial oxygen demand. In addition, through its coronary vasodilating effects, it may augment the supply of oxygen and substrate to the myocardial area at risk.
These intrinsic cardiovascular properties of diltiazem are dose-related[6,21]. Also, in humans, coronary vasodilatation and increments in coronary flow only become apparent when relatively high dosages are used[3,5-11]. Thus, to

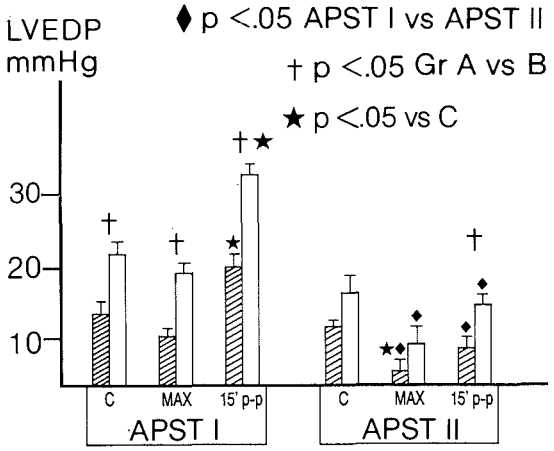


*Fig. 4: Effect of diltiazem on left ventricular end diastolic pressure (LVEDP) in Gr. A (hatched bars) and Gr. B (open bars).*
*Values are mean ± SEM.*
*APST = atrial pacing stress test; C = control; MAX = maximal pacing rates; ' = minute(s); p-p = post-pacing.*

achieve optimal antiischemic efficacy, not only through a reduction in myocardial oxygen demand by unloading of the left ventricle, but also by improving coronary flow and myocardial oxygen supply, a high dose rather than a low dose administration appears preferable.
Alternatively, the intrinsic negative inotropic (and chronotropic) effects of diltiazem may be amplified when high dosages are given[16]. Consequently such administration of diltiazem could be hazardous, particularly in clinical settings, where left ventricular function may be already depressed, e.g. during prolonged periods of myocardial ischemia or acute infarction. Thus, whereas diltiazem, at high dosages, may improve left ventricular function through its antiischemic properties, it may alternatively prove to be deleterious by its direct myocardial depressant effect.

**Systemic hemodynamic effects of high dose intravenous diltiazem in impaired versus normal ventricular function:**

In the present study, diltiazem, administered at a high infusion rate, was well tolerated, both by patients with a markedly impaired ventricular function and by patients with normal ventricles. In both groups it equally decreased systemic vascular resistance and caused a moderate but similar reduction in left ventricular systolic and aortic pressures, accompanied by an increase in cardiac output. As, in each group, other systemic hemodynamic variables did not change, at least not during the initial phase of the study, the latter most likely resulted from unloading effects on the left ventricle.
The augmentation of left ventricular pump function was more pronounced in patients with an impaired left ventricular function at baseline. In this group, the increase in stroke index was significantly greater and longer lasting than in patients with a normal left ventricular function.
Given the fact, that the isovolumetric indices of contractility, heart rate and preload were not affected in neither group, this observation is not surprising. In this situation, comparable reductions in left ventricular systolic and arterial pressures by diltiazem should have a greater effect on systolic wall stress in patients with dilated, non-hypertrophied left ventricles (group B in this study), than in patients with normal-sized left ventricles (group A). Besides, although myocardial ischemia was not apparent before diltiazem administration, the subsequent greater reduction in myocardial oxygen demand in patients with enlarged left ventricles (group B) may also explain the greater improvement of left ventricular function in this group.
Augmentation of cardiac performance following intravenous diltiazem has been reported in patients with congestive heart failure[17,22]. The present study is the first to compare the effects of diltiazem in patients without clinical signs of heart failure, but with different degrees of left ventricular systolic (dys)function and to report an augmentation in cardiac performance in patients with asymptomatic ventricular dysfunction.

Despite the high dose used in our study, intrinsic negative chronotropic or inotropic properties of diltiazem, were not apparent. Neither heart rate nor contractility indices decreased during diltiazem administration in either group.
The lack of negative chronotropic and inotropic effects in the present study presumably reflect a continuous interaction between the intrinsic hemodynamic properties of diltiazem and baroreceptor-dependent reflex mechanisms. This balance between primary and secondary cardiovascular effects is well-known with vasoactive compounds such as calcium-antagonists[23]. It not only depends on the type of calcium-antagonist and, subsequently, on the magnitude of vasodilator- versus negative chronotropic and inotropic activity, but also on dosage and means of administration. Intravenous diltiazem, at lower dosages than used in the present study, may decrease heart rate[24] and reduce contractility[25]. That contrac-

tility indices and heart rate did not change in the present study may suggest that the high dose evokes a secondary counteractive reflex mechanism. However, statements concerning contractility changes should be made carefully in this study. The pressure-derived isovolumetric indices of contractility, used in the present study, are load-dependent, albeit less pronounced where the velocity parameters, $VCE_{40}$ and $V_{max}$, are concerned. Although the fact that velocity parameters did not change in the face of reduced arterial pressures does not suggest significantly diminished contractility, these parameters may underestimate this. The latter is suggested by the significant increase in left ventricular end diastolic pressure in group A. That in contrast left ventricular filling pressure did not change in group B most likely reflects a greater unloading effect of diltiazem in these patients with enlarged ventricles compared to group A.

**Coronary hemodynamic effects of diltiazem before pacing**

In this study, diltiazem induced a similar improvement in coronary flow in both groups. Such increase in flow has been reported previously, using equivalent high dosages of diltiazem as in the present study[7]. In contrast, with lower dose administrations, significant effects on coronary flow have not been observed[3,5,6,8-12,27]. Myocardial oxygen extraction decreased only in patients with impaired left ventricular function. As this effect was still present at the end of diltiazem administration, when coronary flow had already normalized, changes in myocardial oxygen demand are most likely responsible. In view of similar changes in rate-pressure product in both groups, the difference in myocardial oxygen extraction is best explained by the increase in left ventricular filling pressure and hence, in diastolic wall stress in Gr. A. The latter may have offset the effect of ventricular unloading on myocardial oxygen demand in the latter group, but not in patients with impaired left ventricular function.

**Hemodynamic changes during pacing with diltiazem in impaired versus normal left ventricular function**

Diltiazem reduced left ventricular systolic pressures during pacing and, subsequently, the double product. This effect was similar in both patient groups. In contrast, the effect on mean arterial pressure was different. In Gr. A mean arterial pressure again increased during pacing after diltiazem, as they did during the first, untreated pacing test. However, in patients with impaired ventricular function, arterial pressures remained unchanged during pacing after diltiazem, in contrast to a significant elevation during the first pacing test. An increase in systemic vascular resistance and in arterial pressures during pacing-induced ischemia has been reported[28,29] and may be proportional to the degree of ischemia[28]. The different response to pacing after diltiazem on arterial pressures in both groups may reflect less ischemia in patients with impaired ventricular function. We have recently reported, that a different calcium antagonist, bepridil, in a similar type study, prevented the increase in systemic resistance and in arterial pressures during pacing-induced stress[29]. As it also reduced left ventricular systolic pressure, we hypothetized that its antiischemic effect was predominantly due to its systemic vasodilating effects. The data of the present study also suggest that the systemic vasodilating effect of diltiazem plays a key role in reducing pacing-induced ischemia and that this effect is greater in patients with enlarged left ventricles. In contrast, its antiischemic effects apparently do not relate to an extra improvement of coronary flow during pacing. Coronary flow actually increased less during the second pacing test in Gr. B. Together with a significant reduction in myocardial oxygen extraction in this group, this reflects a greater reduction in pacing-induced oxygen demand after diltiazem in patients with an impaired ventricular function. Although a regional improvement of poststenotic subendocardial flow, undetected by the coronary sinus flow thermodilution technique, may still have occurred, particularly in this type of patients with enlarged left ventricles. It appears more likely that the greater reduction in wall stress following systemic vasodilation accounts for the difference in flow response and oxygen extraction during pacing.

**Antiischemic effects of diltiazem during pacing in impaired versus normal left ventricular function**

Diltiazem significantly reduced myocardial ischemia in both groups. It had similar effects on symptoms and on several objective signs of ischemia in patients with and without left ventricular dysfunction. However, there were indications, that the antiischemic effect of diltiazem was more pronounced in patients with impaired left ventricular function. Changes in myocardial lactate metabolism, identical in both groups during the first pacing-stress test, were significantly different

during pacing after diltiazem. In group A myocardial lactate production reoccurred during APST II, but not in patients with an impaired left ventricular function. This indicates a more pronounced antiischemic effect of diltiazem in the latter patient group. Even so, the increase in left ventricular end diastolic pressure also was less pronounced during pacing with diltiazem in patients with impaired versus normal ventricular function. Together with the better improvement of contractility in group B patients, this suggests that the antiischemic properties of high-dose diltiazem are more pronounced in patients with left ventricular dysfunction, at least during pacing-induced stress and ischemia.

### Plasma diltiazem levels

Plasma diltiazem levels were comparable in both groups. Despite high initial values, hemodynamic deterioration was not observed in either patient group, and no untoward clinical effect or side-effect was noted. Also, plasma diltiazem levels did not correlate with hemodynamic changes. Moreover, levels assessed just before discontinuation of pacing, were similar in both groups, indicating that the more pronounced antiischemic effects of diltiazem in group B patients did not relate to differences in plasma diltiazem levels.

### Limitations of the study

Pacing was carried out after and not during diltiazem administration. Thus, a potential extra antiischemic effect through drug-induced changes in coronary flow may have been missed. Moreover, diltiazem plasma levels during pacing were not in a steady state, but still declining. Optimally, the bolus administration should have been followed by a maintenance infusion of diltiazem and pacing carried out during steady state conditions. However, this would have increased the duration of this study too much.

### Clinical implications

This study indicates, that high dosages of intravenous diltiazem are safe and that ventricular function improves following such dose administration, in particular in patients with a depressed ventricular function at baseline. Moreover, as the antiischemic effects are more pronounced in the latter patient group, this may favour the use of high-dose diltiazem in patients with prolonged ischemia at rest or infarction. Here, not only the systemic vasodilating effects of diltiazem are important; effects, which result in a reduction in myocardial oxygen demand and, hence, in ischemia, and which may improve ventricular function. Also, the coronary vasodilating properties of high-dose intravenous diltiazem should act to improve the myocardial oxygen supply-demand ratio. Low-dose intravenous diltiazem already induces some beneficial effects in acute myocardial infarction in man[14]. Our study suggests that the potential for extra protection by high-dose diltiazem should be further evaluated.

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# Part 2

## Systemic and cardiac neurohumoral activation during acute myocardial ischemia in man. Rationale for converting enzyme inhibition in ischemia.

### A. Introduction and rationale for the studies.

Myocardial ischemia results in a complex chain of events, which is predominantly but not exclusively of cardiac origin. Apart from the metabolic alterations mentioned in chapter I and their hemodynamic sequelae, a large variety of other changes occur in the heart during ischemia, that are, as yet not touched upon.

**Free radicals.**

Oxygen-derived free radical formation, although directly linked to metabolism, has only been mentioned in passing. Although this phenomenon is generally considered in connection with reperfusion and reoxygenation[1], free radicals may also be generated from various sources during the acute phase of myocardial ischemia[2]. For instance, interruption of mitochondrial electron transport induces a shift from the normal tetravalent reduction of oxygen to water by cytochrome oxidase to univalent reduction and the formation of superoxide radicals, in particular superoxide anions[3-5]. Alternative free radical production during ischemia may occur secondary to phospholipase activation and arachidonic acid accumulation, subsequently metabolized to endoperoxides, and to the conversion of (hypo)xanthine by xanthine oxidase to urate, superoxide anion and hydroxyl peroxide. The latter enzyme is formed during ischemia by conversion from xanthine dehydrogenase, normally present and active during aerobic metabolism[6]. Besides enhanced production of free radicals, ischemia is also characterized by a decline in its normal cellular scavengers[7]. Accumulation of superoxide anion and hydroxyl peroxide may lead to production of the highly reactive hydroxyl radical, which subsequently forms additional toxic products, such as lipid radicals and lipid peroxides. As a result, (phospho)lipases become activated and a detergent action of increased free fatty acids on membranes is observed[8]. This leads to a destruction of the latter, to abnormalities in membranal calcium kinetics and to cellular osmotic swelling. As, in the human heart, significant amounts of hypoxanthine are produced at an early stage of ischemia[9,10], it has been hypothesized that free radical production by way of the xanthine oxidase system may be an important mechanism during acute ischemia in man. However, in contrast to animal species such as the dog, but comparable to the situation in rabbit or pig hearts, the human heart does not produce xanthine oxidase[11,12]. It is doubtful therefore, whether, during ischemia, the system really adds significantly to oxygen derived free radical production in the human heart.
Although recent observations during angioplasty suggested some urate release following ischemia and, hence, the presence of xanthine oxidoreductase in the human heart[13], it remains to be determined whether the xanthine oxidase form is really present during ischemia.
Apart from the myocardium, the vascular endothelium is also a target for free radical damage during ischemia. Besides enhanced permeability, free radicals may promote thrombogenicity, which, in part, depends on the interaction of the endothelial surface with blood elements, such as platelets.
Platelets generate superoxide anions[14], which, in turn, may promote their aggregation, further free radical release and, through serotonin production, may facilitate leucocyte adherence to the endothelium[15].

**Leucocytes.**

Leucocytes may amplify ischemic injury through leucocyte entrapment in microvessels and subsequent activation and release of potentially toxic materials, which may affect the vessels, blood constituents and the myocyte[16-19]. Moreover, accumulation of leucocytes may occur in response to ischemia-induced chemotactic factors, such as leukotriene $B_4$ or the cytokines, interleukin-1 and the tumor necrosis factor[20]. Activation of leucocytes also leads to oxygen-derived free radicals upon reoxygenation[21], to release of vasoconstrictor substances (the peptide leukotrienes $C_4$, $D_4$ and $E_4$ or 12-HETE[22], and to oxydation of EDRF[23].
Clearly, the potential to modulate leucocyte entrapment in the ischemic area, subsequent release of toxic substances and the pathophysiologic effects of the latter does seem an attractive issue, where the protection against ischemic myocardial damage is concerned. Indeed, depletion of circulating neutrophils may significantly reduce the extent of ischemic injury of the heart[24]. However, although pharmacological interventions with non-steroidal agents may induce beneficial effects in the stunned myocardium, not all NSAID's are effective in this respect. Moreover, some may even exacerbate myocardial ischemic injury and enhance scar thinning after

infarction[25,26]. The experimental design generally applied in these studies makes it difficult to dissociate pure ischemia-related events from reperfusion damage, even after short periods of myocardial ischemia[27].
A different mechanism, potentially involved in myocardial ischemia, relates to vasoconstrictor leukotrienes, synthesized by activated leucocytes. The peptide leukotrienes $LTC_4$ and $LTD_4$, administered intracoronary, induce coronary vasoconstrictory effects[28]. Following leucocyte accumulation in the ischemic area, these lipooxygenase products might therefore result in local coronary vasoconstriction. However, a recent study was unable to find appreciable amounts of these leukotrienes in the effluent from the ischemic myocardium[29]. The same authors reported, that changes in coronary vascular resistance were short lasting, only present for 2-4 minutes, despite ongoing leukotriene administration. In addition, some doubt has recently arisen concerning the potential enhancement of ischemia as a result of sustained coronary vasoconstrictor actions of peptide leukotrienes[30].

**Prostaglandins.**

Thus far, in this thesis, the effect of ischemia on prostaglandins has received limited attention. Theoretically, the combined effects of a reduced production of the endothelial vasodilator prostacyclin in the atherosclerotic endothelium and enhanced formation of the potent vasoconstrictor thromboxane $A_2$ from aggregating platelets on the abnormal endothelial surface, should significantly affect both origin and propagation of ischemia.
Whereas, in the intact, endothelialized coronary circulation, thromboxane $A_2$ does not behave as potent vasoconstrictor, possibly through modulatory effects of locally produced vasodilator mechanisms, this is entirely different in diseased arteries. In models of critical coronary narrowing or endothelial injury, thromboxane $A_2$ results in cyclic blood flow reductions coupled to platelet aggregation[31,32]. Acetyl salicylic acid prevents this flow reduction[32]. The major source of thromboxane $A_2$ in ischemia is the aggregating platelet on the abnormal endothelial surface, although some production in different tissues, such as the vascular wall or the ischemic myocardium, cannot be excluded[33]. Enhanced blood flow velocity may amplify this effect of platelet aggregation. Consequently, attention has been paid to the impact of eicosanoid metabolism using models of increased coronary blood flow velocity.
Animal studies apart[34], several human studies have tried to establish the true significance of an abnormal production of the cyclooxygenase products during myocardial ischemia. Back in 1977 Berger and coworkers reported increased release of prostaglandin F from the heart during pacing-induced ischemia[35]. This was later followed by observations on enhanced thromboxane $A_2$ release as a result of vasospastic angina[36,37] and during pacing[38]. However, reports on cardiac prostaglandin synthesis in man have been rather contradictory since, greatly due to the inherent difficulties of sampling platelet-derived eicosanoids through long catheters.
Whereas thromboxane production may be important in unstable angina or after thrombolysis[39,40], it is at present not quite clear whether pursuing this line of research in stable angina pectoris with the aim of finding alternative antiischemic therapies[41,42] is really worthwhile. In this respect, our own preliminary results do not indicate that myocardial ischemia induces platelet aggregation in the ischemic area[43]. Neither does it result in enhanced thromboxane release and/or diminished prostacyclin production. In contrast, we have found evidence for enhanced circulating thromboxane $A_2$ levels in the course of pacing-induced ischemia in patients with simultaneous neurohumoral activation and systemic vasoconstriction (unpublished observations).

**Myocardial ischemia and neurohumoral activation.**

A different area and one which, at least in humans, has received little attention, is that which covers the interaction between myocardial ischemia on the one hand and peripheral and cardiac neurohumoral activation on the other.
Whereas ample attention has been paid to occurrence and effects of sympathetic stimulation and activation of circulating neurohumoral systems in disease entities, such as heart failure or acute myocardial infarction, very little is known of the interaction, if any, of myocardial ischemia and neurohormones.
Studies in animal models have demonstrated unequivocal evidence of profound cardiac sympathetic stimulation in severe and, particularly, in prolonged episodes of myocardial ischemia[44,45]. These studies indicate, that cardiac catecholamine release, generally accepted as proof of sympathetic activity, does increase both as a function of the degree of ischemia, but also of time[44,46], suggesting that a certain period of ischemia is needed before enhanced cardiac sympathetic tone becomes evident.

In fact, these data would indicate that short periods of myocardial ischemia, such as encountered during stress testing in humans, may be insufficient to induce cardiac catecholamine release as evidence of enhanced cardiac sympathetic tone. This may explain why the few investigations carried out in man have never been able to detect an activated cardiac sympathetic tone during pacing-induced myocardial ischemia[47,49]. Also, it is not clear whether myocardial ischemia may affect systemic neurohormones, mainly because the matter has not been given sufficient attention, neither in the animal model nor in man. Published data on the significance of neurohumoral changes in myocardial ischemia relate predominantly to the topic of sudden death or arrhythmias in the context of ischemia and infarction and reflect studies on cardiac sympathetic activity and/or catecholamine balance in animal models of ischemia[50,51].
Certainly, the interaction of moderate, short lasting myocardial ischemia and peripheral neurohumoral mechanisms in man is incompletely understood. Studies have generally concentrated on angina pectoris rather than on the presence of objectivated myocardial ischemia[47-49,52-54]. No effort has been made to relate such changes, if present, with the degree of myocardial ischemia. Still, as in acute myocardial infarction cardiac dysfunction is directly related to the degree of peripheral neurohumoral activation, such a comparison would certainly make sense in ischemia.
Moreover, studies in humans during ischemia have only considered changes in circulating catecholamines. Recent animal studies, however, do suggest that other, albeit related, neurohumoral systems may also become significantly stimulated[55,56] and may contribute to hemodynamic alterations, secondary to this neurohumoral activation. In particular, the renin-angiotensin system could be important in this respect. A coronary constrictive effect of angiotensin II has been suggested[57]. In contrast, arginine vasopressin does not seem to be involved in ischemia[57], whereas the role of ANF is as yet unclear. However, the latter may function as coronary vasodilator in situations, where, in the course of ischemia, coronary vasoconstriction ensues as a result of catecholamine or renin-angiotensin activation.
During the previous four years we have focused on the aspects of systemic and transcardiac neurohumoral changes resulting from myocardial ischemia. Our studies did not concern patients in whom the significance of neurohumoral interactions has been appreciated already, e.g. in heart failure or hypertension. In contrast, we have concentrated on the typical patient with coronary artery disease, e.g. the normotensive individual with stable, exercise-inducible angina without signs of heart failure.

Our studies have been carried out in a sizeable group of patients, in contrast to the small patient studies published thus far, and have concentrated on catecholamines and the circulating renin-angiotensin system. In chapter X, following an introductory article, our first results with respect to systemic and cardiac neurohumoral changes during ischemia and their effect on systemic and coronary hemodynamics are reported.
The next and the last chapter of this thesis, chapters X and XI, deal with the potential antiischemic effects of specific modulation of these neurohumoral alterations, e.g. by converting enzyme inhibition.
Again, this chapter commences with an introductory article on the possible role of ACE inhibition in myocardial ischemia and angina, followed by a description of the acute antiischemic properties of converting enzyme inhibition and the underlying cardiovascular and neurohumoral properties of the compound studied, enalaprilat. This investigation, carried out in our pacing stress test model, is unique as it is the first of its kind to study the acute antiischemic properties of ACE inhibition in relation to its modulating effect on systemic and transcardiac neurohormones in the normotensive individual with ischemic heart disease without signs of heart failure.

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# PART 2

## Systemic and cardiac neurohumoral activation during acute myocardial ischemia in man. Rationale for converting enzyme inhibition in ischemia.

## B. Specific studies.

# Chapter X

# NEUROHUMORAL CHANGES DURING MYOCARDIAL ISCHEMIA.

# X.1.

# Neuroendocrine Activation in Ischemic Cardiomyopathy without Failure and in Acute Myocardial Ischemia.

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# NEUROENDOCRINE ACTIVATION IN ISCHEMIC CARDIOMYOPATHY WITHOUT FAILURE AND IN ACUTE MYOCARDIAL ISCHEMIA

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**SUMMARY. Systemic neurohumoral activation is a common manifestation in congestive heart failure, irrespective of etiology, i.e., resulting from ischemic or other forms of cardiomyopathy. It is also a feature of acute myocardial infarction, particularly when accompanied by heart failure. However, whether systemic and/or cardiac neuroendocrine activation also occurs in other aspects of ischemic heart disease is, as yet, not fully established. Thus far, reports on neurohumoral changes during exercise-induced angina have been conflicting. In contrast, recent observations made during pacing-induced ischemia would suggest significant elevations in circulating catecholamine and renin-angiotensin levels. These neurohumoral changes, which are accompanied by systemic vasoconstriction, do not necessarily reflect the stress of anginal pain, but could also be the resut of ischemia-induced left ventricular dysfunction. In this model of pacing-induced ischemia, enalaprilat has been reported to prevent both myocardial ischemia and the rise in systemic catecholamines and angiotensin II, which may indicate a role for ACE inhibition in myocardial ischemia. Finally, preliminary data are presented that indicate neuroendocrine activation may be an early feature of ischemic cardiomyopathy, before the occurrence of heart failure symptoms. In resting, supine patients with ischemic heart diseasee, arterial angiotensin II levels were increased in asymptomatic patients with left ventricular dysfunction, as compared with patients with a normal cardiac function. Although the clinical significance of this observation is as yet unclear, it may be an additional argument for studying the usefulness of ACE inhibition in asymptomatic ischemic cardiomyopathy.**

**KEY WORDS. ischemic cardiomyopathy, myocardial infarction, myocardial ischemia, catecholamines, renin-angiotensin, ACE inhibition**

In certain aspects of ischemic heart disease, such as heart failure due to ischemic cardiomyopathy or acute myocardial infarction, neuroendocrine activation is well established. Generally it occurs as a result of cardiac dysfunction. As it may impose an additional burden on the already jeopardized ventricle through its vasoconstrictor effects, it has a significant impact on the ultimate cardiovascular profile and clinical condition of the patient. Although attention has been focused predominantly upon the sympathetic nervous system and on circulating catecholamines, it has recently been realized that other systems are involved as well, which are both vasoconstrictor and vasodilator in nature, such as arginine vasopressin and circulating and local renin-angiotensin systems. Moreover, there are indications that neuroendocrine activation may also occur in other manifestations of ischemic heart disease. Before paying attention to neuroendocrine activation in heart failure or acute myocardial infarction, this article will also focus on neurohumoral changes in some of these other aspects, such as acute myocardial ischemia or ischemic cardiomyopathy with left ventricular dysfunction, but without concomitant heart failure.

## Congestive Heart Failure Due to Ischemic Cardiomyopathy

Systemic neurohumoral activation is a well-known phenomenon in congestive heart failure, whether due to ischemic or other types of cardiomyopathy. During the more advanced stages of failure, both the progressive reduction in cardiac pump function, arterial blood pressure, and renal perfusion, as well as the subsequent administration of diuretics and vasodilators, all result in an increase in sympathetic tone, circulating catecholamines, and arginine vasopressin and in activation of the renin-angiotensin system [1–4]. Available data suggest that the magnitude of this secondary neuroendocrine activation is presumably similar in heart failure due to ischemic heart disease or resulting from other forms of cardiomyopathy [5]. Plasma levels of norepinephrine and dopamine are usually elevated in heart failure [1, 2, 6], regardless of etiology. Individual levels vary widely, however, and, although in some studies a correlation with resting hemodynamic impairment has been

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suggested [5, 7], they do not really reflect the severity of heart failure [2, 5, 6]. Whereas systemic renin-angiotensin levels are usually markedly elevated during the acute phase of cardiac decompensation, they are normal or only moderately increased in stable, chronic heart failure [3]. Moreover, spontaneous but important daily fluctuations in renin and aldosterone levels have been reported in heart failure patients [8]. Nevertheless, despite the apparent lack of correlation between the severity of heart failure symptoms and circulating neurohormones, elevated systemic catecholamine and renin-angiotensin levels appear to be negatively correlated with the prognosis *ad vitam* [9, 10].

## Neurohumoral Activation in Acute Myocardial Infarction

Acute myocardial infarction is another aspect of ischemic heart disease in which neuroendocrine activation is observed. It has been known for a long time that acute myocardial infarction leads to enhanced sympathetic nervous stimulation and significant elevations in plasma and urinary catecholamine levels [11–13]. In addition, there is an increase in norepinephrine release from the infarcted area with a gradual, but ultimately complete, loss of myocardial norepinephrine levels during the first 4 days after coronary occlusion [14]. Moreover, myocardiac norepinephrine content also declines in noninfarcted regions, followed by only a very gradual return to control, a process that may continue up to 40 days after infarction.

The rise in systemic and urinary catecholamines occurs very early, within 1 hour of the onset of symptoms [15], and appears to be related to the hemodynamic status of the patient and to the presence of ventricular dysfunction [16, 17]. Data from animal experiments suggests that during global ischemia there is a progressive overflow of norepinephrine from the heart, which starts relatively late [18]. In contrast, early ischemia, i.e., of 10 minutes duration or less, does not result in significant changes in norepinephrine release [18, 19].

Systemic arginine vasopressin levels are also markedly elevated during acute myocardial infarction, in addition to the increase in circulating catecholamine levels [20]. Again, changes are observed early after the onset of infarction. Although vasopressin values are highest in patients with acute left ventricular failure, generally they appear to relate predominantly to the administration of opiates.

Elevated plasma renin levels have been described in some patients with acute myocardial infarction, but only in those complicated by heart failure or by ventricular dysrhythmias [21, 22]. Recently, however, McAlpine and coworkers have indicated that the renin-angiotensin system may become activated in all patients with a myocardial infarction [20]. This generally occurred at a later stage during infarction, from 24 to 48 hours after the onset of symptoms, depending on the absence or presence of left ventricular failure. Although the authors indicated that the magnitude of the renin-angiotensin activation related predominantly to acute diuretic treatment in their study, renin-angiotensin levels also became elevated in patients without heart failure and not receiving diuretic therapy. These neurohumoral changes lasted for at least 10 days after infarction in patients who also developed heart failure symptoms. Unfortunately, no data are available on renin-angiotensin levels after this time.

As beta blockade has the potential to reduce the detrimental effects of enhanced sympathetic stimulation and increased catecholamine levels in acute myocardial infarction, this form of therapy has received much attention. In contrast, the information available regarding the usefulness of ACE inhibition in this setting in humans is very limited. Data from animal studies indicate that this form of therapy may reduce ischemic injury and limit infarct size [23–25]. Up until now, the only information available on the effect of ACE inhibition in humans comes from studies carried out in myocardial infarctions that were complicated by left ventricular failure [26, 27]. The recent observation that the renin-angiotensin system is also activated in uncomplicated infarcts may indicate that the utility of ACE inhibition should also be studied in the latter circumstances to assess whether it may further improve both ventricular function and, ultimately, the prognosis, in addition to its late beneficial effect on the infarcted ventricle during long-term therapy [28, 29].

## Neuroendocrine Activation in Ischemic Cardiomyopathy without Heart Failure

Relatively little is known of neuroendocrine activation in other manifestations of ischemic heart disease than acute myocardial infarction or heart failure due to ischemic cardiomyopathy. Some years ago, Swedberg and coworkers studied resting plasma catecholamine levels and myocardial catecholamine balance in patients with angina pectoris without heart failure

and in subjects with chronic heart-failure symptoms [30]. Patients with angina and, presumably, a normal left ventricular function had normal arterial and coronary sinus norepinephrine levels that were significantly different from the markedly elevated values found in heart failure patients (Figure 1A). No such differences were observed in epinephrine levels. Moreover, in contrast to the marked cardiac norepinephrine release in heart-failure patients, cardiac norepinephrine efflux was not observed in anginal patients (Figure 1B).

At present it is unknown whether neurohumoral levels are still normal in patients with ischemic cardiomyopathy and left ventricular dysfunction but without heart failure symptoms. We have recently studied this question in 45 patients with coronary artery disease, 28 with a normal left ventricular function, eight with left ventricular dysfunction but no clinical signs of heart failure, and nine patients with chronic, mild-to-moderate congestive heart failure. All cardiac therapy was stopped 24–72 hours before the investigation. Only patients with congestive heart failure received diuretics, which were withheld 24 hours before the study. Patients were fasting and had been supine for at least 1 1/2 hours before neurohumoral evaluation. Also, instrumentation was carried out at least 30 minutes before neurohumoral determinations.

The ejection fraction and contractility parameters were reduced, and left ventricular volumes increased in both the asymptomatic patients with left ventricular dysfunction as well as in heart-failure patients (Table 1). However, left ventricular filling pressure and systemic resistance were still in the normal range and cardiac output only moderately diminished in asymptomatic patients with left ventricular dysfunction.

In contrast, these parameters were clearly abnormal in heart failure patients. Asymptomatic patients, therefore, appeared to do rather well, both hemodynamically and clinically, despite their ventricular dysfunction. Nevertheless, there were some early neurohumoral changes in this group that were not observed in patients with a normal ventricular function. This early neuroendocrine activation in asymptomatic patients with left ventricular dysfunc-

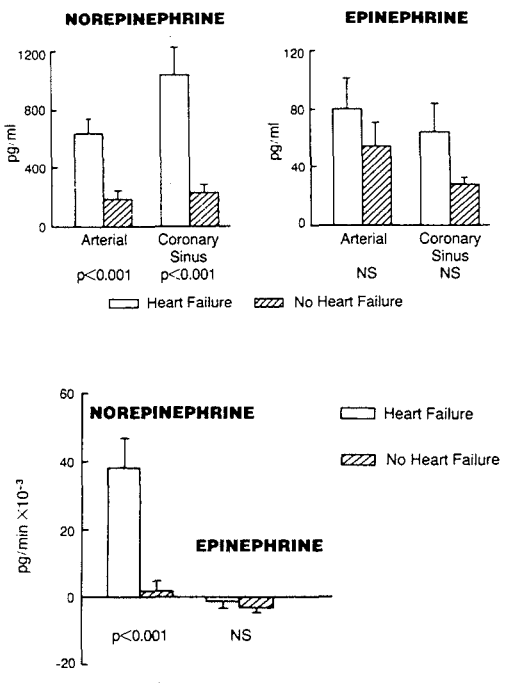


*Fig. 1. A. Plasma norepinephrine and epinephrine levels in patients with heart failure and in patients with angina pectoris without clinical signs of failure. In the latter group plasma catecholamine levels are normal. In contrast, norepinephrine values are significantly elevated in heart failure patients. Adapted from Swedberg et al. [30] with permission. B. Myocardial catecholamine balance in heart failure patients and in patients with angina without signs of heart failure. In the latter patients, there is neither significant catecholamine release nor uptake. In contrast, marked norepinephrine release is present in heart failure patients. Adapted from Swedberg et al. [30] with permission.*

*Table 1. Resting hemodynamic values in patients with coronary artery disease with or without LV dysfunction and heart failure*

| | Normal LV function (n = 28) | Asymptomatic LV dysfunction (n = 8) | LV dysfunction with heart failure (n = 9) |
|---|---|---|---|
| LV ejection fraction (%) | 64 ± 0.03 | 33 ± 0.05[a] | 24 ± 0.03[a] |
| LV end-diastolic volume (ml/min/m²) | 65 ± 5 | 103 ± 15[a] | 120 ± 14[a] |
| Cardiac output (l/min) | 6.2 ± 0.3 | 5.6 ± 0.5 | 4.0 ± 0.4 |
| $V_{max}$ ($sec^{-1}$) | 56 ± 2 | 32 ± 3[a] | 24 ± 2.5[a] |
| LV end-diastolic pressure (mmHg) | 13 ± 1.5 | 15 ± 3 | 24 ± 3[a,b] |
| Systemic resistance ($d.s.cm^{-5}$) | 1380 ± 85 | 1497 ± 140 | 1880 ± 135[a,b] |

x ± SEM: [a]p < .05 vs. normal LV function; [b]p < .05 vs. asymptomatic LV dysfunction. LV = left ventricular.

tion was not evidenced by sympathetic stimulation and increased catecholamine levels. Arterial norepinephrine values were, in fact, only elevated in heart-failure patients, whereas dopamine and epinephrine levels were comparable and normal in all patients. In contrast, arterial angiotensin II levels not only increased in heart failure patients, but also in asymptomatic patients with ventricular dysfunction (Figure 2).

The clinical significance of this early but still modest renin-angiotensin activation in asymptomatic patients with chronic left ventricular dysfunction is as yet unclear. It apparently did not result in systemic vasoconstriction in these patients. Also, in view of a normal cardiac output and blood pressure and in the absence of diuretic treatment, it is not easily comprehended which signal initiates this neuroendocrine activation. Our observations were made in relatively small groups of patients, and data, for the moment, should be considered to be preliminary. However, their potential significance should be further explored in a greater number of asymptomatic patients with ventricular dysfunction, whether or not of ischemic origin. That ACE inhibition may be useful in limiting ventricular dysfunction in the postinfarct period has already been strongly suggested [31, 32]. It may well be that its application should not be limited to this patient group only.

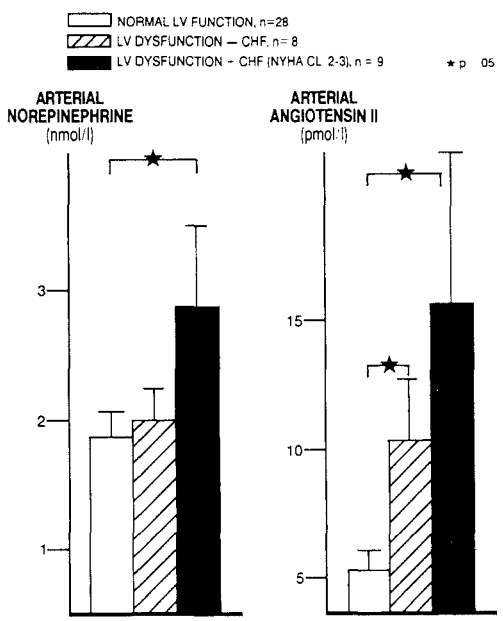


*Fig. 2. Arterial norepinephrine (NE) angiotensin II (A II) levels in patients with coronary artery disease and normal left ventricular (LV) function (LV ejection fraction ≥ 45%, n = 28), LV dysfunction (LV ejection fraction ≤ 45%, n = 8) without and with congestive heart failure (CHF, n = 9). Whereas norepinephrine (NE) levels are only elevated in heart failure patients, A II values are also significantly different in patients with asymptomatic LV dysfunction compared with patients with normal LV function.*

## Neurohumoral Activation During Acute Myocardial Ischemia

Very little attention has been paid to neurohumoral activation as a result of myocardial ischemia, despite the fact that such activation is likely to occur secondary to the stress of anginal pain. In contrast to the observations in acute myocardial infarction, the few data available in stress- or pacing-induced ischemia do not indicate a significant rise in systemic or urinary catecholamine levels [33–35], at least not when compared with the neurohumoral changes found in normal individuals at comparable workloads and when only the more recent studies are taken into account, in which more sensitive and discriminating techniques for the assay of catecholamines were applied [36].

Although early studies did indicate increased catecholamine levels during exercise-induced angina [12], more recent investigations could not corroborate these observations [33]. In contrast, Robertson and coworkers reported a rise in arterial and coronary venous norepinephrine and epinephrine levels, not during stress-induced ischemia, but as a late phenomenon after spontaneous coronary spasm and anginal pain had developed [37]. Thus far, the effect of short periods of myocardial ischemia on systemic and/or transcardiac renin-angiotensin levels has not yet been evaluated in humans. Recent data from our laboratory indicate that during and for several minutes after (pacing-induced) ischemia, a significant elevation of systemic angiotensin II levels occurs without concomitant changes in coronary venous levels [38, 39]. No such changes are observed in patients with coronary artery disease in whom ischemia does not occur, despite identical alterations in the rate-pressure produce during pacing. Moreover, a significant increase in both the arterial and coronary venous norepinephrine values are found during ischemia (Figure 3), again without any such effect in nonischemic patients. These latter observations contrast with previous, similar-type studies in humans [34, 35], which may be due to differences in biochemical techniques or in the study protocols used. In our study specific attention is paid to the direct post-

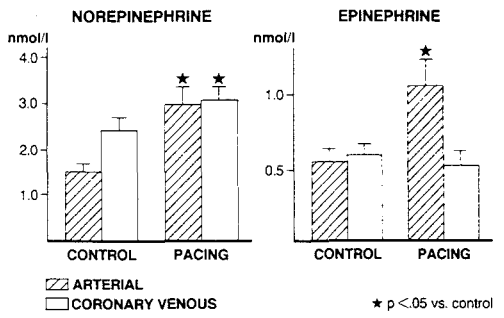


*Fig. 3. Changes in arterial and coronary venous norepinephrine and epinephrine levels during pacing-induced ischemia in patients with coronary artery disease. Compared with control values, arterial and coronary venous norepinephrine and arterial epinephrine levels increase significantly when measured during maximal pacing rates and ischemia.*

pacing period with sequential sampling of blood during the first 2 minutes after pacing. Thus, the largest increase in norepinephrine and angiotensin II levels is observed at 1 minute postpacing and not during maximal rates before the cessation of pacing [39]. The clinical significance of these neurohumoral changes during ischemia relates presumably to the observation that, concomitantly and only in ischemic patients, arterial pressures become significantly increased and that as late as 5 minutes after pacing systemic vascular resistance is still elevated. This systemic vasoconstriction beyond doubt is secondary to the increase in activation of circulating neurohormones and poses an additional burden on the ischemic myocardium.

## Effect of Ischemia on Cardiac Norepinephrine Release

Besides peripheral neurohumoral changes, ischemia-induced alterations in cardiac catecholamine fluxes are also likely to occur. Since during myocardial ischemia and subsequent accumulation of various metabolites reuptake of norepinephrine by nerve endings in the heart may be inhibited, this could result in increased cardiac norepinephrine release. The latter, however, has not been consistently demonstrated [34, 35].

Enhanced cardiac norepinephrine release is invariably present in animal models of infarction or ischemia. However, for this to happen ischemia has to persist for at least 10 minutes [20]. This, presumably, is why in human studies, where (pacing-induced) ischemia is usually present for only a few minutes, the expected increase in cardiac norepinephrine release has never been observed. In contrast, we have recently demonstrated that myocardial ischemia actually results in a short-lasting reversal of norepinephrine release, which is commonly present at baseline, to uptake during the ischemic period [40]. This, however, is only observed in patients who also produce lactate during ischemia (Figure 4). As changes in cardiac norepinephrine balance do not occur in nonlactate producers or in nonischemic patients; this would suggest that norepinephrine release is only affected in the ischemic area. Richardt and coworkers have suggested that local adenosine production may inhibit cardiac noradrenaline release that is evoked by nerve stimulation during the very early phase of myocardial ischemia [41]. This would agree with both of our previous reports on sequential nucleoside production by the human heart in this model of pacing-induced ischemia [42, 43] and our recent observations on cardiac norepinephrine balance in this setting.

## Do Ischemia-Induced Neurohumoral Changes Only Result From Angina?

A plausible way to explain neurohumoral changes during pacing-induced ischemia is to relate these

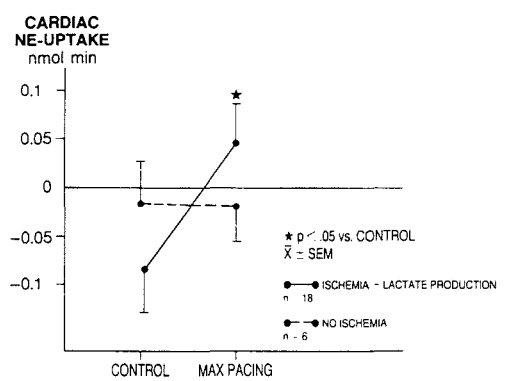


*Fig. 4. Effect of ischemia on cardiac norepinephrine (NE) uptake. In patients who during ischemia produce lactate, a significant change from, cardiac NE release present during control to uptake during ischemia, is observed, which is absent in nonischemic patients.*

effects to the stress of pacing-induced anginal pain. We recently studied patients with symptomatic and asymptomatic ischemia, again with atrial pacing, and compared the results with a nonischemic group. It was clear from this study that neurohumoral activation was only present in patients who developed angina during pacing [44]. In contrast, no changes occurred in either nonischemic or silent ischemic patients. However, symptomatic and silent ischemic patients differed with respect to the absence or presence of angina, and, despite similar electrocardiographic changes, ischemia was clearly more severe in anginal than in asymptomatic patients. This suggests that the neurohumoral changes observed in anginal patients may be the result of ischemia-induced ventricular dysfunction rather than being caused by angina alone.

## Therapeutic Implications of Ischemia-Induced Neurohumoral Activation During Ischemia

The neuroendocrine changes caused by ischemia in our studies indicate that measures limiting this neurohumoral (over)activation and its effect on vascular tone may be of therapeutic value. It is of interest that bepridil, a compound with predominant calcium-entry blocking properties, effectively inhibits ischemia-induced vasoconstriction in the same type of study and patients [45]. As calcium antagonists indirectly attenuate postsynaptic alpha$_2$-receptor-mediated vasoconstriction [46], their usefulness as vasodilating agents may be particularly important during acute ischemia through these mechanisms. However, compounds that directly affect neurohumoral production may be of even greater value in this respect. Thus, it may be useful to evaluate agents that, through presynaptic DA$_2$-receptor modulation or angiotensin-II-receptor inhibition, may reduce norepinephrine release from nerve endings during myocardial ischemia. Likewise, ACE inhibitors may have antiischemic properties through a decrease in local and/or circulating angiotensin II and, subsequently, less activation of this hormone during ischemia. Recently, attention has focused on the potential antiischemic and antianginal effects of ACE inhibition during exercise in normotensive patients with ischemic heart disease. Although in some studies a positive effect was shown [47–49], other investigations were unable to detect a significant antiischemic activity of ACE inhibitors during exercise-induced angina [50, 51]. We have recently demonstrated that enalaprilat reduces pacing-induced ischemia, not through systemic hemodynamic effects, but presumably by limiting neurohumoral activation during ischemia [52]. The significance of the latter effect may be lost during the pronounced stimulation of circulating neurohormones that invariably occurs during exercise [53]. This could also explain the inconsistency of the antiischemic effects of ACE inhibition with exercise-induced stress. In contrast, in situations where ischemia is not exercise related, further exploration of the usefulness of ACE inhibition as an antiischemic therapy is certainly worthwhile.

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# Chapter X.2.

## Systemic Neurohumoral Activation and Vasoconstriction Pacing-induced During Acute Myocardial Ischemia in Patients with Stable Angina Pectoris

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### ABSTRACT

To identify the effect of short periods of myocardial ischemia on systemic neurohormones and vascular resistance, 32 untreated, resting, normotensive patients with coronary artery disease underwent incremental atrial pacing until angina. Arterial and coronary venous lactate levels and arterial values of catecholamines and angiotensin II were determined at control, maximum pacing rates and at 1, 2, 5 and 30 minutes post-pacing. Based on pacing-induced ST segment depression ($\geq$1 mm) and/or myocardial lactate production, patients were selected as ischemic (I, n=25) or non-ischemic (NI, n=7). Baseline clinical, hemodynamic and angiographic data were comparable, except for more severe preexisting angina and coronary lesions in I. During pacing, chest pain was similar [78% (I) vs 72% (NI)] and hemodynamics comparable, except for contractility, which did not improve, and left ventricular end diastolic pressure, which increased significantly by 79% in I. Also, ischemia significantly increased arterial pressures and systemic resistance (13% and 11%, resp.) not observed in NI. Pacing did not affect neurohormones in NI. In contrast, in I arterial norepinephrine increased significantly from 1.7±0.2 (control) to 2.6±0.3 (maximal pacing rates) and further to 3.0±0.4 nmol/l (1 min post-pacing). Concomitantly, angiotensin II levels rose from 6.2±1.4 (control) to 9.3±2.1 pmol/l (1 min post-pacing, $p<0.05$). Arterial epinephrine only increased during maximal rates (0.93±0.13 vs 0.60±0.09 nmol/l at control, $p<0.05$). Thus, pacing-induced myocardial ischemia activates circulating catecholamines and angiotensin II, accompanied by systemic vasoconstriction. As the latter amplifies myocardial ischemia, modulation of this neuroendocrine stimulation may be of therapeutic value.

### INTRODUCTION

In humans, systemic catecholamines and circulating renin-angiotensin levels become activated during acute myocardial infarction, depending on the degree of cardiac dysfunction [1-6]. Whether myocardial ischemia also results in neuroendocrine activation, as a result of an acute, ischemia-induced reduction in cardiac pump function, is less well defined. In humans, available data do not uniformly indicate significant changes in plasma or urinary catecholamines during exercise- or pacing-induced ischemia[2,7-11]. Whereas an early study suggested increased catecholamine levels during exercise-induced angina[2], this could not be confirmed in a more recent investigation in which more sensitive and specific biochemical techniques were applied[7]. Apart from these conflicting reports, it is unknown whether myocardial ischemia affects the circulating renin-angiotensin system. Furthermore, no data exist on the hemodynamic consequences of ischemia-induced systemic neurohumoral activation in man. The present study investigates sequential changes in systemic neurohormones and hemodynamics during pacing-induced ischemia in normotensive patients with coronary artery disease and in similar patients, who did not develop ischemia during the test.

## MATERIALS AND METHODS

### Patient population:

After the protocol was approved by the institutional ethical review board and informed consent obtained, 32 patients, 29 men and 3 women, average age 57 years (range 37 to 69 years) were studied. Subjects were referred for coronary angiography for the evaluation of angina-like chest pain. Objective signs of ischemia or a previous myocardial infarction were not obligatory for inclusion, but patients had to be normotensive without symptoms of heart failure. Exclusion criteria included previous myocardial infarctions <1 month old, unstable angina, valvular heart disease, conduction disturbances or the use of diuretics.
Cardiac therapy was withheld 36-72 hours pre-study. Only short-acting nitroglycerin was allowed until 6 hours before the investigation. Significant coronary artery disease, defined as ≥70% diameter narrowing, was present in 28 patients, 10 with single-, 11 with double- and 7 with triple-vessel disease. Objective signs of ischemia during pre-study ergometry testing (≥0,1 mV ST-segment depression) were present in 22 patients, whereas 18 had ≥1 documented myocardial infarctions.

### Catheterization procedures:

Patients were studied at the same time in the morning after an overnight fast, without premedication. After coronary angiography, using the Seldinger technique, an 8 Fr Sentron pigtail microtip manometer catheter was advanced into the left ventricle through a 9 Fr arterial Desilet introducer system in a femoral artery. The side-arm of this system was used to measure arterial pressures. A 7 Fr triple-lumen thermodilution catheter was positioned with its tip in a pulmonary artery. Finally, a 7 Fr Zucker bipolar pacing catheter or 7 Fr thermodilution pacing catheter was advanced into the mid portion of the coronary sinus, such that the tip of the catheter was at least 3 cm beyond the orifice of the coronary sinus, its position stable and rapid blood sampling was ensured. The position of the catheters was subsequently recorded on video-disc and frequently checked during the study.

### Measurements and calculations

Fluid-filled catheters were calibrated, using Bentley transducers with a zero reference level set at mid chest. The micromanometer pressure was balanced to zero and superimposed on the conventional pressure recording. All pressures, the first derivative of left ventricular pressure and the thermodilution-derived cardiac output signal were recorded on paper at different paper speeds, using a CGR 1000 cathlab system. Pressures and pressure-derived contractility indices were measured on-line from 12 to 15 successive beats, using a Mennen Medical cath lab computer system. Likewise, cardiac output measurements were determined on-line. At the end of the study, the femoral arterial pressure curves were compared with simultaneous recordings from the ascending aorta by the Sentron catheter.

### Hemodynamic variables:

Measured variables included left ventricular peak systolic and mean and end diastolic pressures (mmHg), pressure-derived contractility indices [left ventricular dP/dt (mmHg/sec) and Vmax ($sec^{-1}$)], mean and phasic systemic and pulmonary arterial pressures (mmHg), mean right atrial pressure (mmHg) and cardiac output (l/min). From these, systemic vascular resistance (dynes.s.$cm^{-5}$), stroke index (ml/beat/$m^2$) and stroke work index (g/m/$m^2$) were calculated[12].

### Electrocardiographic parameters:

Leads I, II and V5 were monitored continuously to measure heart rate. ST-segments were determined at a paper speed of 100 mm/sec in 3 successive beats, 60 msec after the J-point of the QRS-complex, using a calibrated magnifying lens.

### Metabolic and neurohumoral parameters:

For the assay of lactate, 1 ml of blood was withdrawn simultaneously from the left ventricle and the coronary sinus in ice cold 8% $HCLO_4$ and analysed enzymatically[13]. For the determination of catecholamines and angiotensin II, 6 ml of blood was collected from the left ventricle in ice cold syringes. Of these, 3 ml were quickly transferred into precooled tubes containing 500 I/U heparin and 3 mg/ml glutathion for the catecholamine assay and 3 ml into precooled tubes containing 4 mg EDTA and 0.06 mg O-fenantrolin for the assessment of angiotensin II. Blood samples were then immediately centrifuged under cooled conditions at 3000 revs/min for 10 minutes and frozen at -20°C. Catecholamines: norepinephrine, epinephrine and dopamine were determined by radioenzymatic assay, using high pressure liquid chromatography to separate the radio-

active products[14]. Angiotensin II was assessed by radioimmunoassay[15].

### Study protocol

Repeated control measurements of all variables were carried out at approximately the same time in the morning, 1.5 to 2 hours after entering the catheterization laboratory, 20 minutes following instrumentation and at least 40 minutes after coronary angiography.
Throughout this period patients were supine. Subsequently, atrial pacing stress testing was performed with increments in heart rate of 10 beats/2 minutes until significant angina-like pain, atrioventricular block or a maximum pacing rate of 170 beats/minute. Significant angina-like pain was defined as the level of pain, at which the patient typically would rest or take nitroglycerin.
All variables were redetermined at maximal pacing rates, just before cessation of pacing, followed by measurements at 1, 2, 5 and 30 minutes post-pacing. In addition, left ventricular end diastolic pressure and lactate were measured at 10 and 15 seconds post-pacing, resp.
Due to the complexity of the study, cardiac output determinations were not carried out until 3-5 minutes post-pacing.

**Table I: Baseline clinical an angiographic characteristics of ischemic and non-ischemic patients**

| | Ischemic (I, n=25) | Non-ischemic (NI, n=7) |
|---|---|---|
| Sex | 25 M / 1 F | 5 M /2 F |
| Age (years) | 55±1.8 | 65±1.2 |
| Positive X-ECG | 19 | 3 |
| Previous Infarct | 15 | 3 |
| Coronary angio (>70% stenosis) | | |
| 0-vessel | 0 | 4 |
| 1-vessel (LCA) | 7 | 3 |
| 2-vessel | 11 | 0 |
| 3-vessel | 7 | 0 |
| Left ventricular ejection fraction | 53±2.9 | 51±6.3 |
| Left ventricular end diastolic volume | 63±4.3 | 69±8.8 |

*Abbreviations: F = Female; LCA = Left Coronary Artery; M = Male; X-ECG = Exercise test.*

### Statistical analysis

Changes from baseline values were calculated for neurohormones in each patient. Statistical differences were assessed using a paired t-test with a p value less than 0.05 considered significant (2-sided). Baseline values for hemodynamic variables and changes from control during pacing were compared in patients, who during the test became ischemic and those who did not, using an unpaired Student t-test with a p value <0.05 to detect significant differences. Values are given as averages ($\bar{x}$) and standard errors of the mean (SEM).

## RESULTS

### Control period

At baseline, none of the patients had clinical signs of ischemia. Values for norepinephrine and epinephrine ranged between 0.7 and 3.15 nmol/l and between 0.17 and 1.55 nmol/l, resp. Angiotensin II varied from <2 (in 2 patients) to 24 pmol/l.

Pacing period

Based on objective signs of ischemia during pacing, e.g. ST-segment depression of >1 mm and/or myocardial lactate production, patients were classified as ischemic (I, n=25) or non-ischemic (NI, n=7). Pacing-induced chest pain was present in 20 patients in I and in 5 in NI. Both groups were comparable as to sex, age, number of previous myocardial infarcts, left ventricular volumes and ejection fraction (Table I). In contrast, objective signs of ischemia during pre-study exercise tests and significant coronary lesions were more prominent in I.

### Sequential hemodynamic changes during and after pacing

Baseline hemodynamic variables were similar in both groups (Table II). Also, during pacing, maximal changes in heart rate, left ventricular systolic pressure and the double product were comparable, as well as pacing-induced alterations in coronary sinus flow, coronary resistance and myocardial oxygen extraction and consumption. In contrast, contractility indices (e.g. Vmax) did not change

**Table II:** **hemodynamic variables at control and maximal pacing rates in ischemic vs non-ischemic patients.**

| Group | | HR b/min | LVSP mmHg | LVEDP mmHg | MAP mmHg | Vmax $sec^{-1}$ | CBF ml/min | DP bpm mmHgx$10^{-3}$ |
|---|---|---|---|---|---|---|---|---|
| I | control | 85±3.5 | 148±6 | 14±1.5 | 112±3.9 | 53±2.6 | 109±8 | 12.2±8.6 |
| | maximal pacing | 152±3.6† | 143±7 | 15±1.9* | 127±4.0† | 57±2.8 | 193±15 | 21.5±1.1† |
| | 10 seconds p-p | | | 25±2.9*† | | | | |
| NI | control | 81±3.6 | 142±6 | 12±1.0 | 110±6.5 | 56±4.8 | 122±14 | 11.5±0.6 |
| | maximal pacing | 147±7.1† | 140±9 | 7±0.7† | 115±8.2 | 71±6.6 | 186±31† | 20.7±1.8† |
| | 10 seconds p-p | | | 13±1.4 | | | | |

*Abbreviations: BPM = beats per minute; CBF=coronary venous blood flow; DP=double product; HR=heart rate; I = ischemic; LVEDP=left ventricular end diastolic pressure; LVSP=left ventricular systolic pressure; MAP=mean arterial pressure; NI = non-ischemic. p-p = post-pacing. *p<.05 group I vs group NI. † p<0.05 vs control.*

during pacing in I, compared to a significant 27% improvement in NI (Fig. 1), whereas left ventricular end diastolic pressure was significantly elevated in I [25±3 versus 13±5 mmHg (NI) at 10 seconds post-pacing]. In I, mean arterial pressure increased by 13% during maximal pacing, whereas systemic resistance was still elevated at 5 minutes post-pacing (1632±76 versus 1470±60 dynes.s.cm-5 at control, p<0.05, Fig. 2). No such changes occurred in NI.

### Sequential electrocardiographic and metabolic changes during pacing

By design, significant repolarization disturbances only occurred in I, persisting until 2 minutes post-pacing (Fig. 3). Pacing did not affect myocardial lactate metabolism in NI. In contrast, in I, myocardial lactate extraction at control changed to production during maximal pacing rates and for 2 minutes after pacing with a maximal value of -71±16% at 15 seconds post-pacing (Fig. 3).

### Effect of pacing-induced ischemia on systemic neurohormones

Pacing did not affect arterial catecholamine or angiotensin II levels in NI (Table III). In contrast, significant neurohumoral changes were observed during myocardial ischemia. In I, arterial norepinephrine increased from 1.7±0.2 (control) to 2.6±0.3 (maximal pacing) and further to 3.0±0.4 nmol/l at 1 minute post-pacing, both p<0.05 versus control (Fig. 4). Concomitantly, arterial angiotensin II levels rose significantly by 39%, from 6.2±1.4 (control) to 8.6±2.0 (maximal pacing) and to 9.3±2.1 pmol/l (1 minute after

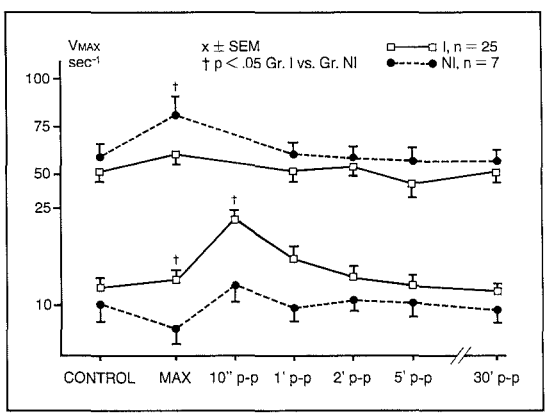


*Fig. 1: Effect of pacing-induced ischemia on contractility (Vmax) and left ventricular end diastolic pressure (LVEDP). Vmax significantly increases during maximal pacing rates (MAX) in non-ischemic patients (Gr NI), but not in the ischemic group (Gr I). In contrast, LVEDP is elevated in I, but not in NI, at 10 seconds (") post-pacing (p-p). ' = minute; C = control*

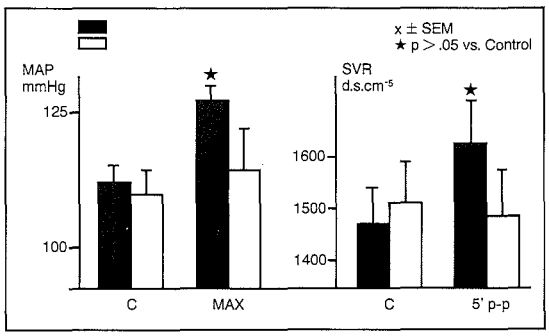


*Fig. 2: Effect of pacing and ischemia on mean arterial pressure (MAP) and systemic vascular resistance (SVR). Both MAP and SVR increase significantly in patients, who become ischemic during pacing (I), but not in non-ischemic patients (NI). ' = minute; C = control; MAX = maximal pacing rates; p-p = post-pacing*

pacing, Fig. 5). By contrast, epinephrine levels only increased during pacing [0.93±0.13 (maximal pacing) versus 0.60±0.09 nmol/l (control), p<0.05, Fig. 6]. Norepinephrine and angiotensin II levels returned to baseline values between 2 and 5 minutes post-pacing. Dopamine levels did not change.

## DISCUSSION

Systemic neurohumoral activation frequently accompanies acute myocardial infarction, particularly when complicated by cardiac pump failure[1-6], whereas, in animal models, long lasting, severe myocardial ischemia also increases neuroendocrine activation[16]. In contrast, it is not at all clear, whether short periods of moderate ischemia in man may equally affect peripheral neurohumoral systems. Thus far, data on catecholamine stimulation during exercise- or pacing-induced ischemia have been conflicting[2,7-11,17], whereas no information exists on other neurohormones in this context. The present study clearly demonstrates that myocardial ischemia, induced by atrial pacing in the resting patient with coronary artery disease, results in significant activation of systemic catecholamines and the circulating renin-angiotensin system together with a significant increase in systemic vascular resistance and arterial pressures. As it may be anticipated that afterload subsequently increases, imposing an extra burden on the already ischemic myocardium, this systemic neurohumoral activation may be of clinical relevance.

### The effect of pacing-induced stress and of anginal pain on circulating neurohormones

Pacing per se does not significantly affect neurohormones. In the present study, non-ischemic patients were paced to the same level as the ischemic patients with similar increases in myocardial oxygen demand and consumption. Nevertheless, arterial neurohumoral levels did not change significantly, despite the fact that 72% of patients in Gr. NI complained of retrosternal pain during pacing. The latter indicates that angina is not a prerequisite for neurohumoral activation. Unfortunately, this cannot be proven in our study, as very few ischemic patients were asymptomatic. However, other studies, carried out during pacing-induced stress, also did not observe significant changes in systemic catecholamines[8,10,11]. In view of the elusive link between transient myocardial ischemia and angina[18], neurohumoral activation most likely does not depend on the presence or absence of angina, but rather on myocardial ischemia and, possibly, on the severity hereof.

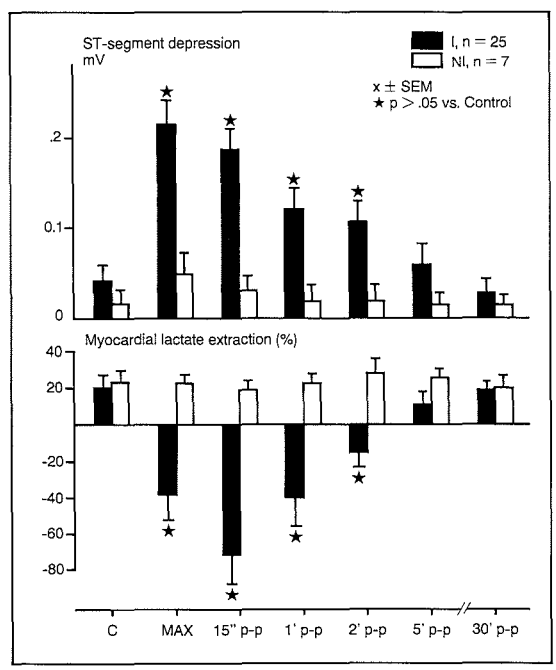


*Fig. 3: Sequential changes in ST-segment and myocardial lactate extraction in ischemic patients (Gr I), persisting until 2 minutes (') post-pacing (p-p), but not in non-ischemic patients (Gr NI). C = control; MAX = maximal pacing rates; " = seconds*

**Table III: Effect of pacing on arterial neurohormones in ischemic versus non-ischemic patients.**

| | C | MAX | 1' P-P | 2' P-P | 5' P-P | 30' P-P |
|---|---|---|---|---|---|---|
| NOREPINEPHRINE (nmol/l) | | | | | | |
| Group I | 1.7±0.2 | 2.6±0.3* | 3.0±0.4* | 2.4±0.3 | 2.4±0.3 | 2.1±0.2 |
| Group NI | 2.0±0.4 | 2.3±0.3 | 2.1±0.3 | 2.1±0.3 | 2.1±0.4 | 2.0±0.6 |
| EPINEPHRINE (nmol/l) | | | | | | |
| Group I | 0.60±0.09 | 0.93±0.13* | 0.71±0.10 | 0.67±0.09 | 0.70±0.13 | 0.63±0.08 |
| Group NI | 0.54±0.09 | 0.62±0.10 | 0.57±0.13 | 0.61±0.13 | 0.58±0.14 | 0.66±0.19 |
| DOPAMINE (nmol/l) | | | | | | |
| Group I | 0.50±0.07 | 0.54±0.08 | 0.49±0.09 | 0.35±0.07 | 0.43±0.12 | 0.53±0.05 |
| Group NI | 0.35±0.08 | 0.33±0.08 | 0.21±0.07 | 0.24±0.08 | 0.36±0.08 | 0.40±0.08 |
| ANGIOTENSIN II (pmol/l) | | | | | | |
| Group I | 6.2±1.4 | 8.6±2.0* | 9.3±2.1* | 5.5±0.9 | 6.0±0.9 | 6.9±1.8 |
| Group NI | 5.0±1.5 | 4.9±1.4 | 4.7±1.4 | 4.3±1.4 | 4.4±1.3 | 5.8±1.6 |

*Abbreviations: '=minute(s); C=control; I=ischemic; MAX=maximal pacing rates; NI=non-ischemic; P-P=post-pacing*
**p<.05 vs control*

Only few patients in Gr. I did not produce lactate, which implicates predominant anterior wall ischemia. Besides stimulation of mechanoreceptors in this area by hemodynamic or metabolic stimuli and subsequent excitation of the vasomotor centre in the medulla oblongata[18,19], a more pronounced reduction in cardiac pump function could also explain the enhanced sympathetic outflow in this study.

### Comparison with other studies of pacing- or exercise-induced ischemia

Thus, the inconsistency in observations on neuroendocrine activation in the available patient studies may be explained by differences in degree of ischemia. Alternatively, they may be explained by differences in sensitivity and specificity of the biochemical analytical techniques available at the time of study. Nevertheless, several relatively recent investigations, all performed in a similar model of pacing-induced stress, have been equally inconsistent in their observations on neuroendocrine activation during ischemia[8,10,17]. As study protocol, type of patients and the catecholamine assay techniques were basically comparable, the variability in study-results is difficult to explain. Our study differs from these investigations in two aspects. First, the objective in the present study was to analyze neurohumoral alterations during ischemia per se, irrespective of angina, but confirmed by objective signs of ischemia. Only Schwartz et al.[8] stipulated the presence of objective evidence for ischemia in their anginal patients. Secondly, the combined radioenzymatic assay and high pressure liquid chromatography technique may significantly improve the detection of small changes in catecholamine levels. Moreover, our study differs from previous investigations in man, as it also investigated alterations in other neurohormones and concomitant hemodynamic changes.

### Effect of pacing-induced ischemia on circulating angiotensin II levels

In the present study, arterial angiotensin II levels increased significantly during pacing-induced Fig.

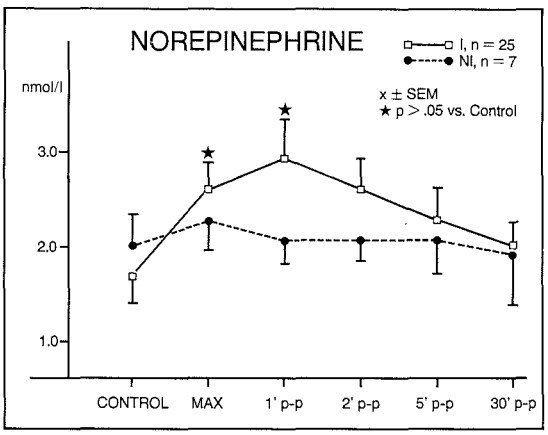


*Fig 4. Effects of pacing and myocardial ischemia on arterial norepinehrine levels. In ischemic patients (Gr I) levels increase significantly from control to maximal pacing rates (MAX) and further, at 1 minute (') post-pacing (p-p). In contrast, in non-ischemic patients (Gr NI) pacing does not affect arterial norepinephrine levels.*

ischemia, but not during equivalent increments in heart rate in non-ischemic patients. Thus far, no data are available on the effect of ischemia on the circulating renin-angiotensin system. Recent animal studies indicate, however, that this system may indeed be influenced by myocardial ischemia[20,21]. Ertl et al. demonstrated a significant increase in arterial renin and, to a lesser extent, in arterial angiotensin II following 10 minutes of coronary occlusion in a canine model[20]. Also, 30-second occlusions of the left anterior descending artery result in significant elevations of plasma renin and angiotensin II, whereas fast atrial pacing in the presence of a coronary artery stenosis induces a 50% increment in arterial renin[20,22]. As this activation of the circulating renin-angiotensin system by ischemia was not modulated by beta-adrenergic blockade, but prevented by nephrectomy, may be initiated by renin release from the kidney as a result of renal underperfusion, secondary to ischemia-induced left ventricular dysfunction. This, in turn, suggests that stimulation of the circulating renin-angiotensin system depends on the severity of myocardial ischemia.

### Ischemia-induced neurohumoral activation may result in systemic vasoconstriction

A significant increase in systemic vascular resistance and a rise in arterial pressures did occur during ischemia, but was not observed in non-ischemic patients. As ischemia-induced cardiac dysfunction and the subsequent decrease in cardiac output primarily will result in a reduction of arterial pressures, secondary counterregulatory forces must supervene. Whether the increase in systemic catecholamines and/or activation of the circulating renin-angiotensin system is causally involved cannot be determined from our studies, as specific antagonists of these neurohumoral systems were not administered. Hence, the concomitant change in systemic resistance and neurohormones only suggests such an interrelationship, but could be coincidental.

### Clinical implications

Our observations were made in the resting, supine patient and do not necessarily apply to the model of exercise-induced ischemia. Exercise by itself results in a marked increase in circulating neurohormones, also in normal individuals[23]. In contrast, in the resting patient, with stable and low baseline neurohumoral levels, subsequent activation during ischemia may be clinically significant.

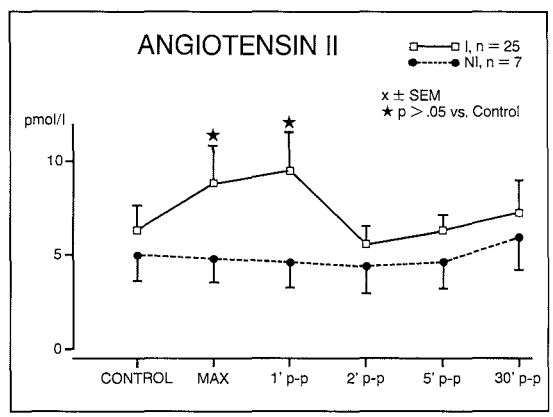


*Fig. 5: Effects of pacing and myocardial ischemia on arterial angiotensin II levels. In the ischemic group (Gr I) a 39 % increase is observed, persisting until 1 minute (') after pacing (p-p). Pacing per se does not affect angiotensin II in the non-ischemic group (Gr NI).*
*MAX = maximal pacing rates.*

Hence, in patients with ischemia at rest, e.g. unstable angina or the early phase of myocardial infarction, a similar or even more pronounced neuroendocrine activation than in our study, may be present with systemic, and possibly coronary

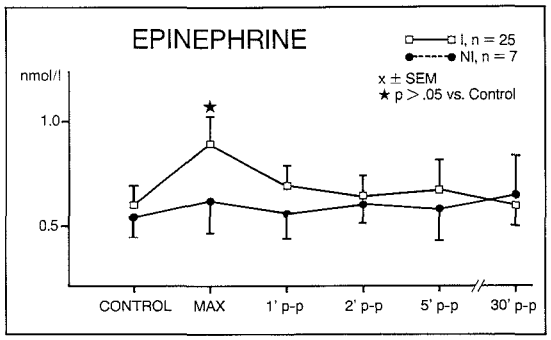


*Fig. 6: Effects of pacing and myocardial ischemia on arterial epinephrine levels. A significant elevation occurs in ischemic (Gr I), but not in non-ischemic patients (Gr NI) and only at maximal pacing rates (MAX). '=minute; p-p=post-pacing.*

vasoconstriction, amplifying the ischemic process. Although their interrelation is still speculative, interventions aimed at modulating neuro-humorally-induced vasoconstriction, i.e. alpha-adrenergic antagonists or inhibitors of the renin-angiotensin system, may be useful as (additional) antiischemic therapy in these situations.

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# Chapter X.3.

## Systemic and Cardiac Neuroendocrine Activation During Pacing-Induced Myocardial Ischemia in Humans

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## ABSTRACT

Systemic and cardiac neuroendocrine activation is prominent in myocardial infarction, depending on concomitant cardiac dysfunction. Its occurrence and clinical significance in myocardial ischemia is less well defined and was studied in 56 fasting, supine, untreated subjects with stable angina-like chest pain during incremental atrial pacing. Coronary venous and arterial neurohormones (catecholamines and angiotensin II) and lactate were determined at control, maximal pacing rates and at 1, 2, 5 and 30 minutes post-pacing (p-p). Based on objective signs of ischemia during pacing, patients were grouped as ischemic and non-ischemic (NI,n=11). To facilitate identification of neurohumoral changes in the ischemic myocardium, ischemic patients were then separated in lactate and non-lactate producers (LP and NLP, n=28 and n=17, resp.). Pacing-induced angina occurred in 55%, 82% and 82% of NI, LP and NLP, resp. Baseline hemodynamic and neurohumoral values were comparable in all groups, as were pacing-induced changes in heart rate, double product and coronary hemodynamics. However, in LP, contractility and relaxation did not improve or deteriorate during pacing, left ventricular filling pressure rose significantly, whereas cardiac output was still significantly reduced (10%) and systemic resistance increased (23%) at 5 minutes p-p, in contrast to NI and NLP. As a result, arterial pressures were markedly elevated during and after pacing in LP. Pacing did not change dopamine levels in either group, neither did it affect other neurohormones in non-ischemic patients. In contrast, in LP, arterial norepinephrine increased from 1.9±0.2 nmol/l (control) to 3.2±0.3 nmol/l (1 minute p-p), persisting until 5 minutes p-p. In NLP, arterial norepinephrine only rose by 36% at the end of pacing. Likewise, increases in arterial and coronary venous epinephrine and in coronary venous norepinephrine were less in NLP than in LP. Moreover, arterial angiotensin II increased from 6.8±0.9 pmol/l (control) to 9.3±1.58 pmol/l (1 minute p-p, $p<0.05$), but only in LP. Cardiac norepinephrine release at rest changed to uptake during pacing (-0.05±0.02 nmol/min versus 0.06±0.05 nmol/min, $p<0.05$) in LP, but not in NLP or NI. In contrast, epinephrine uptake increased significantly in all ischemic patients, although more so in LP. Changes in cardiac catecholamine uptake correlated with changes in respective arterial levels in lactate producers. It is concluded that short periods of ischemia may induce significant activation of circulating catecholamines and renin-angiotensin levels, accompanied by systemic vasoconstrictory effects. Changes do not appear to relate to angina, but depend on the severity of ischemia. Moreover, ischemia enhances cardiac epinephrine uptake and reverses basal norepinephrine release to uptake in the ischemic myocardium.

## INTRODUCTION.

Systemic neuroendocrine activation is a consistent observation in heart failure, particularly prominent in advanced stages or during exacerbations of the syndrome[1,2]. It is also well established in the acute phase of myocardial infarction, where circulating levels of catecholamines, renin-angiotensin and arginine-vasopressin appear to relate to

the degree of left ventricular dysfunction[3-5]. Whereas in animal models of severe, prolonged myocardial ischemia cardiac norepinephrine efflux increases, indicative of enhanced sympathetic stimulation [6], the effect of myocardial ischemia on systemic and cardiac neurohormones in humans is less well understood. Although it may be anticipated that acute, ischemia-induced reductions in cardiac pump function may activate sympathetic tone, increase circulating catecholamines and, possibly, enhance renin-angiotensin levels, there is unsufficient evidence in humans to suggest this. Available data in man do not show uniform changes in plasma or urinary catecholamines during exercise- or pacing-induced ischemia [7-14]. Neither is it clear whether, myocardial catecholamine balance is affected by myocardial ischemia [9,11,14]. Animal studies indicate, that cardiac norepinephrine release is enhanced, when ischemia lasts for 10 minutes or more, but not during shorter periods[6]. In humans, the effect of pacing-induced ischemia on cardiac norepinephrine release is not clear[9,11]. Besides insufficient and/or inconsistent data on circulating or cardiac catecholamines in humans, information on the effect of ischemia on different, but related neurohormones, such as the circulating renin-angiotensin system, is practically non-existent. However, animal data indicate the circulating renin-angiotensin system may bome activated following short episodes of artery occlusion[15]. Whether ischemia affects transcardiac angiotensin II fluxes is unknown though.

In the present study, sequential changes in systemic and transcardiac catecholamine and angiotensin II levels were investigated during pacing-induced ischemia in normotensive patients with coronary artery disease without heart failure and compared with the effects of pacing in patients without ischemia. Secondly, the effect of these changes on coronary and systemic hemodynamics were studied. Thirdly, an attempt was made to compare transcardiac neurohumoral fluxes in ischemic versus non-ischemic myocardium.

## MATERIALS AND METHODS

### Patients.

After approval of the study protocol by the institutional Ethical Review Committee and after informed consent was obtained, 56 patients, 4 women and 52 men, age 54 years (varying from 31 to 73 years) were studied. Subjects were selected at random from patients, referred for coronary angiography for the evaluation of stable angina pectoris with documented exercise-induced ischemia and/or a previous myocardial infarction as well as for angina-like chestpain without signs of myocardial ischemia. To be included in the study, patients had to be normotensive without signs or symptoms of congestive heart failure, valvular heart disease or conduction disturbances. A previous myocardial infarction had to be at least 1 month old. Patients with unstable angina were not included as it was believed, that such patients might deteriorate upon withdrawal of cardiac medication. Unstable angina was defined by the occurrence of recent symptoms, increased frequency and/or duration of episodes of angina during the last month or recent prolonged episodes of angina at rest (20 minutes duration), despite medication. In contrast, patients with stable angina had to have an unchanged pattern of exercise-induced symptoms; occasional, short lasting episodes of angina at rest were allowed.

All cardiac therapy was withheld 36-72 hours pre-study, depending on plasma halflives. Beta-blocking agents were stopped between 48 and 72 hours before the investigation. Only short-acting nitroglycerin was allowed until 6 hours pre-study. Oral anticoagulants were stopped 2 to 3 days before the investigation. Anti-platelet therapy, e.g. aspirin, was withheld 10 days prior to the investigation.

Fifty-six patients had significant coronary artery disease, defined as $\geq$70% diameter narrowing of at least one epicardial coronary artery. Seventeen patients had single vessel disease. Of these, 14 patients had either a lesion in the left anterior descending, a proximal stenosis of the left circumflex artery or a lesion in the proximal marginal branches of the left coronary artery. In 3 patients a significant stenosis in the right coronary artery was the only lesion present. Objective signs of myocardial ischemia during exercise testing were present in 32 patients and a documented old myocardial infarction in 24 patients. In the latter group 9 patients had no demonstrable signs of ischemia during exercise.

### Catheterization procedures.

All patients were studied in the fasting state, at the same time in the morning. Patients were not premedicated and local anaesthesia was achieved with 1% lidocaine. Before the study, coronary angiography was performed, using the Seldinger technique. Thereafter, a no 7 Fr Millar or 8 Fr

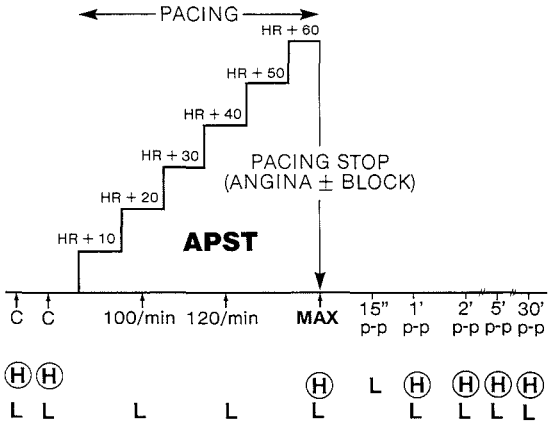



*Fig. 1: Schematic representation of pacing study protocol (APST). At least 40 minutes after coronary angiography and 20 minutes after instrumentation, control measurements of hemodynamic (arrow) and neurohumoral (H) variables and lactate were made. Next, atrial pacing with increments in heart rate (HR) of 10 beats per 2 minutes was carried out. Repeat hemodynamic and metabolic measurements were performed at fixed intervals, i.e. at 100 and 120 beats/minute, at maximal heart rates (MAX) and at 15 seconds, 1, 2, 5 and 30 minutes (') after pacing (p-p). Neurohormones were reevaluated at MAX and at 1, 2, 5 and 30 min p-p.*

Sentron pigtail microtip manometer catheter was advanced to the left ventricle through an 8 or 9 Fr arterial Desilet introducer system in the right femoral artery, to record left ventricular pressures. The side-arm of the introducer system was used to record the arterial pressure curve. A no 7 Fr balloon-tipped triple-lumen thermodilution catheter (Edwards laboratory) was positioned in a pulmonary artery through an 8 Fr Desilet introducer system in the right femoral vein, to measure right atrial pressure and cardiac output. A no 7 Fr coronary sinus thermodilution and pacing catheter (Wilton Webster laboratories) was inserted in a right brachial vein and positioned in the coronary sinus, such that the proximal thermistor was at least 3 cm beyond the orifice of the coronary sinus. Also, the catheter positions had to be stable and allow a rapid sequence of blood sampling. To improve sampling from the coronary sinus, a specially-devised catheter was used, the bol-end Wilton Webster thermodilution catheter [CCS-7U-90B]. Contrary to the conventional end-hole coronary sinus catheter, the sampling orifice of the bol-end version usually stays well clear from the coronary sinus wall. Thus, sampling of relatively large amounts of blood, e.g. 15-20 ml/minute, can be achieved throughout periods of at least 2 hours in approximately 90% of patients (personal experience in more than 250 patients). Absence of atrial reflux into the coronary sinus under the study conditions was confirmed by a bolus injection of 10 ml of saline into the right atrium. After instrumentation, the position of the catheters was recorded on video disc to allow rechecking of their respective positions during the study.

**Hemodynamic and electrocardiographic measurements.**

The hemodynamic parameters in this study included left ventricular peak systolic, mean and end diastolic pressures, left ventricular pressure-derived contractility (peak positive dP/dt, dP/dt/P at 40 mmHg and $V_{max}$) and relaxation indices (peak negative dP/dt, $Tau_1$ and $Tau_2$), mean and phasic arterial pressures, right atrial pressure and coronary sinus blood flow. Cardiac output measurements were performed in a limited number of patients. All fluid-filled catheters were calibrated, using Bentley transducers, with a zero reference level set at midchest. The micromanometer pressure was balanced to zero and superimposed on the conventional pressure tracings. After appropriate calibration, all pressures, coronary flow, cardiac output and the first derivative of left ventricular pressure were recorded on paper, using a CGR 1000 cath lab system. They were also determined on-line by a Mennen cath lab computer system. Through this system, pressures and pressure-derived contractility indices were calculated in 15-20 consecutive beats, to average out respiratory variations. Contractility indices and peak negative dP/dt were calculated and displayed on-line. In contrast, the isovolumetric relaxation indices, $Tau_1$ and $Tau_2$, were measured off-line.

Coronary sinus blood flow was determined during a continuous 30 second infusion of 30 ml glucose at room temperature. Both the mean and phasic coronary flow were recorded. Calculations were made from the mean flow curve, according to the formula: coronary blood flow (ml/min) = $V_i \times [(T_b-T_i)(T_b-T_{cs})-1] \times 1.08$, where $T_b$ is blood temperature before injection, $T_i$ temperature of injectate, $T_{cs}$ temperature of mixture of coronary sinus blood and injectates and Vi the rate of injection (ml/min). Coronary flow was determined with the patient holding his breath in mid-respiration for the last 15 seconds of the recording. As the Mennen cath lab system displays 1-second measurements of coronary flow, it allows

instantaneous estimation of the stability of coronary flow recordings.
At the end of the study, the pressure curves from the femoral artery were compared with a simultaneous recording from the aortic root by the micromanometer catheter, to compensate for any difference between proximal and distal arterial pressures.
Heart rate and ST-segment changes were determined from 100 mm/sec recordings of ECG leads I, II and $V_5$. The ST-segment was measured in 3 to 5 consecutive beats, 80 milliseconds after the J point, using a calibrated magnifying glass.

**Blood sample collection and assay of metabolites.**

Approximately 1 ml of blood was collected simultaneously from the left ventricle and coronary sinus for the immediate determination of oxygen saturation values on an OSM-80 oxymeter (Waters Association). For lactate, simultaneous sampling from the left ventricle and the coronary sinus was carried out. Exactly 1 ml blood was quickly transferred into pre-cooled glass tubes, containing 2 ml of icecold 0.6 M $HClO_4$, mixed thoroughly and kept on ice. Immediately after the study, the samples were weighed and centrifuged for 20 minutes at a speed of 2,000 xg. Thereupon, the supernatant was frozen for subsequent assay of lactate, which was carried out in triplicate by the enzymatic technique as described by Guttman and Wahlefeld [16]. The standard deviation of this determination in our laboratory, carried out in approximately 2.500 samples per year, is 0.012 mmol/l[17]. To prevent mistaking arterial for venous blood samples or vice-versa during fast repetitive sampling procedures, blood was collected in colour-coded tubes.

**Neurohumoral measurements.**

In this study, the levels of angiotensin II and the catecholamines norepinephrine, epinephrine and dopamine were assessed by collecting a minimum of 6 ml of blood simultaneously from the left ventricle and coronary sinus in pre-cooled syringes. For the assay of angiotensin II, 3 ml was immediately transferred into ice-cold tubes containing 4 mg EDTA and 0.06 mg O-fenantroline. The remaining 3 ml was transferred into ice-cold tubes containing 500 I/U heparin and 3 mg/ml gluthathion. Samples were immediately centrifuged at 3000 xg under coooled conditions and the supernatants frozen at -20° C. Catecholamines were determined by radio-enzymatic assay and high-pressure liquid chromatography to separate the radio-active products, whereas angiotensin II was assessed by radioimmunoassay[18,19].

**Calculations.**

From the measured variables the following parameters were derived. Coronary vascular resistance (mmHg/ml/min) was calculated as the difference between mean arterial pressure (mmHg) and left ventricular mean diastolic pressure (mmHg) divided by coronary sinus blood flow (ml/min). Systemic vascular resistance (dynes.sec.$cm^{-5}$) was derived as [mean arterial pressure (mmHg) - mean right arterial pressure (mmHg) / cardiac output (l/min)] x 80. Stroke work index (g.m.$m^2$) was calculated as stroke index (ml/beat/$m^2$) x [mean arterial pressure (mmHg) - left ventricular end diastolic pressure (mmHg)] x 0.0136. Myocardial oxygen extraction ($mlO_2$/ml) was calculated as arterial oxygen content ($mlO_2$/ml) - coronary venous oxygen content ($mlO_2$/ml), and myocardial oxygen consumption (ml/min) as the product of myocardial oxygen extraction ($mlO_2$/ml) and coronary blood flow (ml/min). Percentage myocardial lactate extraction was calculated as 100 x [arterial lactate content (mmol/l) - coronary venous lactate content (mmol/l)] / arterial lactate content (mmol/l). Myocardial lactate uptake (mmol/min) was determined by multiplying the difference in arterial and coronary venous lactate content (mmol/ml) with coronary blood flow (ml/min).
Myocardial catecholamine and angiotensin II uptake (nmol/min and pmol/min, resp.) were calculated by multiplying the respective differences in arterial and coronary venous catecholamine (nmol/ml) or angiotensin II (pmol/ml) levels with the instantaneous coronary sinus blood flow (ml/min).

**Study protocol.**

Patients were studied at least 45 to 60 minutes after the last coronary angiogram and 25 to 30 minutes after instrumentation. Hence, all studies were performed between 10.30 and 11.30 AM with the patient resting and supine for approximately 2 hours. First, multiple control measurements of all hemodynamic variables were performed to ensure stable baseline values. Before control, arterial and coronary venous blood

**Table I: Baseline clinical and angiographic criteria.**

| | Gr NI (n=11) | Gr LP (n=28) | Gr NLP (n=17) |
|---|---|---|---|
| Sex (male/female) | 9/2 | 28/0 | 15/2 |
| Age (yrs) | 49±3.1 (range 31-66) | 55±1.7 (range 37-73) | 54±2.4 (range 36-71) |
| Previous infarcts (ant/inf) | 4/1 | 8/4 | 2/5 |
| Positive exercise test | 3 | 20 | 10 |
| Coronary angiography (≥70% diameter stenosis) | | | |
| 0-vessel | 4 | 0 | 0 |
| 1-vessel (L/R) | 3L/1R | 7L/1R | 4L/1R |
| 2-vessel | 1 | 12 | 6 |
| 3-vessel | 2 | 9 | 5 |
| Left ventricular ejection fraction (%) | 54±3.5 | 52±2.6 | 53±3.1 |
| Left ventricular end diastolic volume ($ml/m^2$) | 74±6.7 | 69±4.8 | 67±3.7 |

*Abbreviations: ant/inf = anterior/inferior; LP = lactate producers; L/R = left/right; N = number; NI = non-ischemic; NLP = non-lactate producers. All values $\bar{x}$ ±SEM.*

samples for metabolic and neurohumoral parameters were collected, and repetitive coronary venous oxygen saturation values were carried out. Arterial and coronary venous blood samples were collected in duplicate. Then, an atrial pacing stress test was carried out, during which heart rate was elevated by 10 beats/2 minutes until anginal pain or atrioventricular block occurred or a maximal heart rate of 170 beats/minute was reached (Fig. 1). The patient always determined which level of anginal pain he or she would endure and, hence, the duration of the atrial pacing stress test. However, we tried to continue pacing until the severity of angina was at least comparable to that, at which the patient would normally discontinue exercise or take sublingual nitroglycerin. Repeated determinations of all hemodynamic and metabolic parameters were carried out at fixed intervals during pacing, e.g. midway the 100, 120, 140 and 160 beats/minute period. During maximal heart rates, just before cessation of pacing, all variables, including neurohormones, were reassessed, followed by repeated measurements of all variables during the first, second and fifth minute after pacing. The sequence of blood sampling after pacing was such, that exactly at 15 seconds post-pacing the collection of blood for lactate commenced. This was followed immediately by the collection of blood for neurohormones. This sequence was repeated during the second and fifth minute after pacing. Additional arterial and coronary venous blood samples for lactate were collected following the second minute after pacing. These will be referred to as the 2-minute post-pacing lactate samples.

After pacing, all hemodynamic variables were reassessed at the 1-, 2- and 5-minute post-pacing period, except for coronary flow, which was measured just preceding the 5-minute post-pacing period. In 39 patients variables were reassessed at 30 minutes after pacing.

During this interval patients had been resting without further instrumentation or investigational procedures. Throughout the entire study, approximately 60 ml of blood was collected for metabolic and neurohumoral assay. Although,

**Table II: Systemic hemodynamic variables during and after pacing.**

| | Control | 120/min | Max | 1' P-P | 5'P-P | 30'P-P |
|---|---|---|---|---|---|---|
| ***LVSP mmHg*** | | | | | | |
| NI | 138±6.6 | 136±4.5 | 130±4.0† | 136±6.5 | 134±5.2 | 135±5.5 |
| LP | 153±5.3 | 147±6.3 | 149±6.0 | 162±6.6*† | 153±5.3 | 144±7.1 |
| NLP | 143±6.5 | 142±6.6 | 139±7.1 | 143±6.9 | 141±7.3 | 137±5.5 |
| ***DP (HRxLVSPx10-3)*** | | | | | | |
| NI | 10±0.7 | 15±0.9* | 17±1.2*† | 10±0.7 | 10±0.7 | 9±0.6 |
| LP | 12±0.5 | 18±0.9* | 22±0.9* | 13±0.8 | 12±0.5 | 12±0.6 |
| NLP | 11±0.7 | 17±0.9* | 20±1.2* | 11±1.0 | 11±0.9 | 10±0.5 |
| ***LVEDP mmHg*** | | | | | | |
| NI | 13±2.3 | 9.6±2.4 | 7±1.4* | 12±2.3 | 12±2.4 | 13±3.0 |
| LP | 14±1.3 | 10.9±1.0 | 16±2.0† | 18±2.1† | 13±1.2 | 11±1.3 |
| NLP | 13±0.9 | 8.9±0.9 | 7±1.0* | 13±0.9 | 11±1.0 | 11±1.0 |
| ***MAP mmHg*** | | | | | | |
| NI | 101±2.8 | 105±4.2 | 103±4.8 | 104±5.3 | 102±3.6 | 100±1.6 |
| LP | 111±3.2 | 119±4.1* | 127±4.7* | 124±4.2* | 115±4.0 | 109±4.9 |
| NLP | 106±4.9 | 112±5.4 | 117±5.3* | 114±4.8 | 111±4.9 | 103±3.5 |
| ***LVdP/dt+mmHg.s$^{-1}$*** | | | | | | |
| NI | 1511±134 | 1876±136* | 1897±138* | 1567±124 | 1547±92 | 1326±69 |
| LP | 1779±80 | 2078±95* | 2241±126* | 1840±117 | 1796±65 | 1686±108 |
| NLP | 1715±102 | 2229±175* | 2363±125* | 1740±130 | 1753±103 | 1720±106 |
| ***Vmax s$^{-1}$*** | | | | | | |
| NI | 45±3.5 | 62±6.9* | 59±3.8* | 44±3.8 | 45±3.6 | 41±2.7 |
| LP | 50±2.6 | 57±3.0* | 56±2.7*# | 51±2.9 | 49±2.5 | 49±13.3 |
| NLP | 50±2.4 | 64±4.2* | 68±3.5* | 52±2.7 | 50±2.4 | 51±2.5 |
| ***VCE$_{40}$ s$^{-1}$*** | | | | | | |
| NI | 30±2.6 | 42±4.8* | 40±2.9* | 30±2.4 | 30±2.2 | 27±1.7 |
| LP | 35±1.7 | 40±2.0* | 40±2.0*# | 34±1.9 | 34±1.6 | 33±2.2 |
| NLP | 33±1.7 | 45±3.3* | 47±2.4* | 35±1.8 | 34±1.6 | 34 ±1.8 |
| ***LVdP/dt-mmHg.s$^{-1}$*** | | | | | | |
| NI | 1769±127 | 2032±200 | 1999±121* | 1776±137 | 1958±147 | 1852±150 |
| LP | 1914±86 | 2011±105 | 1971±118 | 1960±103 | 1980±100 | 1875±136 |
| NLP | 1883±89 | 2081±117 | 2111±115* | 1963±125 | 1858±104 | 1788±74 |
| ***Tau$_1$ msec*** | | | | | | |
| NI | 53±4.7 | 41±5.4 | 43±3.9* | 52±4.9 | 56±7.2 | 56±5.9 |
| LP | 50±4.5 | 47±4.3 | 47±4.7# | 51±4.9 | 51±4.8 | 50±7.0 |
| NLP | 46±1.7 | 41±1.8* | 39±2.2* | 46±2.3 | 48±2.1 | 50±1.4 |
| ***Tau$_2$ msec*** | | | | | | |
| NI | 42±3.9 | 38±6.8 | 42±4.7 | 43±4.1 | 41±4.2 | 43±4.2 |
| LP | 44±4.7 | 50±7.2 | 55±5.5*# | 48±5.1 | 44±5.2 | 38±4.0 |
| NLP | 37±1.2 | 35±2.3 | 39±3.1 | 38±2.5 | 38±1.7 | 38±1.5 |
| ***CO l/m*** | | | | | | |
| NI | 5.0±0.4 | – | – | – | 4.8±0.4 | 4.7±0.5 |
| LP | 5.9±0.3 | – | – | – | 5.3±0.3* | 6.0±0.4 |
| NLP | 5.4±0.3 | – | – | – | 4.9±0.2 | 5.7±0.5 |
| ***SVI ml/b/m$^2$*** | | | | | | |
| NI | 40±2.8 | – | – | – | 38±3.3 | 38±2.7 |
| LP | 39±1.7 | – | – | – | 35±2.4 | 39±1.8 |
| NLP | 39±1.9 | – | – | – | 36±2.2 | 41±3.8 |
| ***SVR d.s.cm$^{-5}$*** | | | | | | |
| NI | 1545±114 | – | – | – | 1646±145 | 1684±175 |
| LP | 1723±132 | – | – | – | 2087±197* | 1376±97 |
| NLP | 1475±99 | – | – | – | 1670±158 | 1418±83 |
| ***PAM mmHg*** | | | | | | |
| NI | 17±1.7 | 17±2.6 | 18±2.1 | 16±1.6 | 16±1.6 | 16±1.8 |
| LP | 17±0.8 | 17±0.8 | 24±1.3* | 19±1.4 | 17±1.1 | 14±1.0 |
| NLP | 17±0.9 | 17±1.3 | 21±1.3* | 18±1.4 | 17±0.9 | 15 ±1.1 |

*Abbreviations: CO = cardiac output, DP = double product, LVEDP = left ventricular end diastolic pressure, LVSP = left ventricular systolic pressure, MAP = mean arterial pressure, Max = maximal pacing rates, min = minutes PAM = mean pulmonary artery pressure, P-P = post-pacing, SVI = stroke volume index, SVR = systemic vascular resistance.*
*All values x ± SEM; * $p<.05$ vs control; † $p<.05$ vs other groups; # $p<.05$ $\Delta$APST versus other $\Delta$APST.*

**Table III: Coronary hemodynamic variables during and after pacing.**

| | Control | 120/min | Max | 5'P-P | 30'P-P |
|---|---|---|---|---|---|
| ***CSBF ml/min*** | | | | | |
| NI | 137±15 | 143±25 | 202±32 | 149±15 | 144±25 |
| LP | 117±12 | 149±14 | 205±19* | 126±14 | 112±19 |
| NLP | 122±16 | 144±17 | 175±24* | 116±9 | 110±12 |
| ***CVR mmHg/ml/m*** | | | | | |
| NI | 0.71±0.08 | 0.67±0.11 | 0.58±0.13 | 0.74±0.13 | 0.87±0.18 |
| LP | 1.19±0.12† | 1.00±0.13 | 0.73±0.06* | 1.19±0.13 | 1.32±0.14 |
| NLP | 0.90±0.10 | 0.95±0.10 | 0.75±0.09 | 0.95±1.01. | 00±0.11 |
| $\Delta$***A-CS $O_2$ ml/min*** | | | | | |
| NI | 14±0.6 | 12±1.0 | 12±0.9 | 13±0.8 | 13±0.8 |
| LP | 12±0.5 | 12±0.7 | 12±0.6 | 11±0.6 | 11±0.6 |
| NLP | 12±0.7 | 13±0.7 | 12±0.7 | 12±0.7 | 13±0.8 |
| ***$MVO_2$ ml/min*** | | | | | |
| NI | 19±2.8† | 17±4.2 | 23±4.9 | 20±2.7† | 20±5.5 |
| LP | 12±1.1 | 15±1.7 | 22±2.0* | 12±1.0 | 11±1.5 |
| NLP | 14±1.6 | 17±2.5 | 21±2.5* | 13±1.3 | 15±2.0 |

*Abbreviations: CSBF = coronary sinus blood flow; CVR = coronary vascular resistance; $\Delta$A-CS $O_2$ = myocardial oxygen extraction; Max = maximal pacing rates; min = minute; $MVO_2$ = myocardial oxygen consumption; P-P = post-pacing. All values $\bar{x}$ ± SEM; *p<.05 vs control; † p<.05 vs other groups.*

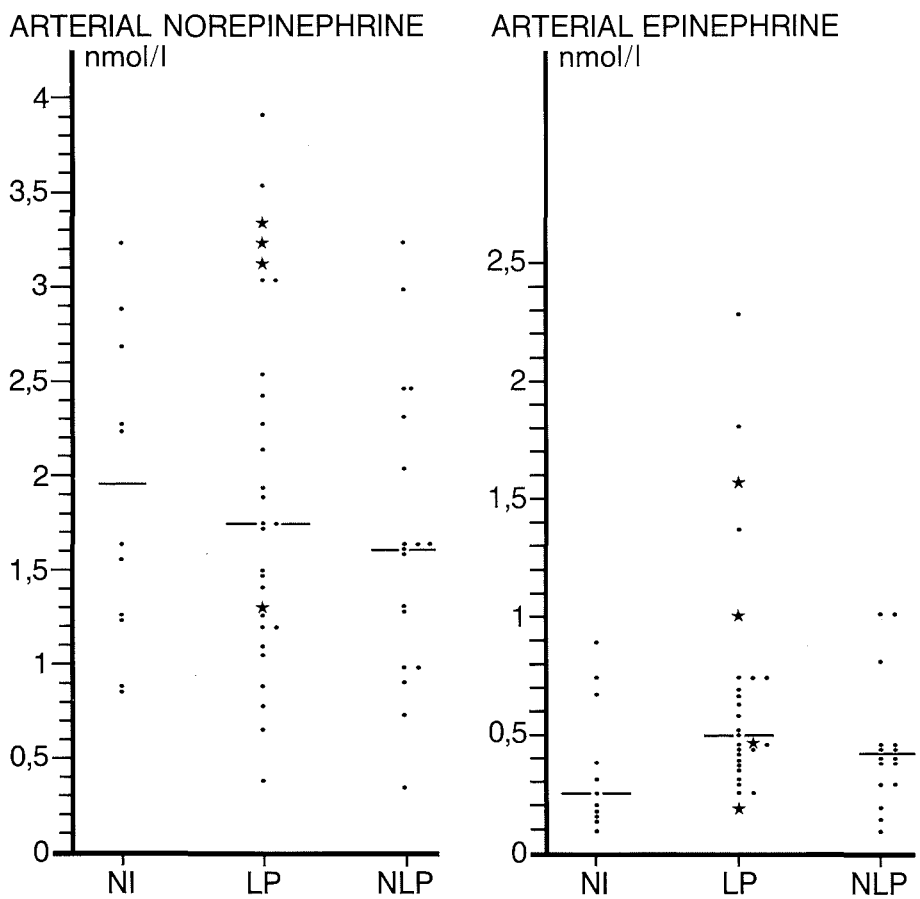



*Fig. 2: Individual values and medians of arterial norepinephrine and epinephrine at baseline. Median values and range are similar in the non-ischemic group (NI) and in ischemic patients with (LP) or without lactate production (NLP). Values of patients with ischemia at rest (lactate production asterix) are not different from non-ischemic patients.*

**Table IV: Arterial and coronary venous neurohormones during and after pacing.**

| | | Control | Max | 1'P-P | 2'P-P | 5'P-P | 30'P-P |
|---|---|---|---|---|---|---|---|
| ***Norepinephrine*** | | | | | | | |
| Arterial | LP | 1.9±0.18 | 2.8±0.26* | 3.2±0.34* | 2.7±0.2* | 2.4±0.25 | 2.1±0.21 |
| | NLP | 1.6±0.19 | 2.1±0.28* | 2.1±0.27* | 2.2±0.26* | 2.0±0.26 | 1.7±0.24 |
| | NI | 1.9±0.25 | 2.0±0.36 | 1.8±0.25 | 1.9±0.26 | 1.7±0.32 | 2.0±0.32 |
| Coronary venous | LP | 2.6±0.29† | 3.0±0.36* | 3.2±0.3* | 2.8±0.32 | 3.1±0.48 | 2.7±0.3 |
| | NLP | 1.7±0.17 | 2.3±0.36 | 1.9±0.21 | 2.1±0.28 | 2.1±0.31 | 1.8±0.21 |
| | NI | 1.8±0.17 | 1.7±0.29 | 1.5±0.24 | 1.9±0.30 | 1.5±0.38 | 1.6±0.19 |
| ***Epinephrine*** | | | | | | | |
| Arterial | LP | 0.65±0.09 | 1.0±0.16* | 0.8±0.1 | 0.7±0.09 | 0.9±0.14 | 0.7±0.14 |
| | NLP | 0.45±0.06 | 0.6±0.08* | 0.5±0.07 | 0.5±0.06 | 0.6±0.09 | 0.5±0.09 |
| | NI | 0.4±0.08 | 0.4±0.07 | 0.3±0.05 | 0.4±0.08 | 0.3±0.09 | 0.3±0.03 |
| Coronary venous | LP | .45±0.07† | 0.7±0.10* | 0.65±0.10 | 0.5±0.09 | 0.6±0.11 | 0.5±0.10 |
| | NLP | 0.2±0.04 | 0.4±0.06* | 0.3±0.06 | 0.25±0.04 | 0.3±0.04 | 0.3±0.07 |
| | NI | 0.2±0.05 | 0.4±0.15 | 0.2±0.05 | 0.2±0.06 | 0.2±0.06 | 0.2±0.03 |
| ***Dopamine*** | | | | | | | |
| Arterial | LP | 0.4±0.07 | 0.4±0.07 | 1.0±0.62 | 0.9±0.57 | 0.4±0.1 | 0.5±0.06 |
| | NLP | 0.2±0.04† | 0.3±0.12 | 0.2±0.03 | 0.2±0.05 | 0.09±0.04 | 0.2±0.04 |
| | NI | 0.5±0.12 | 0.6±0.15 | 0.5±0.11 | 0.6±0.14 | 0.4±0.17 | 0.6±0.17 |
| Coronary venous | LP | 0.55±0.08 | 0.5±0.08 | 0.5±0.08 | 0.8±0.42 | 0.5±0.14 | 0.5±0.07 |
| | NLP | 0.2±0.04† | 0.25±0.05 | 0.2±0.03 | 0.25±0.06 | 0.1±0.06 | 0.3±0.05 |
| | NI | 0.6±0.12 | 0.7±0.16 | 0.4±0.09 | 0.5±0.09 | 0.8±0.47 | 0.5±0.08 |
| ***Angiotensin II*** | | | | | | | |
| Arterial | LP | 6.8±0.93 | 9.1±1.57* | 9.7±1.60* | 8.5±1.13* | 7.8±1.10* | 8.3±1.38 |
| | NLP | 5.8±0.84 | 5.7±0.84 | 5.7±0.81 | 5.4±0.99 | 5.9±1.60 | 5.5±0.45 |
| | NI | 4.5±0.78 | 4.2±0.71 | 4.7±0.51 | 3.9±0.56 | 3.5±0.89 | 5.2±0.87 |
| Coronary venous | LP | 6.4±0.89 | 7.4±1.02 | 7.3±0.72 | 5.9±0.65 | 5.9±0.9 | 6.8±1.2 |
| | NLP | 5.3±0.75 | 5.1±0.62 | 5.0±0.75 | 4.6±1.18 | 4.8±1.12 | 6.1±0.83 |
| | NI | 4.0±0.66 | 4.2±0.77 | 4.5±0.69 | 3.6±0.59 | 2.6±0.89 | 5.1±1.0 |

*Abbreviations: Max = maximal pacing rates; LP = lactate producers; NI = non-ischemic patients; NLP = non-lactate producers; P-P = post-pacing. All values x ± SEM; * $p<.05$ vs control; † $p<.05$ vs other groups.*

before each sample, catheters were emptied to obviate the collection of residual saline and blood, the latter were always returned into the patient after sampling.

### Statistical analysis.

Data are presented as mean and the standard error of the mean. The change in values between measurements during and after pacing and control were calculated. Group differences at control and for calculated changes were examined using a one-way analysis of variance. Within groups comparisons with control values were evaluated using a two-tailed paired t-test. Coefficients of correlation were calculated where appropriate. A p-value of <.05 was regarded as significant.

## RESULTS

Based on the occurrence of objective signs of myocardial ischemia during pacing, e.g. myocardial lactate production and/or significant (>0.1 mV) ST-segment depression, patients were grouped as ischemic (45 patients) or non-ischemic (NI, 11 patients). To facilitate identification of transcardiac neurohumoral changes in ischemic versus non-ischemic myocardium, the ischemic patients were subsequently divided into lactate- and non-lactate producers (LP, n=28 and NLP, n=17, resp.), the rationale being, that lactate production at least ensured that (part of the) coronary venous blood, collected during the study, represented the ischemic area. The presence of angina during pacing-induced stress was not considered as true

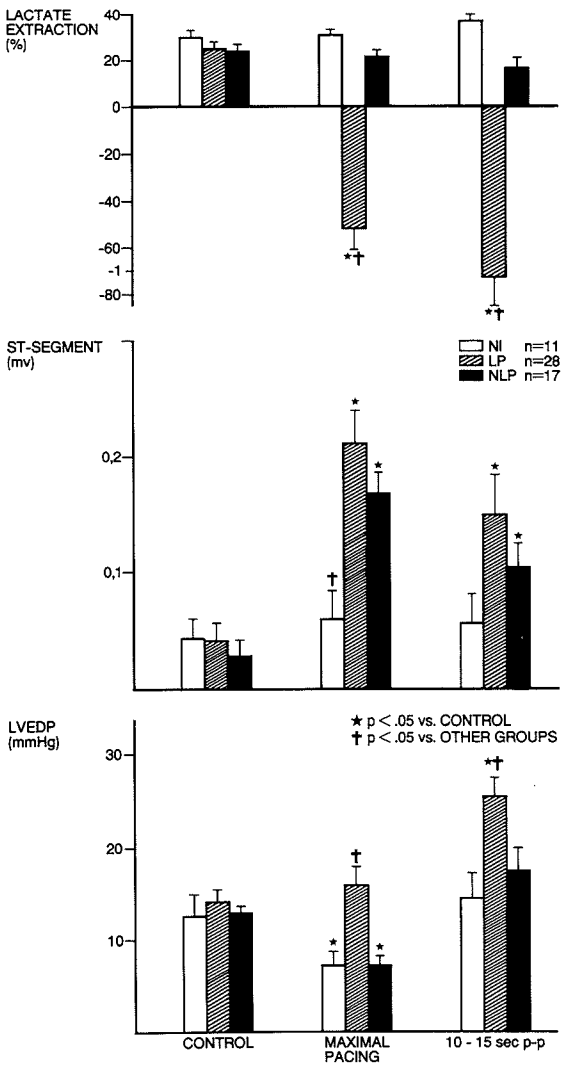



*Fig. 3: Metabolic, electrocardiographic and hemodynamic effects of pacing in non-ischemic patients (NI) and ischemic patients with (LP) and without myocardial lactate production (NLP). Although ST-segment changes were not different between LP and NLP, hemodynamic changes were. Left ventricular filling pressure (LVEDP) decreased during pacing in NLP and in NI, but remained unchanged in LP. Moreover, in the latter group LVEDP rose markedly after pacing, but not in NLP. Values are* $\bar{x} \pm SEM$.

representation of myocardial ischemia. Angina-like symptoms occurred in 6 (55%), 14 (82%) and 23 (82%) patients in groups NI, LP and NLP, respectively.

Clinical and angiographic characteristics of the 3 groups are presented in Table I. Groups were comparable as to sex, age, number of old infarcts and left ventricular volumes and ejection fraction. In contrast, objective signs of ischemia during exercise occurred more often in groups LP and NLP than in group NI. Alternatively, the severity of coronary artery disease was significantly less in group NI than in both ischemic groups. Of the non-ischemic patients, 4 had no significant coronary lesions. In contrast, the 2 ischemic groups were similar with respect to the number of vessels diseased and the ratios of left versus right coronary artery lesions.

**Hemodynamic, metabolic and neurohumoral values at control.**

At baseline, none of the patients had clinical signs or symptoms of myocardial ischemia. However, retrospectively, 4 patients, all in group LP, had myocardial lactate production, indicative of asymptomatic myocardial ischemia before pacing. In none of these patients were significant electrocardiographic changes present, compared with the initial recordings upon entry in the catheterization laboratory. As a result, average myocardial lactate uptake was significantly reduced in group LP, compared with the other two groups. In contrast, myocardial lactate extraction values were not different between the three patient groups.

Baseline hemodynamic values of the silent ischemic patients were not different from the other patients in group LP. Neither arterial nor coronary venous neurohumoral levels were outside the range of values in the other patient groups at control, although arterial norepinephrine and epinephrine values in 3 of the 4 asymptomatic patients were in the upper range (Fig. 2).

Average values for systemic and coronary hemodynamic variables were comparable between the three groups (Tables II and III).

Only myocardial oxygen consumption was greater in group NI, whereas coronary vascular resistance was significantly higher in group LP, irrrespective of the 4 patients with lactate production at baseline.

Also, the control values of most neurohormones did not differ between the groups (Table IV). Only coronary venous norepinephrine and epinephrine levels were higher in group LP, whereas arterial and coronary venous dopamine values were lower in group NLP. Again, the 4 patients with lactate production at baseline had no effect on the difference in coronary venous catecholamine levels

between LP patients on the one hand and NI and NLP patients on the other.

**Metabolic and electrocardiographic changes during and after pacing.**

By design, only group LP had abnormal myocardial lactate metabolism with maximal production values at 15 seconds post-pacing (lactate extraction -76±15%, Fig. 3), persisting until between 2 and 5 minutes after the test. ST-segment depression, however, was similar in both ischemic groups, 0.21±0.03 mV in LP and 0.17±0.02 mV in NLP patients (Fig. 3), still being significantly abnormal during the second minute post-pacing.

**Coronary and systemic hemodynamic variables during and after pacing (Tables II and III).**

Maximal pacing heart rates were comparable in both ischemic groups. In contrast, non-ischemic patients were paced to a lower heart rate (130±6.4 beats/min) compared with groups LP and NLP (152±4.1 and 147±5.1 beats/min, resp., both $p<0.05$ versus NI). As left ventricular pressures did not differ during pacing between groups, maximal values for the double product were likewise less in ischemic patients. However, when changes in heart rate and the double product between control and maximal pacing were compared, instead of maximal values, all groups were comparable.
Also, maximal values of, as well as changes from control in coronary hemodynamic variables were similar between patient groups.
In contrast, there were significant differences in pacing-induced changes in contractility and relaxation. Whereas all pressure-derived contractility and relaxation parameters improved markedly in groups NI and NLP, changes in lactate producers were minimal or non-existent (Table II). Vmax increased by 14±3.0 $s^{-1}$ and by 19±2.2 $s^{-1}$ in NI and NLP, resp., compared with only 5.3±1.2 $s^{-1}$ rise in group LP ($p<0.01$). Similar differences were present for $VCE_{40}$, although changes in the peak positive derivative of dP/dt were comparable. Moreover, whereas LV dP/dt negative and $Tau_1$ shortened significantly in NI and NLP patients, this was less in LP. Furthermore, $Tau_2$ lengthened markedly in lactate producers, but not in the other groups.
These data indicate a marked disturbance in both contractility and relaxation, but only in patients who produced lactate during pacing. As a result, left ventricular end diastolic pressure (LVEDP) fell in NI and NLP during pacing, but remained unchanged in LP, whereas at 10 seconds after pacing, LVEDP increased significantly more in lactate producers than in non-lactate producers (Fig. 3). Similar differential results were obtained with mean pulmonary artery pressures. Also, cardiac output decreased significantly from 5.9±0.35 l/min (control) to 5.3±0.37 l/min (5 min post-pacing) in group LP. Concomitantly, systemic vascular resistance increased by 21% in these patients. No such changes were observed in the other groups. Arterial pressures also increased significantly in both ischemic groups, however, more profoundly in group LP than in NLP (Fig. 4).

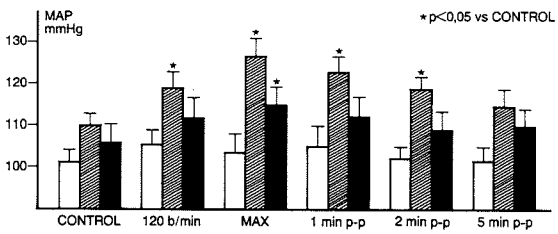



*Fig. 4: Effect of pacing-induced ischemia on arterial pressures. In lactate producers (LP) a marked rise is observed in mean arterial pressure (MAP), starting at 120 beats/minute (min) and persisting until after 2 minutes post-pacing (p-p).*

*In contrast, in non-lactate producers (NLP) a moderate increase occurs only at maximal pacing (MAX). Arterial pressures do not change in non-ischemic patients. All values are $\bar{x}$ ± SEM.*

Also, the rise in mean arterial pressure started earlier in LP patients, at 120 beats/minute, and persisted up to 2 minutes after pacing, whereas the increase in group NLP was only observed during maximal pacing rates. In contrast, arterial pressures did not change at all in non-ischemic patients.

**Neurohumoral changes during and after pacing.**

Both arterial and coronary venous neurohumoral levels remained unchanged during and after

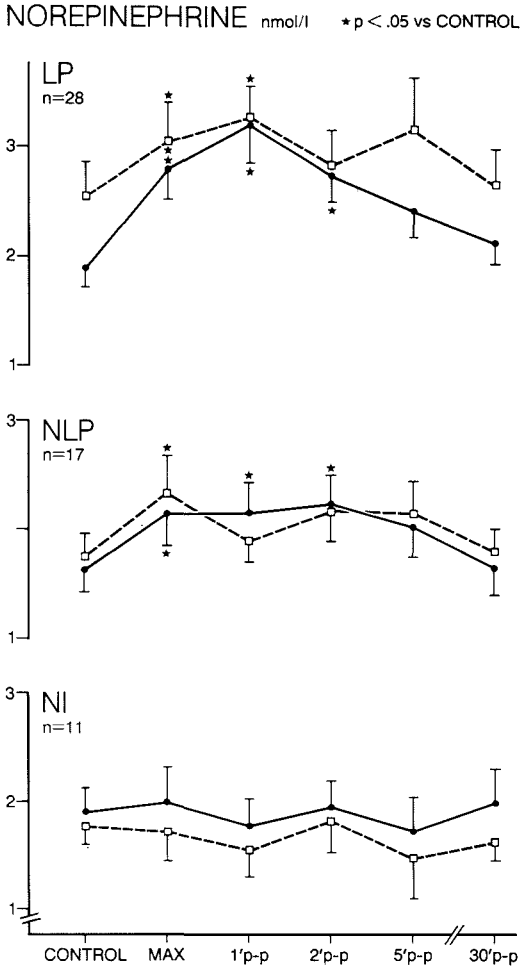
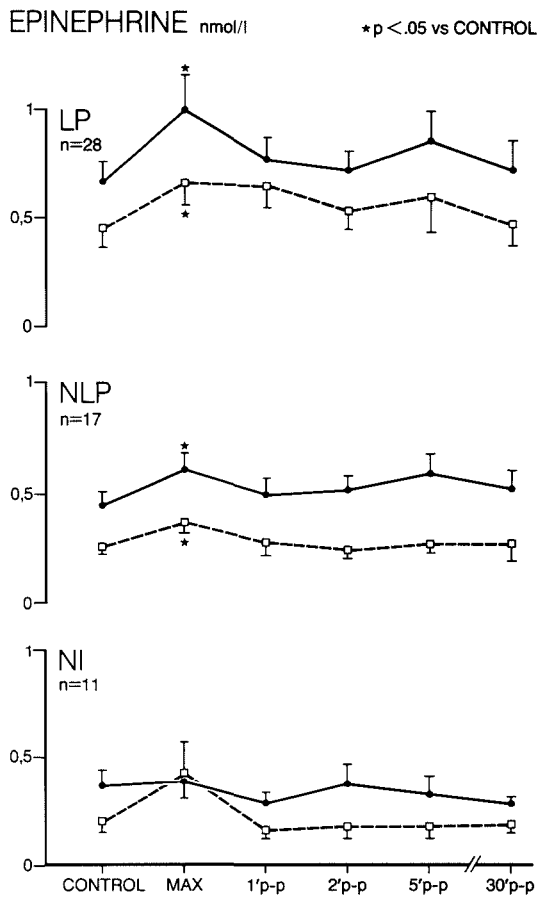



*Fig. 5: Effect of pacing-induced ischemia on arterial (closed symbols) and coronary venous (open symbols) norepinephrine levels in non-ischemic (NI), ischemic with (LP) and ischemic patients without lactate production (NLP). Pacing does not affect catecholamines in NI. In contrast, a significant increase in arterial and coronary venous levels is observed in LP at maximal pacing rates (MAX), persisting for several minutes after pacing (p-p). In NLP, only a moderate rise in norepinephrine levels is observed.*

pacing in the non-ischemic group. Furthermore, ischemia neither affected arterial nor coronary venous dopamine levels in groups LP and NLP.
In contrast, arterial norepinephrine increased significantly from 1.91±0.18 nmol/l at control to 2.8±0.26 nmol/l at the end of pacing with a further rise to 3.21±0.34 nmol/l at 1 minute post-pacing in group LP, accompanied by equally significant, albeit less pronounced increases in coronary venous norepinephrine levels. Thereafter, norepinephrine values levelled off, remaining just not significant at 5 minutes post-pacing (Fig. 5). Both arterial and coronary sinus norepinephrine levels increased in non-lactate producers, but not as profound and consistent as in the LP group. Compared to a 68% increase in arterial norepinephrine in LP, this neurohormone rose with 36% in the NLP group. Moreover,

*Fig. 6: Effects of ischemia on epinephrine levels. In ischemic patients with lactate production (LP) both arterial (closed circles) and coronary venous (open squares) rise significantly during maximal pacing rates (MAX). In non-lactate producers (NLP) changes are in the same direction, but significantly less than in LP, whereas in non-ischemic patients (NI) epinephrine does not change at all. All values are $\bar{x} \pm SEM$.*



whereas in the latter group coronary venous norepinephrine levels were elevated at maximal rates, they did fluctuate thereafter, in contrast to a sustained rise in the lactate production group. Epinephrine only changed during maximal pacing rates. Elevated arterial and coronary venous levels were found in both ischemic groups, again more so in lactate producers (Fig. 6).
Arterial angiotensin II levels also increased, but only in lactate producers, from 6.78±0.93 pmol/l at control to 9.13±1.58 pmol/l at maximal pacing rates and further to 9.7±1.68 pmol/l, 1 minute after pacing (both $p<0.05$ versus control). Values were still significantly elevated at 5 minutes post-pacing (Fig. 7). Coronary venous levels did not change, neither did arterial nor coronary venous angiotensin II levels change in the ischemic group without lactate production or in non-ischemic patients.
Neurohumoral changes did not correlate with lactate production values. However, a significant correlation was observed between changes in arterial norepinephrine and left ventricular end diastolic pressure in group LP ($p<0.05$).

**Cardiac neurohumoral balance during pacing and ischemia.**

Data on transcardiac neurohormones and coronary flow were available in 19 patients in group LP, in 15 patients in group NLP and in 9 patients in group NI.

**Catecholamines**

At control, transcardiac catecholamine fluxes were not statistically different between the three groups, although patients in groups LP and NLP showed a net release, and non-ischemic patients showed a net uptake pattern for norepinephrine (Table V). In contrast, transcardiac epinephrine and dopamine fluxes were similar in the three patient groups with uptake predominantly of the first neurohormone and release of the latter. Again, during and after pacing, no changes were found in cardiac dopamine balance. Likewise, myocardial fluxes of the other catecholamines remained unaltered in non-ischemic patients. In contrast, in lactate producers, net myocardial norepinephrine release, present at control, changed significantly to net uptake during maximal pacing rates (Fig. 8). No such changes occurred in non-lactate producers. However, the latter group showed a small but significant increase in epinephrine uptake during maximal pacing (Fig. 9). A significant increase in cardiac

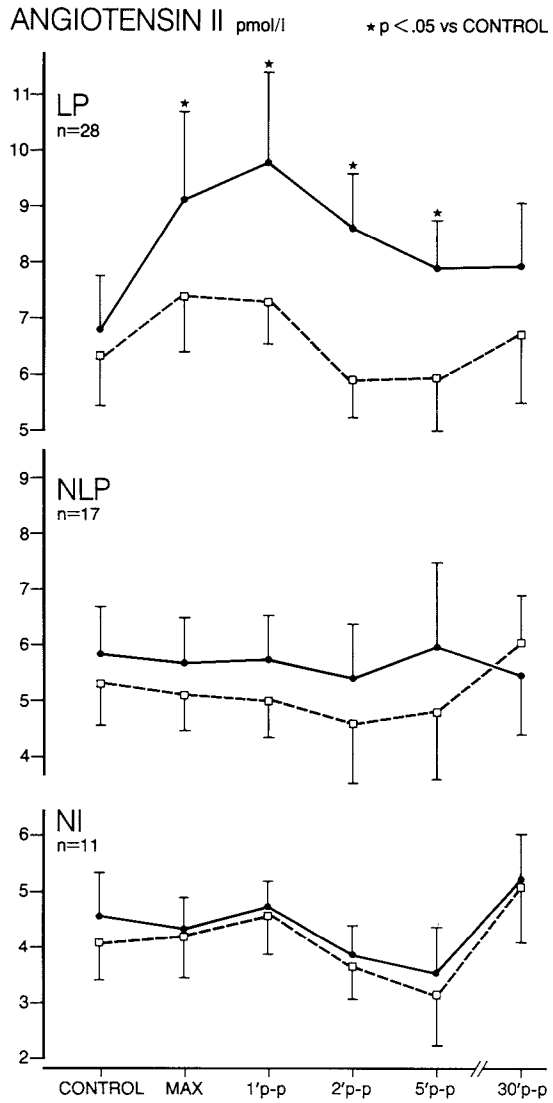



*Fig. 7: Arterial angiotensin II levels (closed symbols) increase significantly by 45% in lactate producers (LP) during maximal (MAX) and after pacing (p-p). Changes persist until 5 minutes p-p. Coronary venous levels (open squares) do not change. Also, angiotensin II remains unaltered in non-ischemic patients (NI) and in ischemic patients without lactate production (NLP).*

epinephrine uptake also occurred in group LP and was more pronounced than in group NLP.

**Angiotensin II**

At rest, control average values for angiotensin II

uptake were positive in all groups (Table V). However, individual values showed a large variation with relatively numerous patients producing angiotensin II from the heart. Pacing did not alter this pattern significantly, although lactate producers tended to have a greater angiotensin II uptake by the heart during pacing-induced ischemia (Fig. 8). Also, changes in arterial angiotensin II levels correlated significantly with cardiac uptake values during pacing ($p<0.01$).

### Hemodynamic, metabolic and neurohumoral variables after a 30-minute rest period

Patients were re-evaluated following a 30 minute resting period after cessation of pacing. All metabolic and electrocardiographic parameters were comparable to the respective control values in the three patient groups. Of the hemodynamic and neurohumoral variables, only arterial norepinephrine and mean arterial pressure in group LP were different, significantly increased with respect to their baseline values.

## DISCUSSION

The effect of myocardial ischemia on systemic and transcardiac neurohumoral levels in man has been disputed where catecholamines are concerned[7-14], whereas the effect on other neurohumoral systems, such as the circulating renin-angiotensin, is unknown.
Moreover, no attempts have yet been made to differentiate cardiac neurohumoral balances in ischemic versus non-ischemic myocardium, to relate changes in neurohormones with either systemic or coronary hemodynamic alterations during ischemia, or to estimate whether neurohormones may be differentially affected by variable degrees of ischemia.
Although the present study cannot claim to have addressed all of these arguments exhaustively, it provides several answers and clues to the complicated issue of myocardial ischemia-induced neurohumoral activation. First, it is clearly shown that systemic catecholamines become stimulated during ischemia and that the level of activation may depend on the degree of ischemia. Secondly, the circulating renin-angiotensin system may become activated as well, depending on the severity of ischemia. Thirdly, these systemic neurohumoral changes do not appear to relate to pacing per se or to the presence of angina. However, they do result in an augmentation of systemic vascular resistance and of arterial pressures, again as a function of the level of ischemia.
With respect to cardiac neurohumoral balance, ischemia results in a change of cardiac norepinephrine release to uptake, but only in lactate producers, presumably reflecting alterations in the ischemic area. In contrast, epinephrine uptake is enhanced in all ischemic patients. Finally, whereas angiotensin II in this study was shown to be released from the heart at rest in a sizeable number of patients, both pacing and ischemia did not significantly affect the average uptake pattern. Furthermore, no significant alterations in the cardiac pattern of neurohumoral fluxes were observed in non-ischemic patients.Ischemia-induced changes in systemic catecholamine levels: In our study arterial norepinephrine and epinephrine levels increased significantly in both ischemic groups during pacing-induced myocardial ischemia. By contrast, this did not occur in non-ischemic patients, despite equivalent increments in heart rate. As such, this is the first study to show systemic catecholamine activation in patients with myocardial ischemia, not necessarily accompanied by angina. Previous investigations by different groups have nearly always been carried out in anginal patients[9,11-13], thus making it fairly impossible to dissect the effect of stress, caused by angina from that of ischemia per se. Also, the presence of objective signs during ischemia was not stipulated in all studies [11,13,20]. The only study, which reported increased levels of norepinephrine and epinephrine during pacing in a well-defined patient population, did in fact include several patients without objective or subjective signs of ischemia[10].
Thus, reported changes in catecholamine levels may represent the occurrence of angina-like pain rather than of ischemia per se, which may be one of the reasons for the observed differences in results from previous studies.

### Is angina the cause for systemic neurohumoral stimulation during ischemia?

Intuitively, angina pectoris and the inherent stress may be associated with enhanced sympathetic stimulation and elevated levels of circulating catecholamines. Also, blood pressure elevations, such as observed in the present study, have been reported during anginal pain[21]. However, the majority of studies, carried out during angina, do not support this assumption[9,11,12,14]. Although early studies reported a significant increase in plasma epinephrine and norepinephrine during

**Table V: Transcardiac neurohumoral changes during and after pacing.**

| | | Control | Max | 2'P-P | 5'P-P | 30'P-P |
|---|---|---|---|---|---|---|
| ***Norepinephrine*** | | | | | | |
| nmol/min | | | | | | |
| | LP | -0.051±0.024 | 0.062±0.054* | -0.041±0.039 | -0.072±0.024 | -0.048±0.036 |
| | NLP | -0.019±0.018 | -0.039±0.023 | -0.011±0.027 | -0.020±0.023 | -0.020±0.018 |
| | NI | 0.031±0.023 | 0.060±0.061 | 0.022±0.058 | 0.044±0.021 | 0.053±0.035 |
| ***Epinephrine*** | | | | | | |
| nmol/min | | | | | | |
| | LP | 0.025±0.006 | 0.070±0.019* | 0.023±0.011 | 0.032±0.011 | 0.037±0.013 |
| | NLP | 0.022±0.008 | 0.043±0.011 | 0.026±0.005 | 0.034±0.012 | 0.022±0.008 |
| | NI | 0.027±0.009 | 0.006±0.018 | 0.025±0.019 | 0.024±0.014 | 0.021±0.017 |
| ***Dopamine*** | | | | | | |
| nmol/min | | | | | | |
| | LP | -0.014±0.009 | -0.014±0.015 | -0.014±0.008 | -0.010±0.008 | -0.003±0.010 |
| | NLP | -0.011±0.006 | 0.004±0.011 | 0.006±0.010 | 0.001±0.008 | -0.005±0.006 |
| | NI | -0.029±0.019 | -0.007±0.043 | 0.029±0.016 | -0.058±0.070 | 0.024±0.019 |
| ***Angiotensin II*** | | | | | | |
| pmol/min | | | | | | |
| | LP | 0.045±0.040 | 0.357±0.162 | 0.151±0.050 | -0.001±0.087 | 0.136±0.064 |
| | NLP | 0.052±0.04 | 0.071±0.054 | 0.101±0.064 | 0.098±0.080 | -0.085±0.078 |
| | NI | 0.053±0.035 | -0.005±0.026 | -0.044±0.044 | 0.061±0.034 | 0.032±0.028 |

*Abbreviations: LP = lactate producers; Max = maximal pacing rates; NI = non-ischemic patients; NLP = non-lactate producers; P-P = post-pacing; All values $\bar{x}$ ± SEM. *p<.05 vs control.*

exercise-induced angina[7,8], similar responses were also observed in normal individuals[8]. Alternatively, whereas one of these studies reported that exercise did not influence catecholamine levels in normal subjects[7], later investigations in normal individuals have provided unequivocal evidence for the marked stimulation of circulating catecholamines during exercise in normal persons[22,23]. This suggests, that the value of early studies may be limited, presumably as the analytic techniques available were less sensitive and specific. Whether this argument also holds as explanation for the different results with respect to catecholamine changes during angina in more recent (pacing) studies is questionable, as all make use of a more advanced radioenzymatic assay technique[24], in one instance in combination with HPLC[14]. Nevertheless, in four of these studies, no significant changes in norepinephrine nor epinephrine levels were observed during angina[9,11,12,14]. Only the study by Emmanuelson et al. reported a rise in circulating catecholamine levels[13]. Our data too, do not support an important role for angina as the predominant causative factor in catecholamine activation during myocardial ischemia. In the first place, 6 out of the 11 non-ischemic patients, in whom neurohumoral activation did not occur, had very definite angina-like complaints during the pacing test. Secondly, whereas the level and duration of catecholamine activation was different in lactate versus non-lactate producers, the percentage of patients developing angina during pacing, was identical in both groups. Moreover, if angina is a major determinant, it does not explain why nearly all studies, carried out in anginal patients only, have been negative in the neurohumoral response to pacing-induced angina, whereas the present investigation does not necessarily concentrate on angina, but nevertheless indicates that ischemia does result in neurohumoral activation. One explanation for this difference in results may be, that in our study, the addition of HPLC separation of the radioactive products improved the sensitivity of the radioenzymatic assay for catecholamines. In the only other study, where a similar technique was used[14] and in which arterial norepinephrine levels did not change, the pacing protocol was adapted to allow for long pacing periods without causing severe angina. As a result, maximum heart rates were relatively low and objective signs of ischemia only found in 9 out of 15 patients. In any case, the explanation given above does not alter the fact that other mechanisms than angina may be responsible for the effect of ischemia on neurohormones, such as observed in our study.

**Neurohumoral activation depends on the degree of ischemia.**

To explain neurohumoral activation in the sense of enhanced sympathetic stimulation and elevated circulating catecholamines in the absence of angina is no simple task. The concomitant increase in arterial pressures may reflect a cardiogenic hypertensive chemoreflex, described by James et al.[25-27], elicited by serotonin injection in the proximal part of the left coronary artery. A similar hypertensive response has been described following bradykinin injection in the left coronary artery[28]. How relevant these observations are to our study is unclear though. Few data are available on serotonin or bradykinin release from the heart during ischemia in man. In contrast, a preliminary study from our laboratory, carried out under identical circumstances as the present investigation, was

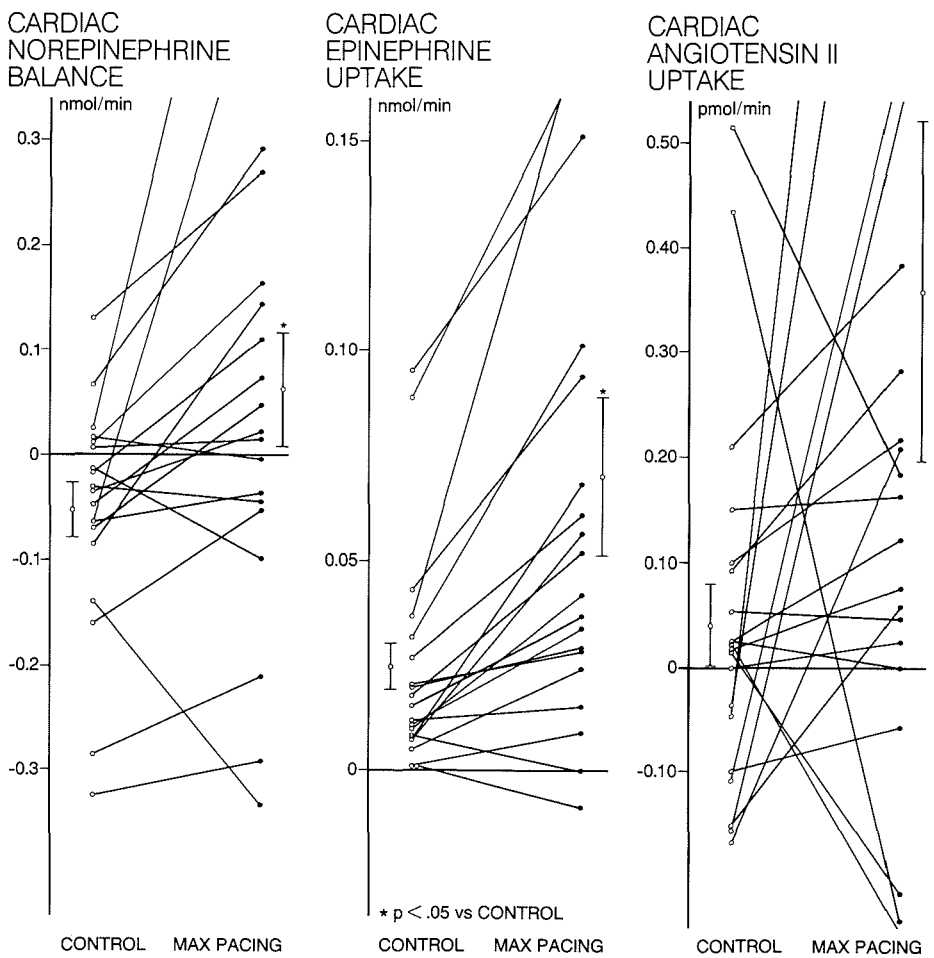



*Fig. 8: Individual changes in cardiac norepinephrine balance and in epinephrine and angiotensin II uptake by the heart in ischemic patients with myocardial lactate production. During maximal pacing rates norepinephrine release, present at control, reverses to uptake. Also, epinephrine uptake increases significantly. In contrast, angiotensin II fluxes are too variable, although there is a tendency towards enhanced uptake.*

unable to identify enhanced platelet aggregation in blood from the ischemic heart[29].
Stimulation of mechanoreceptors with afferent nerves, that travel to the tractus solitarius in the medulla oblongate, has been implicated as explanation for the tachycardia and systemic hypertension, which may accompany anterior wall ischemia[30,31]. Besides mechanical stimuli, lactate may activate (some of) these receptors with subsequent excitation of the vasomotor centre and efferent sympathetic impulse formation. Of importance is, that the majority of these receptors are in the anterior wall, which may, in part, explain the sympathetic stimulation, elevation of circulating catecholamines and blood pressure rise in the group with lactate production. By definition, ischemia in these patients must arise from the anterior, high septal or anterolateral wall of the heart. It does not explain why, however, in our study, patients without lactate production also had some degree of catecholamine stimulation and increase in blood pressure. Apart from the receptors just mentioned, receptors with vagal C fiber afferent nerves are also present in the heart, predominantly in the inferoposterior part. Stimulation of these receptors, by changes in preload or volume, depresses the activity of the vasomotor centre in the medulla oblongata. This mechanism has been implicated as explanation for the vagal overactivity with hypotension observed in interior wall ischemia or infarction[31,32]. If, in our patients without lactate production, ischemia had been confined to the inferoposterior or posterolateral wall, as the absence of lactate changes in the coronary venous effluent during ischemia may suggest, a reduction in blood pressure without elevated catecholamine levels was to be expected. This did not occur. Moreover, at the time that systemic catecholamine activation and increased arterial pressures were first observed in this group, at maximal pacing heart rates, left ventricular end diastolic pressure was reduced, which makes a significant increase in preload less probable. Thus, myocardial ischemia, exclusively located in the inferoposterior part of the heart, may not be the sole explanation. More likely, the non-lactate group, of which 88% has significant left coronary artery disease, had either predominant anterior wall ischemia comparable to lactate producers, but significantly less extensive, indicated by the absence of lactate production, or mixed ischemia of both anterior and inferior areas with relatively small ischemic regions in the sampling area of the coronary sinus catheter. In the latter instance, dilution with coronary venous effluent from non-ischemic areas would make changes in lactate metabolism in the ischemic region undetectable.
Either way, irrespective of electrocardiographic changes being similar in the two groups, the ischemic reponse would be far more pronounced in the lactate producers. This was suggested by marked depression of contractility and relaxation during pacing in the latter group. Moreover, in lactate producers, cardiac output was still significantly impaired, 5 minutes after pacing. In contrast, none of these changes occurred in the group without lactate production. Also, left ventricular end diastolic pressure increased significantly more in patients, who produced lactate during and immediately after pacing.
The significant and prolonged reduction in cardiac pump function is most likely an important factor in the more pronounced neurohumoral activation during ischemia in this group, compared with the non-lactate producers. A reduction in cardiac output during the development of ischemia may reset arterial baroreceptors, stimulating the vasomotor centre in the medulla oblongata. Whether this results in a secondary overshoot, or mainly helps to amplify the stimulus for systemic vasoconstriction derived from sympathetic afferent nerves, is unknown. The pronounced reduction in myocardial function and cardiac output in lactate producers may also explain why a significant and sustained elevation of angiotensin II levels was observed in these patients, and not in the other ischemic group.

**Circulating angiotensin II levels and degree of myocardial ischemia.**

The present data suggest, that stimulation of the circulating renin-angiotensin system is directly related to the degree of myocardial ischemia and subsequent cardiac dysfunction. Our observations indicate, that such a stimulation only occurs when ischemia is sufficiently severe to reduce cardiac and stroke output and, possibly, diminish renal flow. Whereas no further data on ischemia-induced alterations of the circulating renin-angiotensin system exist in man, two recent studies in an ischemic dog model have suggested that the various components of the circulating renin-angiotensin system, e.g. renin, angiotensin I and II, are subject to changes as a result of ischemia[15,33]. A significant elevation of arterial renin and angiotensin II levels is already observed after 30 seconds of coronary occlusion[34]. Interestingly, these changes are absent in nephrectomized animals, but not affected by beta-blockade[34]. This underscores the importance of ischemia-induced ventricular dysfunction, rather

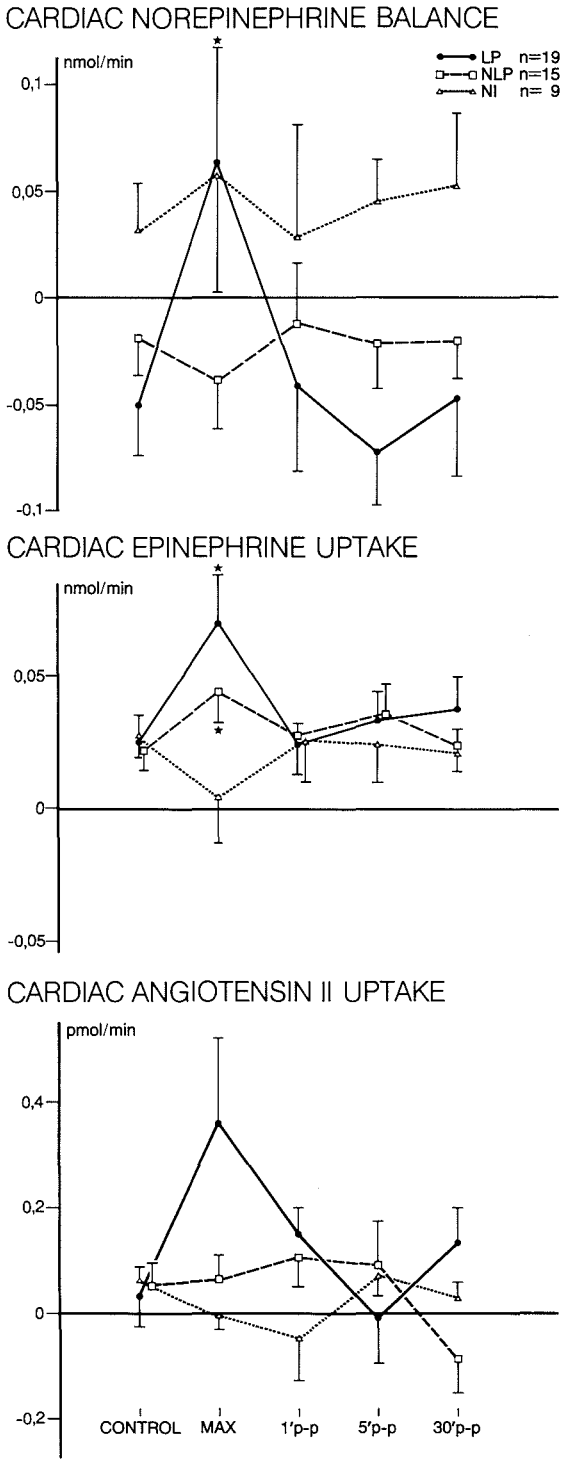



*Fig. 9: Sequential alterations in cardiac neurohumoral balance. Changes in catecholamine balance in ischemic patients with (LP) and without lactate production (NLP), if present, only occur during maximal pacing rates (MAX) and return quickly to control values after pacing (p-p). Values are $\bar{x} \pm$ SEM. NI = non-ischemic group.*

than a direct stimulatory effect through enhanced sympathetic stimulation and/or elevated circulating catecholamine levels.

**Cardiac neurohumoral balance at rest and during myocardial ischemia.**

Although, on average, angiotensin II was taken up by the heart at rest, a number of patients released the octapeptide. Recent data on gene expression of several components of the system, including renin and angiotensinogen, by the rodent heart[35] indicate, that the heart has its own renin-angiotensin system. Also, there is evidence, that (part of) the system becomes activated during cardiac hypertrophy and myocardial infarction[36]. Whether ischemia has similar effects is unknown, although our data do not support this concept.

By contrast, ischemia did significantly affect cardiac catecholamine balance. In patients with lactate production, the pattern of net cardiac norepinephrine release, present at baseline, was reversed to net cardiac norepinephrine uptake. This was very consistent in patients in this group with marked ischemia, but short lasting (Fig. 9). Although data on the 2-minute post-pacing period are only present in a small number of patients, they indicate that, even after this short period, norepinephrine balance was already returning to control values.

As no such changes were found in ischemic patients without lactate production, the observed alterations in norepinephrine balance in lactate producers presumably reflect changes in norepinephrine kinetics in the ischemic area.

Whether ischemia results in diminished regional norepinephrine spillover or enhanced clearance cannot be deduced from our methods, but would have implied tracer techniques with long equilibration times[14,37]. In animal experiments, myocardial ischemia produces overflow of norepinephrine(6). This is generally taken to reflect enhanced sympathetic activity, secondary to stress and anginal pain, as well as to enhanced autonomic reflexes, following stimulation of chemoreceptors and impaired hemodynamics[38]. However, during short lasting periods of ischemia, comparable to patient study models of pacing-induced stress,

norepinephrine overflow is either minor[6] or absent[39,40]. Thus far, patient studies have not been able to show net enhanced norepinephrine release from the heart [7,11,14]. The present study therefore is the first to indicate that during myocardial ischemia net norepinephrine release is not only absent but that in addition, its usual release pattern is reversed to net uptake in the ischemic area. The difference in results between our study and other investigations may relate to different sensitivities of the catecholamine assay[9,11] or to varying degrees of ischemia in the different studies.

The change in net norepinephrine release to uptake during ischemia is not readily explained. Merely a reduction in efflux from the ischemic area as a result of diminished venous outflow is unlikely, in view of the increased coronary venous lactate levels. Enhanced neuronal re-uptake of norepinephrine during ischemia[41] could theoretically prevent the wash-out of norepinephrine, which has been shown to accumulate in the interstitial space in the ischemic myocardium, following sympathetic stimulation [42]. Whether the relatively moderate rise in arterial catecholamine levels, found in our study, relates to the cardiac sympathetic stimulation at all, achieved under experimental conditions[42], is doubtful, however. The change in circulating neurohormones certainly did not originate from the heart. Local norepinephrine release from stimulated cardiac sympathetic nerves (exocytotic release) is dependent on the presence of high-energy phosphates in the neuron [43]. A decrease in norepinephrine release therefore may result from reduced availability of high-energy phosphates during ischemia. However, this process will take some time and cannot account for the early change, observed in our studies. More likely, presynaptic inhibition of exocytotic norepinephrine release by adenosine could be an important mechanism in addition to others, such as enhanced neuronal re-uptake[44]. Adenosine accumulation in the ischemic area has been well documented[45]. Moreover, in a similar model as used in the present study, the human heart has been shown to release significant amounts of hypoxanthine early during pacing-induced ischemia[17,46], indicative of enhanced production of its precursor adenosine in the ischemic area. Thus, this mechanism, presynaptic modulation of norepinephrine release by adenosine through coupling to adenosine A1-receptors, may well be important during early myocardial ischemia. In addition to factors influencing production and metabolism of norepinephrine, enhanced binding to cardiac or vascular adrenergic receptors during ischemia may also be considered.

In contrast to the specific changes in cardiac norepinephrine balance, only occurring in severe ischemia with lactate production, enhanced epinephrine uptake was observed in all ischemic patients, irrespective of the degree of ischemia. There was no significant difference in this respect between lactate and non-lactate producers. Enhanced extraction of epinephrine has been described in association with increased arterial epinephrine levels during relatively short periods of ischemia in a canine model[40]. In contrast, in human models with pacing-induced ischemia, significant changes in epinephrine uptake have not yet been detected[9,11]. The fact, that in our study these changes were found, may relate to a different assay technique. More importantly, the fact, that changes were also detected in the coronary venous effluent from myocardial areas, which were presumably non-ischemic, may indicate that epinephrine uptake by the heart is an unspecific event, possibly reflecting attempts to improve function in normal and ischemic parts of the heart.

**Neurohumoral stimulation and hemodynamics in myocardial ischemia.**

The enhanced cardiac uptake of epinephrine in the non-lactate group may have attributed to the fact that the hemodynamic profile was identical in this group to non-ischemic patients, despite the presence of ischemia. In contrast, neither the increase in cardiac epinephrine uptake nor the reversal of net norepinephrine release to uptake, significantly counteracted the ischemia-induced impairment of contractility and relaxation, observed in lactate producers.

Also, when the overall coronary sinus flow measurements are considered, there was no indication that enhanced norepinephrine uptake had any effect on coronary flow. If anything, coronary vascular resistance decreased more in lactate producers than in other groups. Several studies indicate that during progressive coronary flow reduction, cardiac sympathetic stimulation may reverse metabolic dilatation to alpha-adrenergic vasoconstriction[47-49]. Also, human studies, conducted in identical circumstances as the present investigation, suggest a limitation of coronary flow increase or a decrease in the post-stenotic area, preceding the manifestation of ischemia[17,50]. Whether adrenergic mechanisms are involved in the latter phenomenon is unknown. In this study, neurohormones were only determined after ischemia was established. Moreover, the thermodilution flow technique does

not allow for a precise determination of regional, sequential changes in flow. It does not exclude the possibility that a local decrease in coronary flow did indeed occur, following the changes in cardiac norepinephrine balance in patients with severe ischemia.
In contrast, our study clearly indicates that systemic vasoconstriction occurs concomitantly with the activation of systemic neurohormones. Elevated systolic arterial pressures during pacing-induced ischemia have been reported before, but only in a limited number of patients with coronary artery disease and severe myocardial ischemia[51]. When a rise in arterial pressures is taken to reflect enhanced systemic vasotone, ischemia-induced changes in catecholamines and in circulating angiotensin II levels could explain the latter. In the group without lactate production and moderate ischemia, the small increase in arterial pressures during maximal pacing rates was accompanied by a moderate rise in arterial catecholamines, but not in angiotensin II. Our data indicate that, depending on the severity of myocardial ischemia, the increase in systemic vascular resistance and arterial pressures may be sustained following ischemia and could be clinically important. Although we have no data on ventricular volumes during pacing-induced ischemia, it is reasonable to assume that systolic wall stress will be elevated following the rise in arterial pressures. Subsequently, myocardial oxygen demand will increase, this may suggest a role for specific neurohumoral modulators, such as alpha-adrenergic antagonists and, possibly, converting enzyme inhibitors[55], during ischemia.

**Limitations of the study.**

By necessity, several assumptions have been made in this study, which may not be entirely correct.
To differentiate coronary venous effluent from ischemic versus non-ischemic areas, a distinction was made between lactate and non-lactate producers. Here it was assumed, that the presence of myocardial lactate production values in the coronary venous effluent at least ensured, that blood was sampled from the ischemic area. The number of patients with myocardial lactate production may have been underestimated, though it stands to reason that in those patients, where the coronary effluent from the ischemic area was diluted to such an extent that lactate release was no longer detectable, the same was assumedly true for the neurohormones. Moreover, the distinction between lactate versus non-lactate producers served to differentiate between mild versus more severe ischemic patients. Again, the absence of appreciable lactate release in the coronary venous effluent either indicates small ischemic areas in the sampling region of the coronary sinus catheter or predominant inferoposterior ischemia. In both instances the hemodynamic (and neurohumoral) consequences of ischemia are mitigated compared with conditions, where lactate production is clearly present.
Theoretically, the lower absolute maximal pacing values in non-ischemic patients may limit the interpretation of the study. Ideally, pacing conditions should have been identical in the three groups. No data are present in humans, which suggest that elevating heart rate increases circulating catecholamines or activating cardiac sympathetic tone. In contrast, preliminary data from our laboratory indicate that fast atrial pacing to similar heart rates, which induces marked circulating neurohumoral activation in ischemic patients has no effect on these systems when ischemia is absent[53]. Moroever, in the present study, the difference in heart rate between baseline and the end of pacing was comparable in all three groups. Therefore, the (absence of) changes in neurohormones in the non-ischemic group in this study has been taken to truly represent the absence of myocardial ischemia rather than the result of a mismatch between study populations.

**Implications.**

This study indicates that acute myocardial ischemia not only results in activation of systemic catecholamines and the circulating renin-angiotensin system in man, but also in significant changes in cardiac catecholamine balance. Whereas the first is associated with a sustained increase in systemic vascular resistance, the second may influence post-stenotic coronary vasotone, although further detailed studies will have to be conducted to substantiate the latter hypothesis. Both mechanisms may significantly influence the severity of the ischemic attack, which originally provoked this neurohumoral response. Although the clinical importance of systemic and transcardiac neurohumoral changes, resulting from pacing-induced ischemia, have to be further specified, acute myocardial ischemia at rest, e.g. during unstable angina or the early phase of infarction, may well reflect this condition. The significance of specific therapy, focussing on modulation of these neurohumoral changes, such as alpha-adrenergic blockade, converting-enzyme inhibitors or renin-antagonists as an additional form of antiischemic therapy, should be further

evaluated in these clinical settings.
Our observation, that enalaprilat diminishes pacing-induced myocardial ischemia through a modulating effect on systemic neurohumoral activation[52] emphasizes the importance of these therapeutic modalities as antiischemic therapy.

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# Chapter XI

# ANTIISCHEMIC EFFECTS OF CONVERTING ENZYME INHIBITION THROUGH MODULATION OF ISCHEMIA-INDUCED NEUROHUMORAL ACTIVATION.

# XI.1.

# Neurohumoral Activation during Acute Myocardial Ischemia. Effects of ACE Inhibition.

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# Neurohumoral activation during acute myocardial ischaemia. Effects of ACE inhibition

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KEY WORDS: ACE inhibitors, myocardial ischaemia, catecholamines, renin-angiotensin.

*ACE inhibition may be useful in several manifestations of ischaemic heart disease, such as heart failure due to ischaemic cardiomyopathy. Recent evidence suggests that these effects may also be present in normotensive patients with ischaemic heart disease without heart failure. Theoretically, converting-enzyme inhibition, through coronary and systemic vasodilating effects and negative inotropic properties, should have a favourable effect on the myocardial oxygen supply/demand ratio and, hence, affect the incidence and severity of myocardial ischaemia. It is doubtful, however, whether these cardiac and extracardiac properties of ACE inhibitors really underlie its potential antiischaemic effects, at least in the average patient with ischaemic heart disease without concomitant heart failure and hypertension. Recent animal and human studies indicate that converting-enzyme inhibitors may modulate myocardial ischaemia by reducing ischaemia-induced circulating neurohumoral activation. It has been shown that, depending on the severity of ischaemia, the circulating renin-angiotensin system may become activated together with an increase in circulating catecholamine levels. ACE inhibition suppresses this neuroendocrine stimulation during ischaemia and modulates subsequent systemic and, presumably, also coronary vasoconstriction. In addition to these effects on circulating neurohormones, ACE inhibition could affect myocardial ischaemia through a number of local actions, e.g. modulation of tissue (cardiac) angiotensin II formation and bradykinin breakdown, stimulation of prostaglandin synthesis and, in the use of sulphydryl compounds, by affecting EDRF formation. Whether ACE inhibitors have clear antiischaemic effects in all clinical conditions is uncertain. Their efficacy to limit exercise-induced ischaemia has been questioned. In contrast, pacing-induced ischaemia in patients at rest is clearly prevented by ACE inhibition. This differential effect may be related to a more pronounced difference in circulating neurohormones during exercise per se. It also suggests that ACE inhibitors may be particularly useful as (additional) antiischaemic therapy in patients with angina at rest, e.g. unstable angina and the acute phase of myocardial infarction.*

## Introduction

The usefulness of ACE inhibition in heart failure and hypertension has been well established. Also, recent data indicate beneficial effects of ACE inhibition on ventricular dilation and remodelling after myocardial infarction[1]. Moreover, animal studies strongly suggest that converting-enzyme inhibiton may limit the occurrence of ventricular dysrhythmias during sudden reperfusion following prolonged periods of ischaemia[2–5].

There are a number of theoretical considerations which suggest that, besides these beneficial cardiovascular effects, ACE inhibitors may have direct antiischaemic properties as well. Thus far, however, converting-enzyme inhibitors have not been uniformly successful in reducing myocardial ischaemia, despite their potential to reduce myocardial oxygen demand and to improve coronary flow. Following the rationale that converting-enzyme inhibition may have a beneficial effect on the myocardial oxygen supply/demand ratio through a reduction in pre- and afterload, a negative inotropic effect and by way of coronary vasodilatation, a number of studies have been carried out in patients with ischaemic heart disease to prove the antiischaemic and antianginal efficiency of ACE-inhibition. Following high dosages of oral

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captopril, Daly and coworkers observed a small, but significant reduction in arterial pressures and in myocardial oxygen consumption in patients with angina[6]. As these effects were not accompanied by systemic or transcardiac neurohumoral changes, the authors speculated that the drug might have potential antianginal effects through a lack of reflex increase in myocardial sympathetic tone. A subsequent, placebo-controlled study in the same patients did indeed show an improvement of exercise duration, but not of anginal threshold, and only in those patients in whom captopril decreased systolic blood pressure. However, the use of additional antianginal medication, including beta-blockers, as well as diuretics obviates a clear interpretation of the exact role of captopril as an antiischaemic agent in this study. Further studies in patients with angina pectoris, also carried out during exercise-induced stress, have not been able to clarify whether ACE-inhibitors have antiischaemic effects. Whereas a number of these investigations did indicate some antiischaemic efficacy of the ACE inhibitor under study[7–11], others did not[12,13]. Moreover, antiischaemic effects, when present, do not appear necessarily to relate to changes in systemic haemodynamics and myocardial oxygen consumption[7–9] as suggested by Daly and coworkers[6]. One problem with interpreting the data is that most studies were uncontrolled and of relatively short duration. Recently, another mechanism has emerged, through which ACE inhibition may affect ischaemia in humans. During (pacing-induced) myocardial ischaemia a short-lasting, but significant systemic neuroendocrine activation has been demonstrated, accompanied by systemic vasoconstriction and a rise in arterial pressures[14,15] As it is likely that the latter mechanism may amplify myocardial ischaemia, ACE inhibition, theoretically, could attenuate this by preventing the activation of circulating neurohormones.

This article focuses on the potential antiischaemic properties of ACE inhibition in relation to its effect on neuroendocrine stimulation during ischaemia.

## Neurohumoral activation in ischaemic syndromes

Significant neuroendocrine stimulation is commonly observed during acute myocardial infarction. Early following the onset of infarction, both plasma and urinary catecholamine levels are significantly elevated[16,18] The magnitude of these changes is directly related to acute alterations in cardiac function, with marked elevations prominent in patients with a low-output state or cardiogenic shock[19,20]. Likewise, circulating renin-angiotensin levels increase significantly during myocardial infarction complicated by acute failure[21]. These early neurohumoral changes during infarction apparently relate to cardiac dysfunction and, therefore, are likely to be secondary to the subsequent reduction in cardiac output, blood pressure and tissue renal perfusion. Whether the stress of anginal pain, commonly present during the initial phase of infarction, exerts additional stimulatory effects on neurohormones is not clear. Likewise, it is uncertain, whether anginal pain has any effect on circulating neurohormones during short periods of myocardial ischaemia per se without infarction. Studies carried out during exercise-induced stress, have demonstrated similar increases in circulating catecholamines in normal individuals and in patients with myocardial ischaemia and angina[22]. Also former investigations carried out during pacing-induced stress, which in itself does not affect circulating neuro-hormones[14], have not consistently indicated significant alterations in systemic catecholamine levels during anginal pain[23–25]. In contrast to the variable and conflicting effects of stress- or pacing-induced angina on neurohormones, a significant elevation of norepinephrine and epinephrine levels has been demonstrated during angina following spontaneous coronary artery spasm[26]. Besides these conflicting observations on the relationship between anginal pain and circulating neurohormones, it is also not clear whether ischaemia per se, not necessarily accompanied by anginal pain, affects systemic catecholamines. The few data available in humans do not indicate that ischaemia results in an extra activation of circulating catecholamines, at least not when compared with normal individuals subjected to identical stress[22]. Moreover, it is not known whether, in humans, myocardial ischaemia affects circulating renin-angiotensin levels, as this aspect has not yet received much attention at least not in subjects without hypertension or heart failure.

**Systemic neurohormones in normotensive patients with coronary artery disease at rest and during pacing-induced stress**

Ertl and coworkers have demonstrated, that in canine models of myocardial ischaemia, arterial renin activity is significantly increased, following 15 min of coronary artery occlusion[27,28] (Fig. 1). These effects were accompanied by changes in arterial angiotensin II, but not by increments in coronary venous renin-angiotensin levels. Interestingly, whereas beta-blockade did not blunt the activation of circulating renin-angiotensin, nephrectomy prevented it completely. The authors also observed, that fast atrial pacing which, in the absence of a coronary artery narrowing, had no effect resulted in a 50% increase in arterial and coronary venous renin levels in the presence of a significant stenosis.

Recently, we evaluated the response of the circulating renin-angiotensin system to myocardial ischaemia in humans. To this purpose, normotensive patients with coronary artery disease referred for coronary angiography, were studied. Changes were compared with simultaneous effects of ischaemia on circulating catecholamines. At present, only limited data are available concerning the normal values of

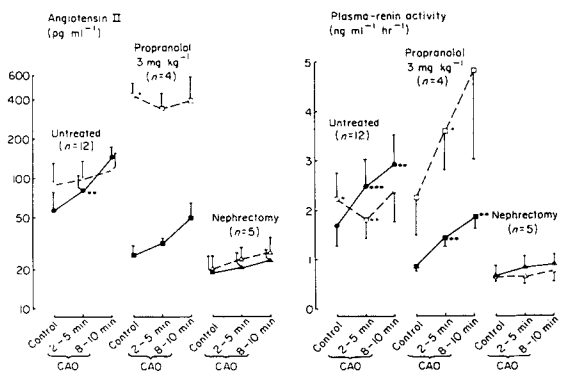


*Figure 1* Changes in plasma-renin activity (PRA) and angiotensin II concentrations during coronary artery occlusion (CAO) in open-chest, anaesthetized dogs. In untreated and propanolol pretreated animals arterial (filled symbols) PRA levels increase significantly. No such changes are observed in nephrectomized animals subjected to CAO. Arterial angiotensin II levels parallelled the effect of CAO on PRA levels, but changes were less significant, due to large scatter. (Reproduced from Ref 28, with permission.)

arterial angiotensin II, the hormone under study, in normotensive patients with coronary artery disease, without clinical signs of heart failure. Hence, we first compared resting circulating neurohumoral levels in these patients and in patients with ischaemic cardiomyopathy and (moderate) heart failure. A significant difference was observed in arterial norepinephrine and angiotensin II levels, but not in arterial epinephrine and dopamine values[29] (Fig. 2). Interestingly, when patients with coronary artery disease without heart failure were subsequently divided into patients with normal vs depressed left ventricular function, a difference was also observed in arterial angiotensin II levels between the two patient groups; a significantly higher arterial angiotensin II level was present in patients with impaired left ventricular function than in those with normal ventricular function. This early activation of the renin-angiotensin system apparently does not affect vasotone. In our study, systemic vascular resistance was comparable in patients with a normal and with an impaired left ventricular function without heart failure[29].

Pacing-induced stress does not affect systemic or coronary venous catecholamine and angiotensin II levels, at least not in normal individuals or in patients with coronary artery disease who do not become ischaemic during pacing[14]. In contrast, during pacing-induced ischaemia, a significant elevation in systemic angiotensin II is observed[30]. This observation compares well with the data from Ertl's studies on renin-angiotensin changes during coronary artery occlusion and subsequent fast atrial pacing[27,28]. The main difference is that, in our studies we did not observe a rise in coronary venous angiotensin II levels. Consequently, in our study, there was a tendency for angiotensin II to be taken up by the heart during the ischaemic period (Fig. 3). These changes were not significant, however, due to a wide variability of individual values[31]. Nevertheless, our observation on cardiac angiotensin II balance does not support an enhanced angiotensin II release by the heart during ischaemia.

Myocardial ischaemia also significantly increases circulating catecholamines in normotensive patients with coronary artery disease. In our studies, an elevation of both arterial and coronary venous epinephrine levels was observed druing myocardial ischaemia (Fig. 4).

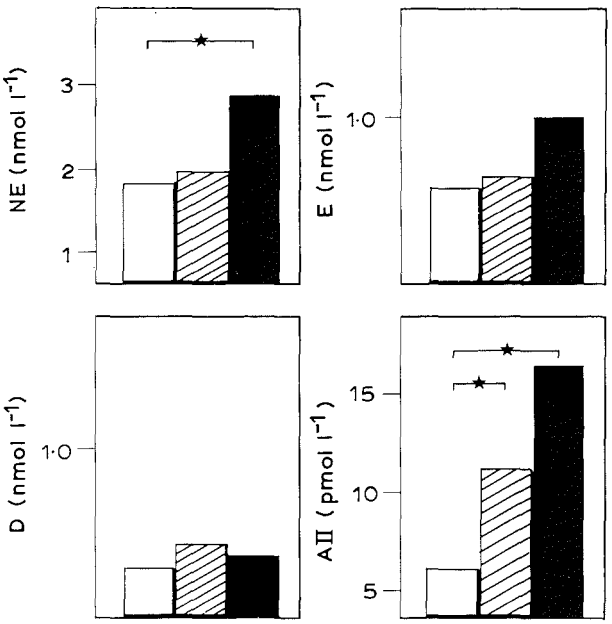


*Figure 2* Arterial catecholamines and angiotensin II (AII) levels in patients with coronary artery disease and normal left ventricular (LV) function (LV ejection fraction ≥45%, $n = 28$, □), LV dysfunction without (LV ejection fraction <45%, $n = 8$, ▨) and with congestive heart failure (CHF, $n = 9$, NYHA Class 3, ■). Epinephrine (E) and dopamine (D) levels are similar in all groups. Whereas norepinephrine (NE) levels are only elevated in heart failure patients, AII values are also significantly different in patients with asymptomatic LV dysfunction compared with patients with normal LV function.* $P < 0{\cdot}05$ vs control. (Adapted from Ref 29, with permission of the publishers).

However, only during pacing itself and not following pacing-induced stress. In contrast, arterial and coronary venous norepinephrine levels were significantly elevated for a longer period, not only during pacing but also for several minutes thereafter[15]. The magnitude of this neuroendocrine activation during myocardial ischaemia is variable and appears to relate to the severity of ischaemia and subsequent ventricular dysfunction[32,33]. Nevertheless, increased arterial pressures, due to enhanced systemic vasoconstriction, are a consistent finding during pacing-induced ischaemia[15]. It is not illogical to assume a direct relationship between the observed changes in neurohormones and increased arterial pressures during pacing-induced myocardial ischaemia. Secondary to the effect of ischaemia on cardiac pump function, arterial pressures are not expected to increase, but rather decrease. Our findings, therefore, suggest counterregulatory forces.

Furthermore, it is not illogical to assume that the increase in arterial pressures and afterload and, hence, in myocardial oxygen demand, will amplify the severity of myocardial ischaemia already present. Whether this additional form of myocardial ischaemia is substantial enough to warrant extra antiischaemic therapy in patients who are already ischaemic is, as yet, unknown.

## Reduction of myocardial ischaemia through inhibition of secondary neurohumoral stimulation. Which agent to choose?

Theoretically, a number of options exist to affect myocardial ischaemia by reducing

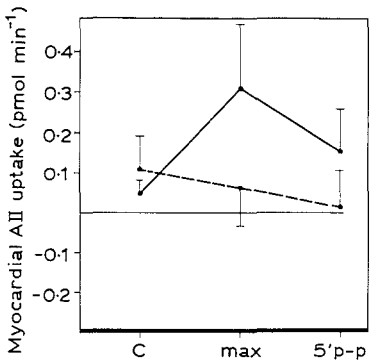


*Figure 3* Cardiac angiotensin II balance in normotensive patients with coronary artery disease without heart failure. Data are given during control (C) before atrial pacing and during maximal pacing rates (max). Patients are divided into those who become ischaemic during atrial pacing with subsequent myocardial lactate production (GR I + L, $n = 26$, ●–●) and those who do not (GR NI, $n = 7$, ●– – –●). In GR NI cardiac angiotensin II balance remains unchanged whereas, in ischaemic patients, there is a tendency towards enhanced cardiac angiotensin II uptake. However, due to large individual variability, changes are not significant. Data are mean ± SEM.

ischaemia-induced neurohumoral overactivation and/or the accompanying systemic vasoconstriction. Vasodilating agents in general may reduce the increase in arterial pressures following myocardial ischaemia and, hence,

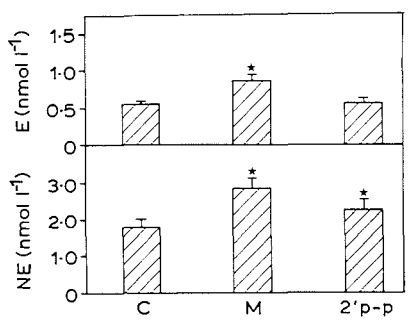


*Figure 4* Effect of pacing-induced ischaemia on arterial catecholamine levels in normotensive patients with coronary artery disease with heart failure. A significant elevation in epinephrine (E) and norepinephrine (NE) is observed during maximum pacing rates (M), compared with control (C) levels. Changes in epinephrine are shortlasting, but the increase in norepinephrine persists for at least several minutes post-pacing (p-p). Data are mean ± SEM $*P < 0{\cdot}05$ vs control. (Reproduced from Ref 33, with permission of the publishers.)

attentuate ischaemia, without any effects on or related to neurohormones. Bepridil, a compound with both fast sodium- and slow calcium-channel blocking properties and calmodulin-inhibiting effects, indeed prevents the increase in systemic vascular resistance and arterial pressures following myocardial ischaemia[34]. Whether this effect relates to vasodilation per se, to modulation of post-synaptic $\alpha_2$ stimulation or primarily to the antiischaemic effects of bepridil, which mainly result from a reduction of myocardial oxygen demand, is unclear. The antiischaemic effects of calcium-antagonists, such as bepridil, are well established. As they affect vasoconstriction, induced by postsynaptic $\alpha_2$ stimulation[35], this property is likely to be of additional importance during ischaemia-induced increments in circulating catecholamine levels.

Alternatively, postsynaptic $\alpha_1$-blocking agents, for similar reasons, could be useful during (pacing-induced) myocardial ischaemia. Supposedly, these agents should be particularly useful in preventing $\alpha_1$-receptor-mediated coronary vasoconstriction during myocardial ischaemia[36]. Unfortunately, no data are available on the effects of selective postsynaptic $\alpha_1$-blocking agents in this setting. In fact, only few data exist on the effects of $\alpha_1$-adrenoceptor blockade on myocardial ischaemia and/or angina pectoris. In patients with variant angina, prazosin does not prevent or reduce ischaemic attacks[37]. In contrast, in patients with chronic, stable angina pectoris, indoramin, in addition to nitrates and beta-blockade, improves exercise duration and increases maximal oxygen consumption during exercise without a change in the maximal double product[38]. Although in the latter study it has been suggested that the antiischaemic effect of the selective $\alpha_1$ adrenoceptor-blocker, indoramin, results from actions on peripheral arterioles, no evidence has been provided for an effect on neurohumoral (over)activation during exercise-induced ischaemia.

Besides $\alpha$-adrenoceptor blockade, converting-enzyme inhibition may afford another approach to induce antiischaemic effects by modulating (the effects on) neurohumoral stimulation secondary to myocardial ischaemia. ACE inhibition, well-established in the treatment of congestive heart failure and hypertension, and now recognized as potentially useful in limiting post-myocardial dilatation and remodelling,

thus far has not been uniformly considered as antiischaemic therapy. A few short-term studies in patients with ischaemic heart disease have recently been conducted with captopril, enalapril, ramipril and cilazapril to establish whether these compounds have any antiischaemic potential[6–13]. The outcome of these studies has been variable. A number have claimed success in this respect[6–11]; others have been unable to show any antiischaemic effect[12,13]. All of these studies were carried out during exercise-induced stress. If limitation of ischaemia-induced neurohumoral activation is the primary mode of action through which ACE inhibition reduces myocardial ischaemia it may be argued that this phenomenon is probably of less importance in exercise- than in pacing-induced ischaemia. Exercise results in a marked increase in circulating neurohormones, at least in catecholamines, even in normal individuals[22]. The extra increase in circulating neurohormones by subsequent myocardial ischaemia may be small in comparison to the initial elevation following exercise alone and, hence, may not result in additional systemic (and coronary) vasoconstriction. In contrast, during pacing-induced stress without ischaemia, with the individual under study resting, systemic catecholamine and renin-angiotensin levels do not change[14]. Here, the subsequent activation during ischaemia becomes clearly evident. It may be anticipated, therefore, that in patients with ischaemia at rest, e.g. with unstable angina or during the early phase of infarction, a similar or even more pronounced neuroendocrine activation is observed.

**Ischaemia-induced neurohumoral activation and the potential antiischaemic effects of ACE inhibition**

Converting-enzyme inhibition presumably affects ischaemia-induced sympathetic stimulation and neuroendocrine activation in a rather complex way. Besides reducing circulating angiotensin II levels and increasing plasma renin and angiotensin I, ACE inhibition also modulates the formation of local tissue angiotensin II. Endogenous renin-angiotensin systems are present in many tissues, including the heart, and exert paracrine and autocrine influences on local tissue function[39–42]. It has been postulated that these tissue renin-angiotensin systems are the primary site of action of ACE inhibitors. It is unknown, however, whether, during relatively short periods of ischaemia, these local tissue renin-angiotensin systems really are important and whether they become activated in these conditions. Cardiac angiotensin II may influence coronary vasotone and coronary flow. In the isolated ischaemic rat heart, angiotensin I and II administration negatively affects myocardial metabolism and high-energy phosphate content and amplifies reperfusion arrhythmias[43]. It is tempting, therefore, to speculate on changes in cardiac renin-angiotensin activation during ischaemia in man. Evidence for this does not exist, however. In contrast, it has been proposed that not the local systems, but rather the circulating renin-angiotensin system is involved in short-term control of cardiovascular regulation[44]. The local tissue systems, on the other hand, would provide for a more tonic regulation of tissue function. Hence, unless regional changes in tissue renin-angiotensin activation during short periods of ischaemia are established for certain, it appears more sensible to consider only overall changes in circulatory renin-angiotensin activation during short periods of myocardial ischaemia.

In this respect, preliminary data from our laboratory indicate, that ACE inhibition with intravenous enalaprilat, at dosages which reduce arterial ACE activity by 90%, abolishes the increase in angiotensin II levels, observed during pacing-induced stress[45]. In addition to these effects on angiotensin II levels, enalaprilat also reduces catecholamine stimulation during ischaemia.

The renin-angiotensin system affects sympathetic adrenergic activity through various central and peripheral actions. Angiotensin II facilitates norepinephrine release from vascular adrenergic nerve endings, limits neuronal reuptake of norepinephrine, promotes norepinephrine biosynthesis in the adrenergic nerve terminal and stimulates central norepinephrine activity[46,47]. Furthermore, it facilitates catecholamine release from the adrenal medulla and potentiates the activity of the sympathetic nervous system in general. Converting-enzyme inhibition has been shown to increase elevated circulating norepinephrine levels in severe hypertension and in heart failure. Probably more applicable to the topic discussed in this paper, Liang and Gavras have demonstrated that ACE-inhibition decreases norepinephrine

release in conscious dogs subjected to acute hypoxaemia[48]. The reduction in catecholamine and renin-angiotensin activation during myocardial ischaemia by enalaprilat, as shown by us, may be of clinical value. With its effect on neurohormones, enalaprilat abolished the rise in arterial pressures commonly observed during pacing-induced ischaemia. Hence, it may be postulated that enalaprilat also reduced myocardial oxgyen demand in this setting of pacing-induced ischaemia. The latter may be (one of) the mechanism(s) through which the compound exerted its antiischaemic properties in this study[45].

### Other mechanisms through which ACE inhibition may affect myocardial ischaemia

Besides its modulating effect on neurohumoral stimulation during ischaemia, ACE inhibition may limit the extent of myocardial ischaemia also through both cardiac and extracardiac pharmacologic effects, not necessarily related to changes in circulating neurohormones. Apart from the latter, converting-enzyme inhibition also modulates cardiovascular function through effects on local renin-angiotensin, prostaglandin synthesis, bradykinin degradation and possibly also on local norepinephrine release. In addition, ACE inhibitors which contain a sulphydryl-group may induce vasodilation through an effect on EDRF. The magnitude of these additional properties varies with each ACE-inhibitor.. Moreover, these effects of ACE-inhibitors in general will vary, depending on duration and magnitude of action, rate of perfusion and distribution of the drug in different tissues and as a result of different physico-chemical properties. Thus, the various ACE inhibitors will contribute differently to the cardiovascular pharmacological profile of converting-enzyme inhibition in general. Some

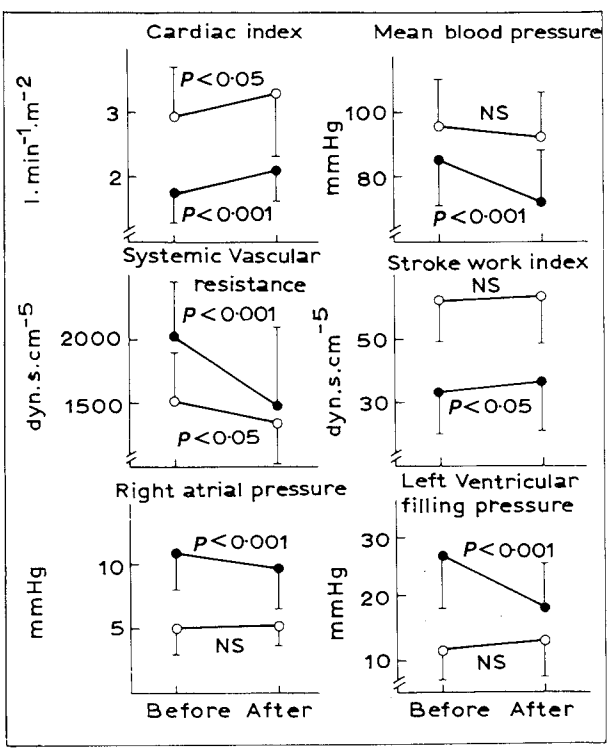


*Figure 5* Systemic haemodynamic effects of ACE inhibition in normal subjects (open circles, $n = 14$) and patients with congestive heart failure (closed circles, $n = 36$). Values given indicate mean ± standard deviation (S.D.). (Reproduced from Ref 50, with permission.)

of these pharmacological properties may influence the myocardial oxygen demand-supply balance and, hence, be instrumental in preventing or alleviating myocardial ischaemia. Arterial and venodilating effects and, subsequently, a reduction in afterload and preload may substantially reduce myocardial oxygen demand. Likewise, coronary blood flow and myocardial oxygen supply may be augmented following converting-enzyme inhibition. Indeed, in heart failure patients, such systemic haemodynamic effects are clearly present. Also, in patients with dilated cardiomyopathy, coronary flow increases with ACE inhibition, given intracoronary[49], but not following intravenous or oral administration of converting-enzyme inhibition[50,51]. However, it is doubtful whether vasodilating effects play a significant role in normotensive patients without heart failure. Faxon *et al.* reported no change in mean arterial, right atrial or left ventricular filling pressures in normal individuals following teprotide[50] (Fig. 5). Likewise, coronary blood flow did not change in their study (Fig. 6). In contrast, Daly and coworkers observed a significant 11% reduction in arterial pressures in normotensive patients with coronary artery disease without heart failure; however, only after high dosages of oral captopril (average 127 mg) were administered[6]. This was accompanied by a significant reduction in myocardial oxygen consumption. It is questionable whether equivalent results are obtained with dosages of captopril, presently considered to be in the 'normal' range[7,8]. We have reported that in similar type patients, enalaprilat (1·25–1·5 mg intravenously) also did not induce significant haemodynamic effects although antiischaemic effects were clearly present[29]. Moreover, coronary flow also did not change in this study. These observations do not support the hypothesis that the antiischaemic effects of ACE inhibitors depend on changes in the myocardial oxygen supply demand ratio. Of course, regional coronary vasodilation in the ischaemic areas through local effects on angiotensin, bradykinin or prostaglandins, cannot be ruled out. In this respect, modulation of cardiac norepinephrine release and its effect on coronary vaostone during ischaemia certainly would seem an attractive hypothesis to explain, at least in part, the antiischaemic effects observed with ACE inhibition.

In conclusion, animal and human data on the effect of ACE-inhibition on myocardial ischaemia do indeed indicate that these agents may have antiischaemic effects, at least in conditions where the patient is resting. These antiischaemic properties appear to relate to modulation of ischaemia-induced neurohumoral activation and subsequent systemic and possibly coronary vasoconstriction. Whether this effect also applies to different situations, such as exercise-induced stress, is unclear. It is also unclear, whether local tissue effects of ACE-inhibitors are relevant during a short period of myocardial ischaemia. The latter aspect needs further clarification, particularly in view of the different effects of the various ACE inhibitors

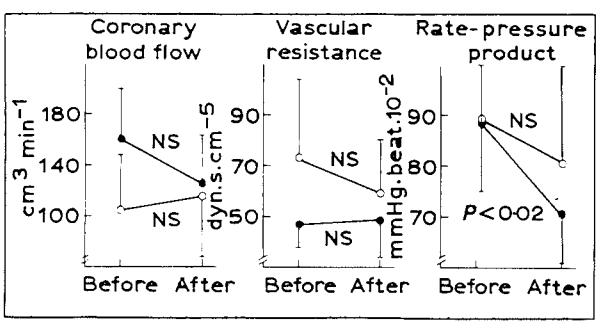


*Figure 6* The effects of angiotensin-converting enzyme inhibiton on coronary blood flow, coronary vascular resistance and rate-pressure product in normal individuals (○) and in patients with heart failure (●). In normal individuals ACE inhibition affects neither coronary flow nor myocardial oxygen demand (rate-pressure product). (Reproduced from Ref 50, with permission.)

on local (cardiac) tissue renin-angiotensin system.

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# XI.2.

# Enalaprilat Acutely Reduces Myocardial Ischemia in Normotensive Patients with Coronary Artery Disease through Neurohumoral Modulation

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## ABSTRACT

Myocardial ischemia may induce activation of the circulating renin-angiotensin system, besides catecholamine stimulation. To investigate whether converting enzyme inhibition has the potential to limit acute myocardial ischemia in relation to modulation of neurohumoral activation, 12 fasting, supine, normotensive patients with ischemic heart disease without heart failure symptoms underwent 2 identical incremental atrial pacing stress tests, 45 minutes before (APST I) and 15 minutes after (APST II) 1.25-1.5 mg enalaprilat (E) i.v. Before, during and for 5 minutes after pacing (p-p), hemodynamic measurements and paired arterial and coronary venous samples for lactate and neurohormones (catecholamines and angiotensin II) were carried out with additional determinations before and 5, 15 and 40 minutes after E. Arterial ACE activity and arterial and coronary venous angiotensin II decreased by 90%, 46% and 38%, resp., 5 minutes after E with changes persisting thereafter. Hemodynamics remained unaltered before and after pacing. Only a moderate 10% reduction in mean arterial pressure, starting 15 minutes after E, and a similar, but later decrease in left ventricular systolic pressure were observed. Also, coronary and systemic hemodynamics were not different during pacing before and after E, apart from a 12% improvement in relaxation. Nevertheless, E resulted in marked anti-ischemic effects, indicated by significantly less lactate production during APST II (extraction -1±9% vs -35±20% during APST I), less ST-segment depression [-0.14±0.03 mV (APST II) vs -0.21±0.03 mV (APST I), $p<0.05$] and a significant reduction in left ventricular end diastolic pressure immediately after pacing [15±3 mmHg (APST II) vs 25±3 mmHg (APST I)]. Also, angina was absent or less in 9 patients during APST II. Whereas during APST I arterial norepinephrine, epinephrine and angiotensin II increased by 93%, 42% and 39%, resp., accompanied by a significant rise in arterial pressures, E abolished this neurohumoral and secondary hemodynamic response. Moreover, it prevented the change from net cardiac norepinephrine release to net uptake, observed during the first, untreated test. It is concluded that enalaprilat may have significant modulating effects on myocardial ischemia at rest, apparently through inhibition of systemic neurohumoral activation during ischemia and subsequent vasoconstriction. As a result it may have important effects on afterload. In addition, it may beneficially affect post-stenotic coronary vascular tone by preventing net cardiac norepinephrine uptake during ischemia. The latter hypothesis should be further evaluated.

## INTRODUCTION

Converting enzyme inhibition may theoretically be useful in the treatment of myocardial ischemia. Through systemic and coronary vasodilating effects and negative inotropic properties it may favourably affect the myocardial oxygen demand/supply ratio and, hence, the occurrence and extent of myocardial ischemia. Systemic vasodilating properties are at the root of ACE-inhibiting therapy in hypertension and heart failure, whereas coronary vasodilatation has been reported in dilated cardiomyopathy following enalaprilat administration[1]. Whether converting enzyme

inhibitors have similar cardiovascular actions in the average patient with coronary artery disease without hypertension or heart failure, is uncertain though.

A different mechanism, which may be more relevant to the potential usefulness of ACE inhibition in this respect, relates to the observation that myocardial ischemia may induce significant neurohumoral activation. Recently, stimulation of systemic catecholamines and angiotensin II has been reported in humans during pacing-induced ischemia[2]. Moreover, in this situation, cardiac epinephrine uptake increases, whereas net cardiac norepinephrine release, present at rest, temporarily reverts to net uptake during ischemia[3]. As these neurohumoral changes are accompanied by significant systemic vasoconstriction and an increase in afterload, they may be clinically relevant. Also, adrenergically-mediated coronary vasoconstriction in the ischemic area has been proposed in animal models of ischemia[4]. These neurohumoral changes therefore may add to the ischemic burden already imposed on the heart.

Modulation of systemic and transcardiac neurohumoral activation could well be the primary mechanism through which ACE inhibitors may result in a reduction of myocardial ischemia[5]. In addition, they may affect ischemia through several local (cardiac) actions, e.g. stimulation of prostaglandin synthesis[6], modulation of bradykinin breakdown[7] and, possibly, of local angiotensin II formation[8-10]. Moreover, compounds which contain sulfhydryl groups may affect EDRF production[11].

Thus far, studies in ambulant patients with ischemic heart disease have been conflicting with respect to limitation of exercise-induced ischemia[5,12-18]. Also, no data are available on the acute effects of ACE inhibition in patients with ischemia at rest. Likewise, it is not known whether antiischemic effects and neurohumoral actions of the compound under study are related. Consequently, whether neurohumoral effects of specific cardiovascular mechanisms of converting enzyme inhibition are responsible for its potential antiischemic activity, is also unknown.

The present study examines the antiischemic efficacy of a non-sulfhydryl ACE inhibitor, enalaprilat, in normotensive patients with coronary artery disease at rest, in relation to its modulating effect on systemic and transcardiac neurohormones and to its systemic and coronary hemodynamic properties.

## MATERIALS AND METHODS

### Patients

Twelve patients participated in the trial, after the study was approved by the institutional Ethical Review Board and after informed consent was obtained. They were selected from patients referred for coronary angiography for the evaluation of stable angina pectoris and/or a previous myocardial infarction. In either case documented exercise-induced myocardial ischemia had to be present. To be selected patients had to be normotensive without signs or symptoms of heart failure, renal insufficiency, valvular heart disease or conduction disturbances. Patients with unstable angina, defined by the occurrence of recent episodes of angina, angina at rest or an increase in frequency and/or duration of anginal episodes despite medication, were excluded. Myocardial infarction had to be at least one month old. All cardiac therapy was withheld 24 to 72 hours before the investigation. Only short acting nitroglycerin was allowed until 6 hours pre-study. Patients on diuretic or ACE-inhibitor treatment were not included. Oral anticoagulation was stopped 2-3 days before the study, whereas anti-platelet therapy and NSAIDs were withheld at least 10 days prior to investigation.

To participate it was essential that patients had at least one $\geq$70% diameter narrowing in either the left anterior descending, a diagonal branch, the proximal part of the left circumflex or a proximal marginal branch.

Patients received 1.25-1.5 mg enalaprilat (depending on body weight, < or > 75 kg, resp.), administered intravenously during 1 minute. This dose was chosen, following a dose-finding, which started with 0.625 mg in the first patient, subsequently doubling this dose in each patient. As the third patient to be studied had a significant (>30% reduction) in mean arterial pressure after 15 minutes, the study dose was set on 1.25 mg. Clinical and angiographic characteristics are given in Table I. All patients were male. By design, all patients had exercise-induced ischemia. In addition, 8 patients had sustained one or more myocardial infarctions. Four patients had single vessel disease of the left anterior or proximal left circumflex artery. Two-vessel disease was present in 6 patients and 3-vessel in 2. Average values for left ventricular (LV) ejection fraction and end diastolic volumes were normal.

**Table I: Patient characteristics**

| | |
|---|---|
| Sex (male/female) | 12/0 |
| Age (years) | 51 ± 2,2<br>(range 42-68) |
| Number of old myocardial infarctions | 8 |
| Coronary angiography<br>(≥70% diameter stenosis): | |
| 1-vessel | 4 |
| 2-vessel | 6 |
| 3-vessel | 2 |
| Left ventricular ejection fraction (%) | 54 ± 3.5 |
| Left ventricular end diastolic volume index (ml/m$^2$) | 74 ± 6.7 |

**Catheterization procedures.**

Patients were studied at the same time in the morning between 10.00 and 12.30 a.m. All had been fasting since the previous night and had been supine for approximately 1.5 hours before the start of the study. None received premedication. All procedures were carried out under local anaesthesia with 1% lidocaine.
First, left and right coronary angiography was performed with non-ionic contrast material, using the Seldinger technique. If the patient then met the inclusion criteria, instrumentation for the study was carried out. This included the positioning of a no 7 Fr thermodilution pacing catheter (Wilton Webster Laboratories) in the coronary sinus via a brachial vein, such that the proximal thermistor was at least 3 cm beyond the ostium of the coronary sinus. Moreover, care was taken that the position of the catheter was stable but at the same time allowed for fast sampling of relatively large quantities of blood. To improve the sampling procedure, a specially designed bol-end variety (CCS-7U-90B) was used. Thus, relatively large amounts of blood (15-20 cc/minute) could be collected repetitively at short intervals throughout periods of at least 2 hours in nearly all patients. After positioning, the absence of reflux was confirmed by bolus injections of saline at room temperature in the right atrium at control and paced heart rates. Next, a no 7 Fr balloon-tipped triple-lumen thermodilution catheter was advanced into a pulmonary artery through a femoral vein for the measurement of right atrial and pulmonary artery pressures and the determination of cardiac output. Again, care was taken that the catheter tip was stable without baseline drift on the thermodilution signal. Finally, a no 8 Fr Sentron pigtail microtip manometer catheter was positioned in the left ventricle through a no 9 Fr arterial Desilet introducer system in the right femoral artery. The side arm of this system was used to record the arterial pressure curve.
The position of the catheters was recorded on video disc and regularly checked throughout the study.

**Hemodynamic and electrocardiographic measurements.**

After instrumentation, all fluid-filled catheters were calibrated, using Bentley transducers with a zero reference level, set at midchest. Next, the micromanometer pressure was balanced to zero and superimposed on the conventional left ventricular pressure curve. After calibration procedures, all pressures, the first derivative of left ventricular pressure, cardiac output, coronary flow and 3 ECG leads (I, II and $V_5$) were recorded on paper at different speeds, e.g. 25, 50 and 100 mm/sec, using a CGR 1000 cath lab system. All hemodynamic parameters, including mean and phasic systemic arterial, pulmonary arterial and right atrial pressures, left ventricular (LV) pressure

derived contractility and relaxation indices (LV peak dP/dt positive and negative, dP/dt/P at 40 mmHg and $V_{max}$), cardiac output and coronary flow, were all determined on-line by a Mennen cath lab computer system. In calculating, the system averages 15-20 consecutive beats to level out respiratory variations. In contrast, isovolumetric relaxation parameters, Tau, $Tau_1$ and $Tau_2$, were determined off-line. Coronary sinus blood flow was measured during a continuous 30-second infusion of 30 ml glucose 5% at room temperature. Although both pulsatile and mean flow curves were recorded, calculations were made from the latter, according to the formula: coronary blood flow (ml/min) = $V_i$ x $[(T_b-T_i)(T_b-T_{cs})-1]$ x 1.08, where $T_b$ is blood temperature before injection, $T_i$ temperature of injectate, $T_{cs}$ temperature of mixture of coronary sinus blood and injectate and $V_i$ the rate of injection (ml/min).
Coronary flow was determined while the patient held his breath at mid-expiration for the last 15 seconds of each recording. As the system displays consecutive 1-second measurements, it allows for proper estimation of the stability of coronary flow recordings.
At the end of the study, the arterial pressure curve was compared with a simultaneous recording by the electrical catheter from the aortic root to compensate for differences between proximal and distal systemic arterial pressures. Heart rate and ST-segment changes were determined from 100 mm/sec ECG recordings. The ST-segment was measured in 3 consecutive beats, at 80 msec after the J point, using a magnifying calibrated glass.

**Calculations.**

From the measured variables, the following parameters were derived. Coronary vascular resistance (mmHg/ml/min) was calculated as the difference between mean arterial pressure (mmHg) and left ventricular mean diastolic pressure (mmHg) divided by coronary sinus blood flow (ml/min). Systemic vascular resistance (dynes.sec.$cm^{-5}$) was derived as [mean arterial pressure (mmHg) - mean right arterial pressure (mmHg) / cardiac output (l/min)] x 80. Stroke work index (g.m.$m^2$) was calculated as stroke index (ml/beat/$m^2$) x [mean arterial pressure (mmHg) - left ventricular end diastolic pressure (mmHg)] x 0.0136. Myocardial oxygen extraction ($mlO_2$/ml) was calculated as arterial oxygen content ($mlO_2$/ml) - coronary venous oxygen content ($mlO_2$/ml), and myocardial oxygen consumption (ml/min) as the product of myocardial oxygen extraction ($mlO_2$/ml) and coronary blood flow (ml/min). Percentage myocardial lactate extraction was calculated as 100 x [arterial lactate content (mmol/l) - coronary venous lactate content (mmol/l)] / arterial lactate content (mmol/l). Myocardial lactate uptake (mmol/min) was determined by multiplying the difference in arterial and coronary venous lactate content (mmol/ml) with coronary blood flow (ml/min).
Myocardial catecholamine and angiotensin II uptake (nmol/min and pmol/min, resp.) were calculated by multiplying the respective differences in arterial and coronary venous catecholamine (nmol/ml) or angiotensin II (pmol/ ml) levels with the instantaneous coronary sinus blood flow (ml/min).
Metabolic and neurohumoral determinations: Approximately 0.5 ml of blood was collected simultaneously from the coronary sinus and left ventricle for the determination of $O_2$ saturation on an OSM-80 oxymeter (Waters Associates). For lactate, exactly 1 cc of blood was collected simultaneously from the left ventricle and the coronary sinus, quickly transferred into tubes, containing 2 cc of icecold 0.6 mol $HClO_4$, thoroughly mixed and kept on ice. After the study, the samples were weighed and centrifuged for 20 minutes at a speed of 2,000 xg. and the supernatant frozen for subsequent lactate assay. This was carried out in triplicate. The assay technique and standard deviation of the technique have been described[19].
For the determination of catecholamines and angiotensin II, 6 ml of blood was collected simultaneously from the left ventricle and coronary sinus in icecold syringes. Of these, 3 ml was quickly transferred into precooled tubes, containing 500 I/U heparin and 3 mg/ml gluthation for the catecholamine assay and the remaining 3 ml into pre-cooled tubes, containing 4 mg EDTA and 0.06 mg O-fenantroline for the assessment of angiotensin II. These samples were then immediately centrifuged under cooled conditions at 3,000 rev/min for 10 minutes and stored at -20°C. Angiotensin II was determined by radioimmunoassay, as previously reported[20]. Norepinephrine, epinephrine and dopamine were assessed by radioenzymatic assay, using high-pressure liquid chromatography, to separate the radio-active products[21].
For the determination of ACE, 3 ml of blood was sampled from the left ventricle. ACE activity was determined by colorimetric assay[22].

**Scoring of anginal pain.**

Before the start of the study, the patients were asked to indicate the moment, when they

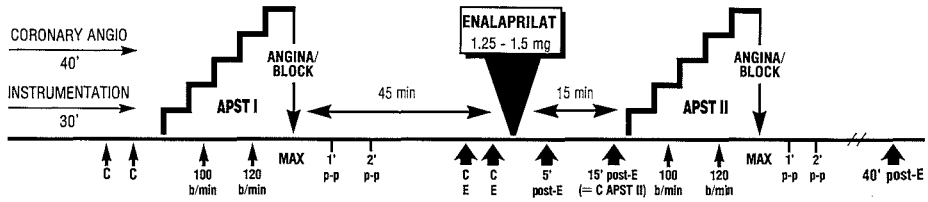



*Fig. 1: Schematic representation of the study protocol. Identical incremental atrial pacing stress tests were carried out 45 minutes before (APST I) and 15 minutes after (APST II) enalaprilat (E) administration. Hemodynamic, metabolic and neurohumoral measurements were carried out at control (C) before pacing, during maximal pacing rates (MAX) and at 1 and 2 minutes post-pacing (P-P). In addition, all variables were determined before (CE) and 5, 15 and 40 minutes after E.*

experienced the beginning of anginal complaints. Moreover, during each pacing test they were asked to score the level of angina, according to a modified Borg scale. Also, patients were prompted to indicate the time of disappearance of angina after pacing.

**Study protocol (Fig. 1).**

Multiple control measurements of all hemodynamic, metabolic and neurohumoral variables were carried out at approximately the same time in the morning, at least 40-50 minutes after coronary angiography and 20-30 minutes following instrumentation. Subsequently, the first, untreated atrial pacing stress test (APST I) was performed with increments in heart rate of 10 beats/2 minutes until a maximal pacing rate of 170 beats/minute, atrioventricular block or significant anginal pain. The latter was defined as the level of pain, at which the patient typically would rest or take nitroglycerin. Hemodynamic, electrocardiographic and metabolic parameters were assessed at fixed intervals during pacing, e.g. halfway the 100, 120 and 140 beats/minute period, followed by repeat measurements of all variables at maximal pacing rates, just before cessation of pacing. All variables were reassessed at 1 and 2 minutes after pacing. In addition, lactate sampling was carried out at 15 seconds post-pacing and determination of left ventricular end diastolic pressure at 10 seconds after pacing. In contrast, due to the complexity of the study at this point in time, cardiac output measurements could only be carried out following the 2-minute post-pacing sampling period in a limited number of patients. For similar reasons, coronary flow was only determined at 5 minutes after pacing and, in a limited number of patients, also at 2 minutes post-pacing. Following the first pacing test, a 30-minute stabilization period was allowed before control determinations of all variables were carried out.
Next, enalaprilat was administered. Subsequently, all variables were redetermined at 5 and 15 minutes following drug administration.
This was followed by a second atrial pacing stress test (APST II), identical in design to the first pacing test, with all measurements performed at exactly the same intervals as during and after APST I. In addition, arterial blood was collected from the left ventricle for ACE assay before drug administration, at 5 and 15 minutes after enalaprilat and at maximal pacing rates during APST II.

**Statistical analysis.**

The data analysis considered two parts of this study. First, measurements made following drug administration but before APST II and at 5 minutes after APST II were compared with baseline measurements before enalaprilat administration. In this evaluation a t-test for paired observations was used with a 2-tailed p value $<0.05$ considered significant. Secondly, changes in the respective variables during pacing were compared between both tests using the same type of analysis. Values are given as averages $\pm$ 1 standard error of the mean.

## RESULTS

Neurohumoral and hemodynamic effects of enalaprilat before and after pacing. Control period: Neurohumoral levels were comparable at baseline before onset of APST I and at control before enalaprilat administration, 30 minutes after APST I (Table II). Likewise, hemodynamic and metabolic variables were comparable between these periods (Table III). None of the patients had clinical signs of ischemia before drug administration or before any of the pacing tests.

Table II: Neurohumoral effects of enalaprilat before and after pacing.

| | | | Minutes after enalaprilat administration | | |
|---|---|---|---|---|---|
| | C-APST I | CE | 5 min | 15 min | 40 min |
| **ACE (U/l)** | | | | | |
| A | -- | 9.9 ±0.6 | 0.9 ±0.2* | 0.9 ±0.2* | 1.1 ±0.3* |
| **Angiotensin II (pmol/l)** | | | | | |
| A | 6.47±0.86 | 6.30±1.05 | 3.39±0.38* | 3.39±0.39* | 3.44±0.69* |
| CS | 6.27±1.10 | 5.48±0.72 | 3.42±0.43* | 3.12±0.30 | 3.32±0.48* |
| **Norepinephrine (nmol/l)** | | | | | |
| A | 1.78±0.28 | 2.01±0.28 | 2.40±0.31 | 2.37±0.35 | 2.35±0.52 |
| CS | 2.30±0.40 | 2.57±0.47 | 2.76±0.48 | 2.93±0.57 | 2.82±0.73 |
| **Epinephrine (nmol/l)** | | | | | |
| A | 0.62±0.09 | 0.66±0.09 | 0.72±0.10 | 0.75±0.08 | 0.68±0.13 |
| CS | 0.37±0.05 | 0.40±0.06 | 0.41±0.05 | 0.43±0.05 | 0.48±0.09 |
| **Dopamine (nmol/l)** | | | | | |
| A | 0.35±0.08 | 0.40±0.06 | 0.42±0.09 | 0.40±0.07 | 0.39±0.12 |
| CS | 0.51±0.07 | 0.53±0.08 | 0.53±0.08 | 0.51±0.08 | 0.57±0.14 |

*Abbreviations: A = arterial; C-APST I = control atrial pacing stress test I; CE = control before enalaprilat; CS = coronary sinus; min = minutes.* $\bar{x} \pm$ *SEM; * p<.05 vs CE.*

**Neurohumoral effects of enalaprilat before and after pacing (Table II).**

ACE activity in arterial blood samples decreased quickly after enalaprilat administration from 9.9±0.6 U/l at control to 0.9±0.2 U/l, at 5 minutes post-drug. Thereafter, ACE-activity remained depressed at approximately the same level (Fig. 2). Simultaneously, arterial and coronary venous angiotensin II levels decreased by 46% and 38%, respectively (Fig. 2) without further changes during the remaining study period. Enalaprilat did not alter circulating catecholamine levels.

**Hemodynamic and metabolic effects of enalaprilat before and after pacing (Table III).**

Enalaprilat did not affect myocardial lactate metabolism, myocardial oxygen extraction nor consumption before or after pacing. Also, the double product, an index of myocardial oxygen demand remained unchanged, despite a moderate, but significant 10% reduction in left ventricular systolic pressure, 40 minutes after drug administration (Fig. 3). A similar, although earlier decrease was observed in mean arterial pressure. Heart rate, contractility and relaxation parameters and left ventricular filling pressure remained unaltered. Also, enalaprilat did not affect cardiac output or systemic vascular resistance. Likewise, coronary flow and resistance did not change significantly, although the latter decreased by 16% at 15 minutes after drug administration (Fig. 3).

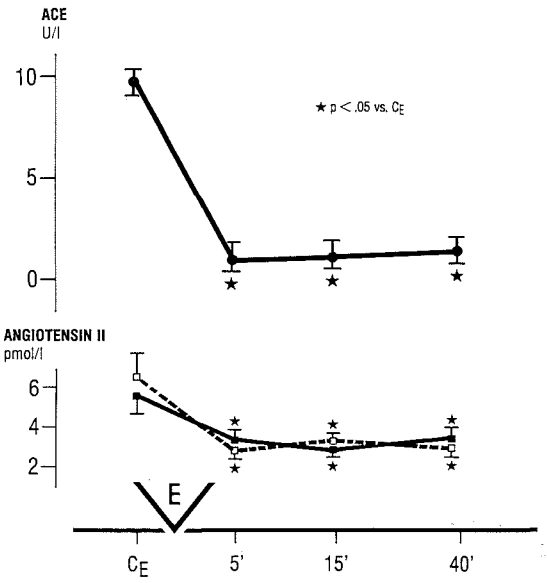



*Fig. 2: Effect of enalaprilat (E) on arterial ACE activity and arterial and coronary venous angiotensin II levels. Already 5 minutes after E. ACE activity had decreased by 90%, accompanied by 46% and 38% reductions in arterial (open symbols) and coronary venous (closed symbols) angiotensin II levels, resp. Values are $\bar{x}$ ± SEM. CE = control before E; ' = minutes.*

**Hemodynamic effects of enalaprilat during pacing (Table IV).**

By design, maximal pacing heart rates were similar during both pacing tests, 157±3.8 and 157±4.06 beats/minute, APST I and II, resp. As left ventricular systolic pressure did not change during pacing before or after enalaprilat, changes in the double product were likewise comparable. Moreover, pacing-induced alterations in contractility were identical during the first and second pace-test.
In contrast, $Tau_1$ shortened by 21% during APST II compared to an 11% reduction during APST I ($p<0.05$). Enalaprilat did not affect coronary hemodynamics during pacing, although myocardial oxygen consumption was 18% less during APST II (ns).
Finally, whereas mean aortic pressure significantly increased during APST I, this did not occur during pacing after enalaprilat (APST I vs II $p<0.05$, Fig. 4).

**Neurohumoral effects of enalaprilat during pacing (Table V).**

During APST I, arterial norepinephrine levels nearly doubled, increasing from 1.78±0.28 mmol/l to 3.44±0.61 mmol/l, 1 minute post-pacing ($p<0.05$). Likewise, arterial epinephrine values increased significantly by 42% at maximal pacing rates. Moreover, arterial angiotensin II levels rose from 6.47±0.86 pmol/l (control) to 9.0±1.65 pmol/l at 1 minute post-pacing ($p<0.05$). Dopamine levels did not change. Coronary venous values of all neurohormones remained unaltered.
During APST II, after enalaprilat, the systemic neurohumoral activation, observed during the first pacing test, was abolished. Arterial values of norepinephrine, epinephrine and angiotensin II did not increase anymore during APST II (Fig. 5). As a result there was a significant difference between maximal neurohumoral activation during both pacing tests. Moreover, whereas during APST I, net cardiac norepinephrine release changed to uptake (Fig. 6), this was prevented by enalaprilat during the second test.

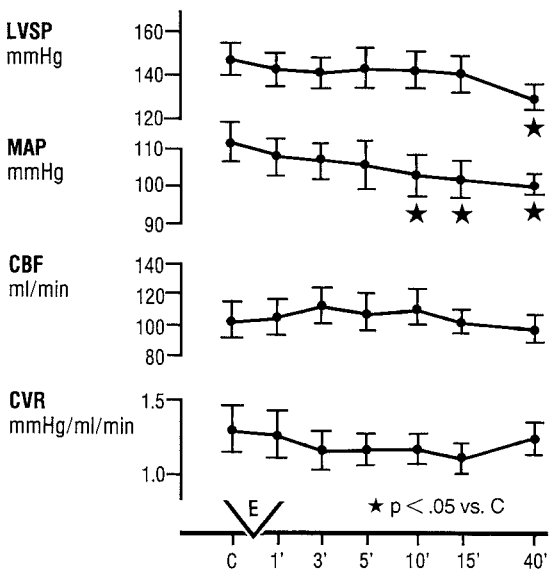



*Fig. 3: Hemodynamic effects of enalaprilat (E) before and after pacing. E only resulted in a relatively late and moderate 10% reduction in left ventricular systolic pressure (LVSP) and mean arterial pressure (MAP). Coronary flow (CSBF) was not affected, although coronary resistance (CVR) decreased by 16% (NI). Values are $\bar{x}$ ± SEM. CE = control before E; ' = minutes.*

**Table III: Hemodynamic and metabolic effects of enalaprilat before and after pacing.**

| | | | Minutes after enalaprilat administration | | |
|---|---|---|---|---|---|
| | C-APST I | CE | 5 min | 15 min | 40 min |
| HR (beats/min) | 78±4.0 | 81±3.6 | 80±4.5 | 82±3.98 | 82±4.2 |
| LVSP (mmHg) | 147±8 | 143±7.7 | 138±8.5 | 136±6.8 | 127±5.4* |
| LVEDP (mmHg) | 12±1.0 | 10±1.2 | 11±1.5 | 10±1.19 | 11±1.35 |
| MAP (mmHg) | 108±5.2 | 111±6.45 | 107±5.8 | 101±5.6* | 98±3.2* |
| PAM (mmHg) | 16±0.9 | 15±1.0 | 14±1.04 | 13±0.78 | 15±1.1 |
| LV dP/dt pos (mmHg.$s^{-1}$) | 1798±106 | 1725±99 | 1735±111 | 1697±107 | 1568±97 |
| Vmax ($sec^{-1}$) | 51.2±3.1 | 49.7±3.3 | 52.6±3.9 | 51±3.8 | 47±3.2 |
| LV dP/dt neg (mmHg $sec^{-1}$) | 1890±111 | 1884±120 | 1764±122 | 1762±124 | 1608±78* |
| $Tau_1$ (msec) | 44.6±1.7 | 45.7±1.5 | 46.7±2.6 | 46.7±1.39 | 48.8±2.27 |
| CO (l/min) | 6.74±0.5 | 6.15±0.4 | 5.89±0.37 | 5.87±0.27 | 5.73±0.57 |
| SVR (dynes $sec.cm^{-5}$) | 1184±85 | 1345±100 | 1343±72 | 1285±72 | 1474±54 |
| CSBF (ml/min) | 104±13 | 99±13 | 106±13 | 99±10 | 96±10 |
| CVR | 1.23±0.19 | 1.29±0.15 | 1.13±0.12 | 1.08±0.10 | 1.18±0.59 |
| $MVO_2$ (ml/min) | 12.2±1.53 | 11.3±4.2 | 11.6±1.54 | 11.1±1.41 | 10.5±1.05 |
| LE (%) | 25±3.8 | 23±3.15 | 23±4.1 | 22±4.0 | 17±4.1 |

*Abbreviations: C-APST I = control atrial pacing stress test I; CE = control before enalaprilat; CO = cardiac output; CSBF = coronary sinus flow; CVR = coronary vascular resistance; HR = heart rate; LE = lactate extraction; LVEDP = left ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; min = minute; $MVO_2$ = myocardial oxygen consumption; PAM = pulmonary artery mean pressure; SVR = systemic vascular resistance.*
*$\bar{x}$ ± SEM. * p<.05 vs CE.*

Likewise, the enhanced cardiac epinephrine uptake, observed during APST I, was not present during APST II.

**Antiischemic effects of enalaprilat.**

Enalaprilat significantly reduced myocardial ischemia. This was most prominently demonstrated by the marked decrease in myocardial lactate production during and immediately after pacing (Fig. 7). At maximal pacing rates, lactate extraction improved from -35±20% (APST I) to -1±9% (APST II) and from -60±19% to -22±17% (APST II) at 15 seconds post-pacing, both p<0.05. Moreover, during APST II there was significantly less ST-segment depression than during APST I,

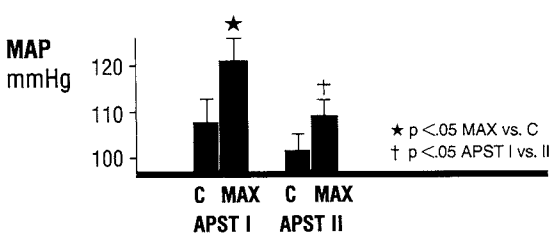



*Fig. 4: Effect of pacing before and after enalaprilat (E) on arterial pressures. Whereas mean arterial pressure (MAP) increased significantly during the first pacing test (APST I) at maximal pacing rates (MAX), this was prevented by E during the second test (APST II). C = control.*

**Table IV: Hemodynamic effects of enalaprilat during pacing.**

| | | Control | Max | 1 min P-P | 2 min P-P |
|---|---|---|---|---|---|
| **LVSP (mmHg)** | | | | | |
| | APST I | 147±8 | 144±7 | 154±9 | 155±9 |
| | APST II | 136±7 | 134±8 | 140±7 | 134±6† |
| **DP (mmHg)** | | | | | |
| | APST I | 11.3±0.8 | 22.6±1.4* | 12.3±1.2 | 12.3±0.9 |
| | APST II | 11.1±0.7 | 21.2±1.4* | 11.8±0.7 | 11.1±0.8 |
| **LV dP/dt pos (mmHg $sec^{-1}$)** | | | | | |
| | APST I | 1798±107 | 2339±196* | 1930±134 | 1956±112 |
| | APST II | 1697±107 | 2159±156* | 1750±94 | 1734±110 |
| **$V_{max}$ ($sec^{-1}$)** | | | | | |
| | APST I | 51.2±3.1 | 60±3.2* | 50±3.4 | 52±3.1 |
| | APST II | 51±3.8 | 61±3.3* | 50±3.6 | 50±1.9 |
| **$Tau_1$ (msec)** | | | | | |
| | APST I | 44±1.7 | 39±1.2* | 51±2.31* | 48±2.7 |
| | APST II | 47±1.4 | 37±1.0*# | 51±2.2 | 48±1.2 |
| **CSBF (ml/min)** | | | | | |
| | APST I | 104±13 | 183±18* | --- | 105±13 |
| | APST II | 99±10 | 157±17* | --- | 96±10 |
| **CVR (mmHg/ml/min)** | | | | | |
| | APST I | 1.23±0.19 | 0.72±0.08* | --- | 1.33±0.26 |
| | APST II | 1.08±0.10 | 0.74±0.07* | --- | 0.96±0.13 |
| **$MVO_2$ (ml/min)** | | | | | |
| | APST I | 12.2±1.53 | 21.8±3.18* | --- | 10.9±1.33 |
| | APST II | 11.3±1.4 | 17.9±1.79* | --- | 10.5±1.05 |

*Abbreviations: APST=atrial pacing stress test; CSBF=coronary blood flow; CVR=coronary vascular resistance; DP=double product; LVSP=left ventricular systolic pressure; Max=maximal pacing rates; min=minutes; P-P=post-pacing.* $\bar{x} \pm SEM$ ** p<.05 vs control; † p<.05 APST I vs APST II; # p<.05 ΔAPST I vs ΔAPST II.*

whereas left ventricular end diastolic pressure was markedly reduced [25±2.6 mmHg (APST I) vs 15±2.6 mmHg (APST II), p<0.05], 10 seconds after pacing (Fig. 8).
Finally, angina, present in 11 of 12 patients during APST I, was absent or less in 9 patients during APST II.

**Adverse effects.**

No side effects were noted with enalaprilat in the dosage used in this study; nor did any untoward hemodynamic or metabolic change occur. Patients did not develop signs of ischemia following drug administration before pacing.

## DISCUSSION

Although ACE inhibition supposedly may be useful as antiischemic therapy based on several theoretical considerations which relate predominantly to its hemodynamic profile, its efficacy in this respect has not been convincingly demonstrated. Previous human studies have concentrated on exercise-induced ischemia and/or

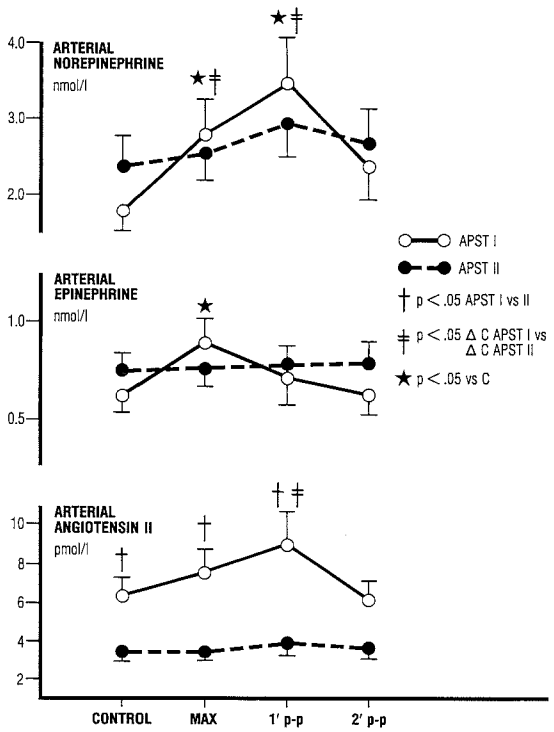



*Fig. 5 (left): Arterial neurohumoral changes during pacing before and after enalaprilat (APST I and II, resp.). During APST I norepinephrine levels increase significantly at maximal pacing rates (MAX) and at 1 minute post-pacing (1'P-P) and epinephrine levels at MAX. In contrast, during APST II changes in catecholamines are prevented by enalaprilat. Also, arterial angiotensin II, already reduced at baseline of APST II, increases significantly less during pacing after enalaprilat. Values are $\bar{x} \pm$ SEM.*

*Fig. 6 (bottom): Effects of enalaprilat on cardiac neurohumoral balance during pacing. During the first pacing test, (APST I, open symbols), norepinephrine release reverses to uptake and epinephrine uptake increases during maximal pacing rates (MAX), both p<.05 vs control. Also, angiotensin II uptake tends to increase. In contrast, changes in cardiac neurohormones are absent during the second test (APST II, closed symbols) after enalaprilat. Values are $\bar{x} \pm$ SEM.*

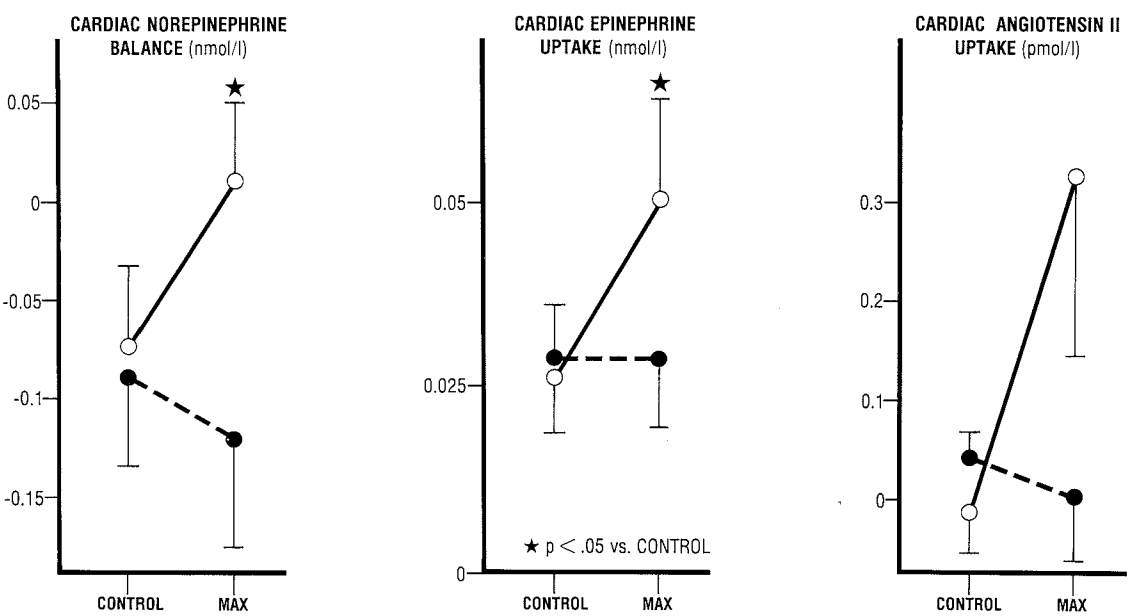


**Table V: Arterial and coronary venous neurohormones during pacing before and after enalaprilat**

| | | Control | Max | 1 min P-P | 2 min P-P |
|---|---|---|---|---|---|
| **Norepinephrine, nmol/l** | | | | | |
| APST I | A | 1.78±0.28 | 2.77±0.49*# | 3.44±0.61*# | 2.31±0.54 |
| | CS | 2.30±0.40 | 2.76±0.51 | 2.92±0.56 | 2.3 ±0.56 |
| APST II | A | 2.37±0.35 | 2.57±0.34 | 2.90±0.39 | 2.68±0.47 |
| | CS | 2.93±0.58 | 3.29±0.57 | 2.98±0.61 | 3.06±0.74 |
| **Epinephrine, nmol/l** | | | | | |
| APST I | A | 0.62±0.09 | 0.88±0.12* | 0.71±0.14 | 0.63±0.11 |
| | CS | 0.37±0.05 | 0.58±0.08 | 0.47±0.07 | 0.40±0.07 |
| APST II | A | 0.75±0.08 | 0.76±0.09 | 0.77±0.09 | 0.79±0.11 |
| | CS | 0.43±0.05 | 0.56±0.06 | 0.48±0.05 | 0.53±0.07 |
| **Dopamine, nmol/l** | | | | | |
| APST I | A | 0.35±0.08 | 0.39±0.08 | 0.35±0.10 | 0.35±0.09 |
| | CS | 0.51±0.07 | 0.48±0.07 | 0.44±0.09 | 0.36±0.08 |
| APST II | A | 0.40±0.07 | 0.37±0.07 | 0.47±0.11 | 0.35±0.13 |
| | CS | 0.51±0.08 | 0.48±0.09 | 0.62±0.07 | 0.57±0.10 |
| **Angiotensin II, pmol/l** | | | | | |
| APST I | A | 6.47±0.86 | 7.38±1.37 | 9.00±1.65# | 6.13±0.97 |
| | CS | 6.27±1.10 | 6.25±1.01 | 6.80±1.30 | 4.75±0.65 |
| APST II | A | 3.39±0.39† | 3.44±0.45† | 3.91±0.60† | 3.52±0.39† |
| | CS | 3.13±0.30† | 3.30±0.45† | 2.85±0.30† | 3.6 ±0.56 |

*Abbreviations: A = arterial; APST = atrial pacing stress test; CS= coronary sinus; max = maximal pacing; min = minutes; P-P = post-pacing.* $\bar{x}$ ± *SEM; * p<.05 vs control; † p<.05 APST I vs APST II; # p<.05 ΔAPST I vs ΔAPST II.*

angina and have not been able to clarify the issue as to whether converting enzyme inhibitors have antiischemic properties. Besides, no data are available concerning the effect of ACE inhibition on ischemia at rest. Neither is there any information, which indicates, that changes in coronary and systemic hemodynamics and, hence, in the myocardial oxygen supply/demand ratio as a result of ACE inhibition, are involved in its potential antiischemic properties in man. In addition, it has not yet been investigated, whether ACE inhibitors may modulate ischemia-induced neurohumoral activation, proposed as an alternative mechanism to limit myocardial ischemia. Consequently, the pharmacological basis for its potential antiischemic activity is still rather speculative.

The present study is the first to address these items in the average type patients with ischemic heart disease, e.g. normotensive patients with stable, exercise-inducable ischemia and a normal left ventricular function. It demonstrates that the non-sulfhydryl-containing ACE inhibitor enalaprilat has the potential to reduce (pacing-induced) myocardial ischemia at rest and that these effects do not

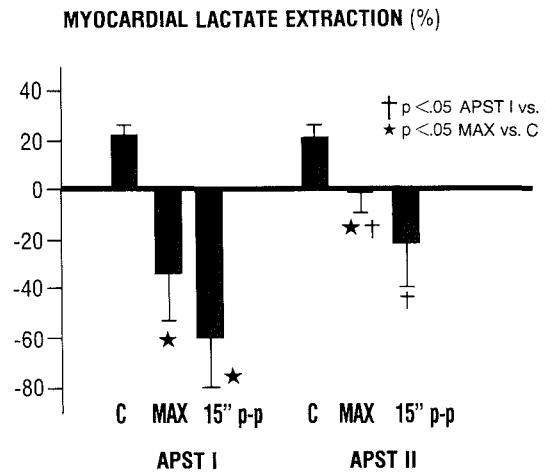


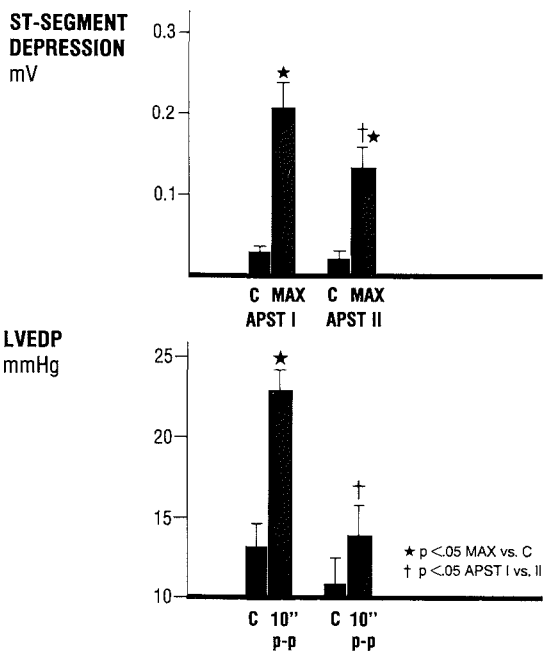

*Fig. 7: Antiischemic properties of enalaprilat as demonstrated by its effect on myocardial lactate metabolism. During pacing after enalaprilat (APST II) myocardial lactate production is significantly less than during the first test (APST I), both during maximal heart rates (MAX) and at 15 seconds post-pacing (P-P). Values are $\bar{x}$ ± SEM. C = control.*

relate to overall coronary or systemic cardiovascular actions of the drug. In contrast, the study strongly suggests that the antiischemic properties of enalaprilat relate to inhibition of ischemia-induced systemic and transcardiac neurohumoral activation and subsequent reduction of systemic vasoconstriction.

**Rationale for the potential antiischemic activity of ACE inhibitors.**

Converting enzyme inhibition has the potential to reduce myocardial oxygen demand and to improve coronary flow[1,3,5,23,24]. Through balanced type vasodilating properties, it may diminish pre- and afterload. Together with the intrinsic negative inotropic activity and the potential coronary vasodilating properties of ACE inhibition, this may beneficially affect the myocardial oxygen supply/demand ratio. Whether these cardiovascular actions are the result of inhibition of local cardiac[25] and/or vascular[26] ACE activity, or of a modulation of circulating ACE, is still unclear.
Other mechanisms, through which converting enzyme inhibitors may affect myocardial ischemia, include modulation of local bradykinin degradation[7] and improved synthesis of prostaglandins[6]. Moreover, converting enzyme inhibitors, which contain sulfhydryl groups, may induce vasodilatation through an effect on endothelium-derived relaxing factor (EDRF). Also, recent data suggest that the latter may act as free radical scavengers[27,28]. An additional and potentially important mechanism, through which ACE inhibition may affect myocardial ischemia, relates to the significant changes in systemic and transcardiac catecholamines and components of the renin-angiotensin system, observed during the acute phase of myocardial ischemia[2,3]. Theoretically, by modulating this neurohumoral stimulation and the ensuing systemic and, possibly, coronary vasoconstriction, converting enzyme inhibitors may result in antiischemic effects.

*Fig. 8: Antiischemic properties of enalaprilat are indicated by the significant reduction in ST-segment depression during the second test (APST II) after enalaprilat, compared with the first, untreated test (APST I).*
*Moreover, left ventricular filling pressure (LVEDP) does not increase anymore at 10 seconds (") post-pacing (P-P) in APST II, in contrast to a marked elevation following APST I. Values are $\bar{x}$ ± SEM. C = control.*



**Ischemia-induced neurohumoral activation and antiischemic properties of ACE inhibition.**

In the present study, myocardial ischemia induced significant changes in several circulating neurohormones, e.g. in norepinephrine and epinephrine, whereas arterial angiotensin II levels rose by 39%. Concomitantly, arterial pressures increased. A similar activation of systemic catecholamines and of the circulating renin-angiotensin system has been previously reported by us[2,29]. In an identical model and similar type patients it was shown, that pacing per se had no such effects in non-ischemic patients. Also,these studies provided strong evidence that neurohumoral stimulation was not necessarily due to the stress of anginal pain. More likely, activation of the circulating catecholamines and, in particular, of the circulating renin-angiotensin system, is directly related to the severity of ischemia[30,31]. In addition, as in the present study, it was shown, that short periods of ischemia do not result in enhanced norepinephrine release from the heart but in temporarily increased net uptake, presumably in the ischemic area. Also, cardiac epinephrine uptake increases during ischemia. In contrast, no significant changes are found in the cardiac angiotensin II balance in normotensive patients, although there is a tendency towards increased uptake[3].

The clinical relevance of the observed changes in systemic neurohormones may relate to the concomitant increase in systemic vascular resistance[29]. In the present study the latter was reflected by a significant increase in arterial pressures during the ischemic event in the first, untreated pacing test. Unfortunately, the few data available on cardiac output after pacing in this study do not allow interpretation of changes in systemic resistance. However, in a similar model as used in the present investigation, we have recently demonstrated that both elevated arterial norepinephrine and angiotensin II levels and the increase in systemic vascular resistance may persist until at least 5 minutes after pacing, depending on the severity of ischemia[31]. It stands to reason to assume, that the resultant increase in afterload will aggravate ischemia already present. As such, modulation of ischemia-induced neurohumoral activation and, hence, of systemic vasoconstriction by specific neurohumoral antagonists may represent a novel, additional form of antiischemic therapy. In this respect, both alpha-1 and alpha-2 adrenergic blocking drugs may prove useful. In fact, several calcium-antagonists have been demonstrated to reduce myocardial ischemia in a similar patient model as in the present study[32-35].

Besides alpha adrenergic blockade, converting enzyme inhibition may provide for an alternative approach to antiischemic therapy. Apart from their direct effect on circulating and local tissue angiotensin II formation, ACE inhibitors may also affect ischemia-induced catecholamine stimulation. The renin-angiotensin system(s) interacts with both central and peripheral sympathetic adrenergic activity. Thus, angiotensin II, besides stimulating adrenal catecholamine release, may promote central norepinephrine activity, facilitate norepinephrine release from adrenergic nerve endings, limit the neuronal re-uptake of this catecholamine and stimulate norepinephrine synthesis in adrenergic nerve terminals. Converting enzyme inhibition, therefore, could limit myocardial ischemia not only through inhibition of angiotensin II stimulation but also by modulating sympathetic activity during ischemia.

In the present study, enalaprilat, during the second pacing test, prevented the increase in arterial catecholamine and angiotensin II levels observed during the first test before drug administration. Simultaneously, it prevented the significant increase in arterial pressures, also observed during the first stress test. Whether this implicates a decrease in wall stress during pacing with enalaprilat is impossible to say in the absence of information on ventricular volumes. However, the reduction in systemic vascular resistance, likely to have occurred during pacing with enalaprilat, suggests that its antiischemic activity in this model may relate to such an effect on left ventricular wall stress. Whether this is the only mechanism involved in its antiischemic effect is uncertain.

Of interest is that, whereas during the untreated pacing stress test net cardiac norepinephrine release changed to net uptake, this was prevented by enalaprilat. Also, the enhanced uptake of epinephrine during pacing without enalaprilat was absent during the second test after medication. Besides mechanisms, such as presynaptic inhibition of exocytotic norepinephrine release or enhanced neuronal re-uptake during ischemia, binding to cardiac and/or vascular adrenergic receptors may be considered to explain the change in cardiac norepinephrine balance during ischemia[31]. Several studies suggest an important role for alpha adrenergic coronary vasoconstriction in the poststenotic area during ischemia[4,36-38]. Under experimental conditions of progressive coronary flow reduction, intracoronary norepinephrine administration or cardiac sympathetic nerve stimulation reverses (metabolic) coronary vasodilatation to vasoconstriction[4,36,39]. The change from net norepinephrine release to uptake in the present

study (Table VI) may reflect coronary vascular alpha adrenergic receptor occupation. In a previous study we have indicated, that enhanced net cardiac norepinephrine uptake during myocardial ischemia may be specific for the ischemic area[31]. Also, in a similar model, post-stenotic coronary flow changes have been observed in humans, preceding metabolic and electrocardiographic indices of ischemia[19]. Although direct evidence is lacking, we speculate that post-stenotic alpha-adrenergic coronary vasoconstriction may also be a feature of pacing-induced ischemia.
Whereas enalaprilat prevented the change from net cardiac norepinephrine release to uptake during pacing, it did not improve overall coronary flow. This does not necessarily implicate that the drug failed to restore coronary flow reserve in the post-stenotic region. Simultaneous reduction of myocardial oxygen demand, for instance by decreasing systemic vascular resistance, will result in less coronary flow increase in non-ischemic areas. Consequently, an improvement of post-stenotic flow may not become apparent with the thermodilution method. Thus, apart from its effects on myocardial oxygen demand, enalaprilat's antiischemic properties may result from a modulatory effect on cardiac norepinephrine kinetics and subsequent alpha adrenergically-induced coronary vasoconstriction in the post-stenotic region.

**Possible alternative mechanisms underlying the antiischemic effects of enalaprilat.**

In this study, emphasis has been on the modulatory effect of enalaprilat on ischemia-induced neurohumoral activation and concomitant vasoconstriction.
Antiischemic properties resulting from a reduction of myocardial oxygen demand by mechanisms other than an effect of the drug on afterload, as suggested above, are unlikely. Enalaprilat did not affect contractility or relaxation in our study. Although Foult et al.[1] reported a reduction in pressure-derived velocity parameters following intracoronary administration of enalaprilat in patients with dilated cardiomyopathy, no such changes were observed in our patients. Neither did isovolumic relaxation parameters change in our study. The decrease in the peak negative value of LV dP/dt at the end of the study followed the moderate reduction in left ventricular systolic pressure and is presumably secondary to this.
An improvement of left ventricular distensibility has been proposed as one mechanism, through which ACE inhibitors may affect cardiac remodelling. Preliminary data suggest improvement following intravenous ACE inhibition in patients with ventricular dysfunction [40]. Whether these effects of converting enzyme inhibition pertain in

**Table VI: Cardiac neurohumoral balance during pacing before and after enalaprilat.**

| | | Control | Max | 2 min P-P (n=5) |
|---|---|---|---|---|
| **Norepinephrine (nmol/min)** | | | | |
| | APST I | -0.074±0.041 | 0.011±0.043* | -0.061±0.037 |
| | APST II | -0.092±0.048 | -0.123±0.055† | -0.054±0.077 |
| **Epinephrine (nmol/min)** | | | | |
| | APST I | 0.026±0.08 | 0.049±0.014* | 0.023±0.010 |
| | APST II | 0.029±0.07 | 0.028±0.010 | 0.046±0.026 |
| **Dopamine (nmol/min)** | | | | |
| | APST I | 0.031±0.039 | -0.017±0.019 | -0.008±0.007 |
| | APST II | -0.009±0.007 | -0.015±0.010 | -0.030±0.021 |
| **Angiotensin II (pmol/min)** | | | | |
| | APST I | -0.020±0.039 | 0.322±0.187 | -0.019±0.084 |
| | APST II | 0.038±0.022 | -0.002±0.068 | 0.003±0.029 |

*Abbreviations: APST = atrial pacing stress test; Max = maximal pacing rates; min = minutes; P-P = post-pacing. $\bar{x}$ ± SEM; * p<.05 vs control; † p<.05 APST I vs APST II. n = number of patients.*

patients with normal-sized, normally contracting ventricles, is not known. As in the present study no information is available on ventricular volumes, extrapolation of our data to (absence of) changes in ventricular distensibility or wall stress is not possible. However, at the beginning of the pacing test, after enalaprilat, systemic vascular resistance had not changed, whereas during pacing neither right nor left ventricular filling pressures differed from those during the first test. Although inconclusive, these data do not suggest that changes in preload or relaxation are important mechanisms in the antiischemic activity of enalaprilat in this model. In contrast, the significant reduction in left ventricular end diastolic pressure immediately after pacing is considered the result rather than the cause of decreased myocardial ischemia.

**Comparison to different models of ischemia.**

A number of short term studies have been carried out with several ACE inhibitors, including enalapril, in patients with ischemic heart disease, with variable outcome[5,12-18]. Although antiischemic efficacy was claimed in most studies [5,12-16], several were unable to show any such effect[17,18]. Apart from these conflicting results, interpretation of these investigations is complicated by the fact that several were conducted in patients who were either hypertensive[5,13] or in whom the circulating renin-angiotensin system was activated by diuretic pretreatment[41]. Moreover, apart from the latter study, the effect of ACE inhibition on neurohumoral activation as a mechanism for its potential antiischemic effects has not been considered in these studies. Yet, the observations in animal models on alpha-1 adrenergically-induced post-stenotic vasoconstriction during exercise and recent publications on exercise-induced coronary vasoconstriction during myocardial ischemia in man[42,43], suggest an antiischemic potential of ACE inhibitors through modulation of neurohumoral activation and subsequent coronary vasoconstriction. In addition, enhanced renin-angiotensin stimulation, as observed in patients with more severe myocardial ischemia [31], may affect coronary reserve[41]. Differential effects of converting enzyme inhibitors on neurohumorally-induced coronary vasoconstriction may explain the divergent effects on ischemia, observed thus far.

**Limitations of the study.**

Several limitations of the study have already been discussed, e.g. the absence of information on regional coronary flow and ventricular volumes or cardiac output during pacing. Also, in this study no information is present on the reproducibility of neurohumoral stimulation during successive periods of pacing-induced ischemia. Preliminary data from a recent placebo comparison indicate, that both the changes in catecholamines and angiotensin II are reproducible when the interval between pacing tests is at least one hour, as in the present study (unpublished observations).

**Clinical implications.**

This study provides evidence that ACE inhibitors may reduce myocardial ischemia, possibly through mechanisms which differ from those underlying currently available antiischemic therapy. Hence, ACE inhibitors may provide for an additional form of therapy. The question is whether this is true for all patients with ischemic heart disease and for all conditions under which ischemia presents itself. With respect to modulation of systemic neurohormones and vasoconstriction, it is questionable whether this aspect of ACE inhibition is important during upright exercise; a situation where neurohormones are already markedly elevated in the absence of ischemia[44].
Our current data indicate that the efficacy of converting enzyme inhibitors may depend on activation of circulating and, possibly, of cardiac neurohormones. As this activation relates to the severity of myocardial ischemia it may well be that ACE inhibitors are particularly effective in supine patients with ischemia at rest, e.g. with unstable angina or during the initial phase of myocardial infarction. It may be anticipated that in these conditions ischemia is more severe than in the present study and, consequently, ischemia-induced neurohumoral stimulation more pronounced. Our data therefore suggest that the clinical usefulness of enalaprilat should be further studied in these clinical conditions.

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# Summary

# Samenvatting

# SUMMARY

This thesis aims at defining the relevance and applicability of some metabolic aspects of acute myocardial ischemia to delineate occurrence and extent of the latter in man. Studies focus on myocardial lactate metabolism and adenine nucleotide catabolism, correlate changes with other markers of ischemia and attempt to define a temporal relation with regional changes in coronary flow. Next, the acute antiischemic properties of different vasoactive compounds are outlined using these metabolites in a properly defined study model. Studies also attempt to differentiate the usefulness of various vasodilator compounds as antiischemic therapy in relation to the underlying cardiac function.
In the second part of this thesis the impact of myocardial ischemia on systemic and cardiac neurohormones, i.e. catecholamines and renin-angiotensin system(s), are discussed.
The relation between degree of ischemia and neurohumoral activation will be emphasized, potential subsequent systemic and coronary vasoconstrictor effects mentioned, and the usefulness of neurohumoral modulation, i.e. by converting enzyme inhibition in the treatment of myocardial ischemia indicated.

## PART I: Myocardial metabolic changes during acute ischemia in man. Relation to coronary flow and potential for evaluating pharmacological interventions.

*Chapter I*

Chapter I of this thesis serves as an introduction to the first theme of this thesis, i.e. the acute metabolic alterations which occur during the early phase of myocardial ischemia. It addresses the normal regulation of cardiac metabolism, followed by a discussion of the derangements herein during ischemia. Particularly those aspects of cardiac metabolism which may be relevant for the detection of ischemia in man, are emphasized.

The heart is a fast metabolizing organ, relying on a continuous supply of oxygen and substrates, such as free fatty acids, glucose or lactate, to provide for its energy production. After processing, these substrates to acetyl-CoA molecules, oxydation takes place in the citric acid cycle and energy in the form of ATP is formed during substrate -and respiratory chain-linked oxydative phosphorylation.
Oxygen supply is central to this and oxydative phosphorylation will immediately slow down or halt when oxygen is not available anymore. Although the role of substrate supply soon becomes crucial, the presence of endogenous substrates, such as glycogen, will provide for fuel, at least for several minutes following the onset of ischemia.
Lack of oxygen and substrates is one aspect of ischemia. Another is the accumulation of intermediates which cannot be further processed, e.g. fatty acid amphiphiles of long-chain acyl CoA and acyl-carnitine, which may affect structure and function of various subcellular components, such as the sarcolemma, and of enzyme systems, leading to a derangement in contractile function, ionic changes and cell swelling. Besides a potentially protective effect by specific fatty acid binding proteins, carnitine is also essential in reducing long-chain acylcarnitines and acyl CoA levels during ischemia. It limits their effect on various enzyme systems, such as pyruvate dehydrogenase or adenine nucleotide translocase, essential for ATP transfer out of the mitochondrion.
During ischemia, cellular pH falls progressively, partly due to accumulation of reduced coenzymes, partly resulting from the activation of anaerobic glycolysis. Enhanced glycolytic flux and glycogenolysis occur early during the ischemic process and provide for 2 mol ATP per mol glucose. Although insufficient energy for cardiac contraction, this additional ATP supply may suffice for basic cellular functions. The endproduct of glycolysis, pyruvate, cannot be further processed in the citric acid cycle and is transformed to lactate. Consequently, myocardial lactate production rather than the normal extraction pattern is encountered during the early phase of ischemia and serves as a specific marker of the latter process. It is also a sensitive indicator of relatively short periods of ischemia, as amply indicated in this thesis. During prolonged periods of ischemia its effectiveness becomes progressively less due to inhibition of glycolysis at the glyceraldehyde-3-P-dehydrogenase and phosphofructokinase level.
Besides lactate, adenine nucleoside release from the heart makes for another metabolic diagnostic tool in the diagnosis of ischemia. Consequently to the inability to resynthesize high-energy phosphates during ischemia, AMP and inorganic phosphate accumulate, followed by dephosphorylation of AMP to adenosine. This nucleoside modulates a variety of physiologic effects in different tissues,

including negative inotropic, dromotropic and chronotropic actions besides its well-known vasodilating effect. From a diagnostic point of view, it is important that this nucleoside is able to pass the cell membrane after which it is quickly deaminated to inosine and further to hypoxanthine. Nucleosides have proved to be significant indicators of ischemia in animal models and in man, as outlined in this thesis. As a byproduct, inorganic phosphate release from the heart could be used to assess ischemia. Other potential markers, to be commented upon in later chapters, include blood gases, potassium and glucose.

Finally, in chapter I attention is paid to the role of citrate and certain amino acids, such as alanine, aspartate and glutamate, in the control of glycolysis by way of the malate-aspartate cycle. Moreover, they may reduce intracellular acidosis during ischemia and remove excess ammonia. Also, a change in the uptake-release pattern of citrate and of the amino acids may reflect the presence of ischemia.

*Chapter II*

The second introductory chapter concerns vascular control and regulation of coronary flow.
Coronary resistance and flow continuously adjust to the instantaneous metabolic demand of the myocardium. This autoregulatory process occurs at the microvascular level on a beat to beat basis and is governed by local metabolic factors, i. e. adenosine, $pO_2$, $pCO_2$ and pH. In addition, coronary flow is under the influence of myogenic mechanisms and of endothelium-dependent control. The endothelium produces several potent vasodilator- and vasoconstrictor substances. Besides the endothelium-derived relaxing factor(EDRF) or nitrous oxide, the endothelium-derived hyperpolarizing factor and prostanoids, such as prostacyclin or the the prostaglandins $F_{2\alpha}$ and -$E_2$, the endothelium also forms endothelins, a family of peptides with long lasting, strong vasoconstrictor properties. EDRF is produced in the intact endothelium by a number of exogenous substances, including acetylcholine, catecholamines, adenine nucleotides, thrombin and bradykinin through stimulation of specific endothelial receptors. Moreover, coronary flow per se may stimulate EDRF production. Apart from these endothelium-dependent processes, vasodilatation may be induced by direct stimulation of vascular smooth muscle cell guanylate and adenylate cyclase systems, similar to the mechanism of action of EDRF. The vascular smooth muscle cell ultimately controls its own contractile state and, hence, vasotone. In chapter II intracellular regulators and signal transduction through smooth muscle cell receptor modulation or by direct intracellular effects are discussed. Emphasis is on adrenergic vascular control in view of our observations of neurohumoral activation during ischemia. Several animal studies suggest an important role for $\alpha$-adrenergic vasoconstriction during ischemia, presumably linked to $\alpha_2$- adrenergic stimulation. During coronary flow reduction, intracoronary norepinephrine or cardiac sympathetic stimulation reverses metabolic dilatation to vasoconstriction. Superimposed on a significant coronary lesion even a small decrease in arterial lumen caused by such vasoconstrictory effects may lead to a severe reduction in coronary flow. In this respect, the abnormal vasodilator response in atherosclerotic arterial segments, but also in hypercholesterolemia without obvious sclerosis, is important. Ample evidence exists that stimuli which would otherwise induce vasodilatation, may actually lead to constriction under these circumstances.
Also, impaired vasodilating prostaglandin synthesis in atherosclerotic arteries leaves the effect of platelet-produced vasoconstricting prostanoids unopposed. In addition to mechanical factors, such as the Bernouilly effect on stenosis resistance, an abnormal regulation of vasomotor tone by mechanisms such as outlined above, may significantly contribute to the syndrome of coronary insufficiency, initially determined by the degree of coronary artery stenosis. Due to the variability of dynamic changes in vasomotor tone superimposed on a fixed arteriosclerotic lesion, different degrees of ischemia may be found in one individual in situations where myocardial oxygen demand is identical. Alternatively, a similar reduction in coronary perfusion may occur in the same individual, but resulting from entirely different stimuli. An abnormal coronary vasomotor tone may indeed be present in the post-stenotic area during exercise or pacing-induced stress. Data on coronary flow distribution during pacing-induced ischemia in this thesis suggest that this abnormal vasomotor tone persists after evidence for ischemia has subsided. Moreover, neurohumoral activation during ischemia indicates that adrenergic mechanisms may indeed be involved.

*Chapter III*

How useful are metabolic markers as indicators of ischemia and are they applicable in man? Chapter III concerns these questions. Specific changes in myocardial metabolism may be detected using

various radionuclide methods or by way of magnetic resonance spectroscopy. Consequently, a number of non-invasive techniques have been developed to monitor these changes during ischemia with obvious advantages over invasive methods. However, a number of important limitations of technical and economic nature (still) restrict their applicability. Most importantly, fast, sequential determinations of metabolic changes, a prerequisite for monitoring myocardial metabolism during the early phase of ischemia, is generally not possible with these methods. The latter is less of a problem with available invasive techniques. Basically, these can be categorized in methods, which use catheter tip electrodes, tracer methods or techniques which rely on the biochemical evaluation of metabolites in the coronary venous effluent. Catheter tip techniques allow for rapid and sensitive determination of specific metabolic alterations. Its main limitation is that only one substrate is measured. In contrast, biochemical evaluation of metabolites, as used throughout this thesis, allows for a multitude of metabolites and other humoral substances to be measured simultaneously with coronary flow. Determinations can be fast, sequential and should allow for reproducible metabolic assessments, provided a number of technical requirements are adhered to.

These are discussed at length in this chapter. In this context, the sensitivity and specificity of several metabolites as indicators of ischemia are considered as well as the optimal stress test for this particular kind of study. With respect to the latter, only two apply in man, i. e intermittent coronary occlusion during angioplasty and the atrial pacing stress test. The main disadvantage of the first, coronary occlusion, may be that strict adherence to the study protocol is not always possible on ethical grounds. The main disadvantage of the second may be that this form of stress, fast atrial pacing, does not necessarily reflect the pathophysiological conditions under which the patient experiences his ischemic attack. On the other hand, the pacing stress test offers a number of advantages, extensively discussed throughout this thesis, not encountered by other methods.
As this thesis concentrates on the usefulness of lactate and hypoxanthine as markers of ischemia in man, available data from the literature are discussed. In fact, it would appear that myocardial lactate production, a certain tell-tale of ischemia and appreciated as such in animal studies, has a relatively low sensitivity in man, in average ranging between 50 and 60%. In this chapter the possible reasons behind these rather disappointing figures are given and suggestions how to improve the assessment of lactate metabolism provided. These suggestions are tested in chapter V. Furthermore, the latter considers whether additional metabolites may improve the detection of ischemia besides lactate. Although it has been suggested that the amino acids alanine and glutamate may be more sensitive as means to assess ischemia, changes are relatively moderate compared with lactate. Likewise, it has been suggested that citrate release during ischemia may add to the detection of the latter, in conjunction with lactate. However, its specificity is uncertain and, at present, insufficient data are available to clarify the real significance of citrate as marker of ischemia.
In contrast, cardiac nucleoside release from the heart provides for an alternative and potentially sensitive means to detect ischemia and one which is specific. In man, hypoxanthine is the nucleoside of choice. In fact, adenosine and inosine are hardly measurable in humans. Studies which have claimed the latter usually have raised questions concerning sampling techniques. Our experience with hypoxanthine release from the heart is discussed in chapters IV and VI.
Finally, chapter III discusses available data on the reproducibility of metabolic markers, specifically of lactate, during successive periods of ischemia, or rather the lack of data in this respect.
Despite widespread use of this metabolite to test the effectiveness of pharmacological interventions in ischemia, little is known of its reproducibility. Moreover, available data are conflicting with respect to the recovery period between ischemic periods needed to provide for reproducible metabolic changes. Furthermore, concerning the reproducibility of other metabolites, no data are available in man.

*Chapter IV*

In this chapter our early experience with respect to adenine nucleoside kinetics in ischemia are discussed. These investigations started off in an open-chested, anesthetized pig model, in which the left anterior descending flow was reduced by 75% for a period of one hour. The porcine heart has several advantages compared with that of other species. One is that it is better comparable to the human situation, e. g. because of the absence of functioning collaterals. In the coronary venous effluent directly draining the ischemic area, an early release of lactate and of the nucleosides inosine and hypoxanthine was found. Maximum changes in all metabolites occurred within the first 10 minutes of

ischemia, lactate preceding inosine only during the initial 2 minutes of occlusion. More importantly, coronary venous levels of both metabolites gradually declined towards the end of the ischemic period. In contrast, the rise in coronary venous hypoxanthine levels remained relatively constant, although maximal changes in the pig were markedly less than inosine. In contrast to the pronounced lactate production and nucleoside release in this model, changes in other metabolites, such as potassium or inorganic phosphate, were moderate. In view of the correlations between lactate and the nucleosides on the one hand and between each of these parameters and hemodynamic changes during ischemia on the other, it was concluded that nucleosides, in particular inosine, were good markers of ischemia. This was further evaluated in humans during incremental atrial pacing. Patients were divided in 2 groups depending on the occurrence of anginal pain, as was usual in those days. In a comparable protocol as described in more detail in chapter V, it was found that patients with angina who had all significant (>50%) coronary disease, did produce significant amounts of hypoxanthine from the heart. Patients without angina, most of whom had no coronary disease, did not. Inosine was not detectable in either group. Of interest, average coronary hypoxanthine levels were still significantly elevated at 5 minutes after pacing. However, when a difference of >0. 4 umol/l was taken to indicate abnormal changes in hypoxanthine levels only 60% of patients with significant coronary lesion had ischemia-induced nucleoside release, compared to 80% with lactate production. Also, in this study, lactate production occurred at an earlier stage than hypoxanthine release. Although the latter did reflect what was observed in our animal studies, the observed difference in sensitivity between lactate and hypoxanthine might also relate to the nucleoside assay technique used at that time.

Apart from the fact that in this study ischemia-induced hypoxanthine release was shown for the first time in man, this investigation also provided clear evidence that other metabolites than lactate and nucleosides were not useful to identify ischemia in humans. Thus, no appreciable changes in coronary venous inorganic phosphate, glucose or blood gases were found, whereas potassium egress from the heart during pacing was very small and not specific.

*Chapter V*

Contrary to our first results presented in chapter IV, most human investigations do not support the assumption that myocardial lactate production is a very useful indicator of ischemia. Percentages of patients with lactate production during pacing-induced stress on average vary between 50 and 60%. This lack of sensitivity not be true but reflect an under-estimation of its value particularly as the metabolite invariably was assessed at the wrong time during the procedure. Moreover, conclusions were generally based on small patient populations, in which angina wrongfully was taken to represent ischemia, and in which the location of coronary artery disease was not always taken into account. In a large prospective trial, described in chapter V, we compared patients with left-sided coronary artery disease with patients without or with minimal coronary lesions, studying lactate metabolism not only during, but also in the post-pacing period. In so doing, it was found that in patients with significant coronary artery disease maximal changes were found between 15 and 30 seconds after pacing and not during maximal pacing rates. Furthermore, the number of patients with lactate production in the post-pacing period only, was significant. The procedure now identified myocardial lactate production as a sensitive and specific marker of ischemia with the difference between lactate and non-lactate producers determined by maximal obtained heart rate and myocardial oxygen demand during pacing. As such it scored appreciably higher than other objective indicators of ischemia, such as electrocardiographic or hemodynamic changes. Moreover, lactate was more specific. In patients without significant coronary lesions, myocardial lactate production was low, in contrast to changes in other objective variables as well as in angina, which proved to be rather unspecific.

*Chapter VI*

To be able to assess the effectiveness of interventions in ischemia through changes in myocardial metabolism one must first address the issue of their reproducibility following repetitive periods of myocardial ischemia. In man this aspect has been given little attention. However, that it is an important issue is clearly shown in chapter VI. 1, where the effect of two relatively severe ischemic episodes of 30 minutes each, separated by a recovery period of 35 minutes, on different metabolites in the pig heart is presented. The main finding here is that changes in both lactate and the nucleoside inosine are not reproducible and that ongoing inosine production throughout the recovery period

suggests persistent ischemia, 35 minutes after onset of reperfusion. The latter may serve to explain why lactate production is not similar during the second compared to the first ischemic period. It is not quite clear why, in the absence of data on myocardial nucleotide content, inosine is not reproducible.
Although the study design does not allow a direct comparison with the pacing protocol used in patients, the results of this investigation certainly caution against the uncontrolled use of lactate or nucleosides in the assessment of repetitive episodes of ischemia. This aspect was further studied with respect to myocardial lactate metabolism in man. The reproducibility of lactate production, compared with electrocardiographic and hemodynamic variables, was evaluated during repetitive atrial pacing stress tests with intervals varying from 15 to 60 minutes. Following the shorter intervals, i. e. 15 and 30 minutes, lactate production was significantly less during the second ischemic episode. In contrast, comparable values were found after 45 or 60 minutes. Also, individual values were reproducible, a few exceptions aside. Again, as with sensitivity or specificity, the reproducibility of lactate appeared better than that of other variables, such as the gold standard, ST segment depression. Also, angina was not at all reproducible, generally being significantly less during the second test. Our results led us to propose that pacing-induced ischemia should be allowed to recover for at least 45 to 60 minutes before a second test is carried out.

*Chapter VII*

Our previous studies suggested that metabolic changes occurred early during pacing-induced ischemia in man. However, they did not provide for data on the temporal relation with changes in coronary flow. The latter necessitates a technique which allows for continuous monitoring of coronary flow distribution over relatively long periods. Available methods generally are not suitable. Thus, a new technique was developed whereby myocardial flow changes were determined by precordial monitoring of krypton-81m distribution in the myocardium.

To this purpose, this short-lived isotope (half-life 13 seconds) was continuously infused in the left coronary artery and mapping performed in regions of interest during 15-second acquisition periods. Early reductions in coronary flow occurred in high-stenosis (>90%) areas preceding changes in 70-90% regions by several minutes. Of importance, they also preceded lactate and hypoxanthine release from the heart by a similar period. Changes in krypton-81m distribution persisted after pacing. Long after angina, electrocardiographic, hemodynamic or metabolic (lactate) signs of ischemia had disappeared, indicative of ongoing flow reduction up to 15 minutes after pacing. As cardiac hypoxanthine release, measured by a more sensitive assay technique than in our earlier investigations, did also persist for periods over and above 5 minutes post-pacing, this not only suggests sustained flow reductions, but also persistent ischemia. Moreover, the improved analysis of hypoxanthine in this study allowed us to show that nucleoside changes were as early as lactate production and that the sensitivity of this marker was as good as lactate in identifying ischemia.
Furthermore, the studies with krypton-81m provided evidence that a coronary artery diameter narrowing of 50 -70% not necessarily has to be significant in terms of flow reductions during pacing-induced stress.

*Chapters VIII and IX*

The issue as to whether metabolic markers, i.e. lactate, are really applicable and useful in assessing the acute antiischemic properties of different pharmacological interventions in man, is addressed in chapters VIII and IX.
To this purpose a number of vasoactive compounds were tested in the model of repetitive atrial pacing stress tests, such as described previous chapters. Based on our experiences with respect to the reproducibility of metabolic, electrocardiographic and hemodynamic indices, a minimum interval of one hour was chosen between tests (see chapter VI). Studies included investigations with amiodarone, bepridil and diltiazem. Although their pharmacological profiles are not identical, these agents share coronary and systemic vasodilating properties besides intrinsic negative inotropic and chronotropic effects. However, which of these cardiovascular properties prevail depends on factors, such as dose as well as route and speed of administration. In general, high dose administration increases the systemic and, particularly, coronary hemodynamic effects. Subsequently, secondary reflex mechanisms tend to counteract the negative chronotropic and inotropic properties of the compound.

Part of our efforts was directed at defining dosage schemes, which would result in optimal cardio-

vascular conditions for the type patient under study, i.e. the patient with acute myocardial ischemia at rest, e.g. with unstable angina or the initial phases of myocardial infarction. Here, marked bradycardia or reduced contractility is unwanted. Instead, vasodilating effects of the agent in question should be such that cardiac pump function is preserved or may even improve together with an improvement of coronary blood flow.
Thus, in each instance high-dose infusions were given with the result that for all compounds tested cardiac output and coronary flow increased, whereas negative chronotropic and inotropic effects were (temporarily) suppressed. In this respect, our observations in patients with significantly impaired cardiac function are interesting. As shown in chapter IX, such patients actually improve better than patients with a normal cardiac function with regards to cardiac - and stroke output. More interesting, the acute antiischemic effects of the agents tested were definitely more pronounced in patients with an impaired ventricular function at baseline. These observations are clinically relevant. Patients, hospitalized with severe ischemia at rest or an acute myocardial infarction generally have a significantly depressed cardiac function. Our studies indicate that relatively high dosages of diltiazem, a calcium-antagonist, or of bepridil, a calcium-antagonist with additional blocking effects of sarcolemmal sodium channels and calmodulin-inhibitory properties, should be administered instead of the low dosages, commonly considered "safe". In this respect, our data on the effect of amiodarone are relevant to the clinical situation where severe ventricular arrhythmias tend to occur in situations where a markedly impaired cardiac function coincides with myocardial ischemia.
The antiischemic properties of diltiazem, bepridil and amiodarone were delineated by a significant reduction in lactate production during the second pacing test. Although in most studies ischemic electrocardiographic and hemodynamic changes were also less during pacing with these compounds, the determination of lactate metabolism proved to be necessary to identify antiischemic efficacy in all events. Also, the magnitude of changes was generally greater for lactate than for ST-changes, which, in addition, indicates the usefulness of this marker of ischemia in this type of investigations. In our study model where the second stress test was started 15 minutes after the onset of drug administration, the antiischemic effects of all compounds tested appeared to relate to their sustained systemic vasodilating properties and the resulting reduction in myocardial oxygen demand. An improvement in coronary flow was not apparent anymore at that time. It may be argued that the significant antiischemic effects of these agents may be more pronounced when these drugs are administered when myocardial ischemia is already present.

Although the possibity of a "steal-effect" in this condition ought to be considered, the potential of these agents to interfere with local coronary vasoconstriction in the ischemic area, brought about by ischemia-induced neurohumoral changes, may be an important argument for the acute intravenous administration of these compounds in the dosages mentioned in this thesis. That such (additional ?) vasodilating effects, based on modulating neurohumorally-induced vasoconstriction, may occur, is suggested by the observation that both bepridil and diltiazem prevented the increase in systemic resistance and in arterial pressures during pacing-induced ischemia.
The neurohumoral aspects of acute myocardial ischemia and the possible relation to ischemia-induced vasoconstriction is discussed in part 2 of this thesis.

## PART 2

### Systemic and cardiac neurohumoral activation during acute myocardial ischemia in man. Rationale for converting enzyme inhibition in ischemia.

*Chapter X*

Myocardial ischemia results in a complex chain of events, only partially reflected by the metabolic changes discussed in this thesis. In this respect, free radical formation and the effect of ischemia on leucocytes, leukotrienes, platelets and prostanoids may be mentioned. In addition, the possibility that local or circulating neurohormones or related systems become activated during myocardial ischemia may be worthwhile to consider; particularly, as such a stimulation could affect systemic or coronary vascular tone and, hence, the ischemic process.
Neurohumoral activation is well known in conditions such as the advanced stages of heart failure or myocardial infarction, particularly when accompanied by acute pump failure. Not only are circulating catecholamines elevated in these situations, circulating arginine vasopressine and renin-angiotensin levels as well as cardiac sympathetic tone may also become increased. Moreover, recent

data suggest that several of these systems are already activated in patients with left ventricular dysfunction, however without the presence of heart failure symptoms. Whether, in humans, myocardial ischemia also results in neurohumoral activation is less well established. Several arguments could be put forward for such an activation, i.e. the stress of anginal pain or acute, ischemia-induced ventricular dysfunction.
However, in contrast to markedly enhanced cardiac sympathetic activity during prolonged, severe ischemia in animal models, data on circulating or cardiac catecholamine changes during short lasting ischemia in man are inconclusive. Futhermore, no information is available on alterations in different systems, such as the renin-angiotensin system(s). That ischemia may affect the latter is indicated by several animal studies.

Part 2 of this thesis relates to our studies on the effect of short periods of relatively moderate ischemia, such as results from pacing-induced stress, on several neurohumoral systems. These investigations focused on circulating and transcardiac catecholamine and angiotensin II levels. As conditions as hypertension or heart failure may affect the results, patients were carefully selected to be normotensive, without heart failure symptoms and not on therapy or diet which could influence baseline neurohormones.
Our studies clearly indicate that during and for several minutes after pacing-induced ischemia arterial norepinephrine and angiotensin II levels become significantly elevated. In addition, epinephrine levels also increase, but only during the stress test. Moreover, net cardiac norepinephrine release, present at baseline, reverts to net uptake in the ischemic area during pacing, accompanied by increased cardiac epinephrine uptake. Our studies further suggest that the magnitude of neurohumoral activation depends on the severity of myocardial ischemia. In contrast, angina is not as clearly related to ischemia-induced neurohumoral stimulation.
Concomitantly with the changes in neurohormones, systemic vascular resistance and arterial pressures also increase significantly. Again, changes correlate with the severity of ischemia. Although no causal relationship with ischemia-induced neurohumoral activation could be established as specific antagonists were not administered in these studies, their association is suggested by this correlation as well as by the temporal relationship. If so, neurohumoral activation during ischemia could very well be clinically relevant. An increase in ventricular wall stress secondary to the rise in arterial pressures undoubtedly will amplify the severity of ischemia already present.
In addition, enhanced catecholamine uptake by the heart may result in adrenergically-induced coronary vasoconstriction. Although our data did not suggest that overall coronary flow decreased during ischemia, regional coronary vasoconstriction in the post-stenotic area may still have occurred. That adrenergic modulation of coronary vascular resistance may occur during pacing-induced ischemia is suggested by the observation that a high, but not a low dose of labetalol limits pacing-induced decrease in resistance, as described in chapter III. Consequently, the high dose with the percentwise greater betablocking profile has less antiischemic efficacy than the low dose of labetalol despite a more pronounced reduction in myocardial oxygen demand with the high dose.
This example illustrates that modulation of adrenergic alterations in coronary tone during ischemia may have therapeutic implications. In the next and last chapter of this thesis the potential clinical usefulness of specific neurohumoral modulators during ischemia is discussed.

*Chapter XI*

The observation that arterial angiotensin II levels increase during ischemia together with changes in catecholamines led us to investigate the antiischemic potential of converting enzyme inhibition.
A number of theoretical considerations suggest that ACE inhibitors may limit myocardial ischemia. Through a reduction in pre- and afterload and negative inotropic effects and by way of coronary vasodilatation, these agents may favourably affect the oxygen supply-demand ratio. These cardiovascular actions not only depend on inhibition of local or circulating angiotensin II formation, but also on their effects on bradykinin degradation, prostaglandin synthesis and their complex interaction with sympathetic adrenergic activity. Furthermore, compounds with SH-groups may affect EDRF production.
Taken together these properties should allow the ACE inhibitor act as an antiischemic agent. However, thus far experiences with chronic treatment of stable, exercise-induced angina have been conflicting and generally disappointing. Several studies have claimed some positive results, others were definitely negative. The reason is not clear. It has been speculated that antiischemic activity in this situation only occurs when hemodynamic

effects in the sense of blood pressure lowering is present as well. However, this also has been disputed in later investigations.
One problem with interpretating the data from these studies is that most of them were uncontrolled and of relatively short duration. In addition, information on the antiischemic effectiveness of ACE inhibitors in humans is lacking.
In chapter XI we describe the acute antiischemic effects of enalaprilat, a non-sulfhydryl containing converting enzyme inhibitor in patients with pacing-induced myocardial ischemia at rest. As in our previous studies, discussed in chapter X, this condition was characterized by activation of circulating catecholamines and of arterial angiotensin II levels together with a significant increase in arterial pressures during the first, untreated pacing test. In contrast, a second test, 15 minutes after enalaprilat, did not result in any form of neurohumoral activation, while arterial pressures remained unchanged. Moreover, enalaprilat significantly reduced myocardial ischemia, indicated by a marked reduction in myocardial lactate production. These antiischemic effects could not be explained by direct systemic or coronary hemodynamic properties of the agent, but appeared to relate to a modulating effect on neurohormones and on arterial pressures. Thus, it has the potential to reduce afterload and myocardial oxygen demand. In addition, as enalaprilat prevented net cardiac norepinephrine uptake during ischemia, a modulating effent on post-stenotic coronary vascular tone cannot be excluded. The results of this study emphasize the potential significance of systemic and cardiac neurohumoral activation during ischemia and also the potential usefulness of specific neurohumoral modulators such as converting enzyme inhibitors and alpha-adrenergic antagonists as an (additional) form of antiischemic therapy.

# SAMENVATTING.

Metabole aspecten van acute myocardischemie en toepasbaarheid van metabole parameters om voorkomen en ernst van dit ziektebeeld bij de mens te kunnen vastleggen staan centraal in het eerste deel van dit proefschrift. Hiertoe werden veranderingen in het lactaatmetabolisme en adenine-nucleotide afbraak in het hart bestudeerd en vergeleken met het effect van ischemie op andere metabole, hemodynamische en elctrocardiografische parameters bestudeerd. Vervolgens werd de tijdsrelatie tot regionale veranderingen in coronaire bloeddoorstroming bepaald. Tenslotte werd het acute anti-ischemische profiel van verschillende vaatverwijdende agentia bestudeerd, gebaseerd op de voordien gedefinieerde metabole aspecten van ischemie.
Het tweede deel van het proefschrift bespreekt het effect van myocardischemie op systemische en cardiale neurohumorale activatie, in het bijzonder het effect op catecholamines en het renine-angiotensine systeem. De relatie tussen de mate van ischemie en neurohumorale activatie en de relatie met gelijktijdige systemische en coronaire vasoconstrictoire effecten wordt in het tweede gedeelte van het proefschrift bediscussieerd, evenals de mogelijke toepasbaarheid van specifieke neurohumorale modulatie, bijvoorbeeld door remming van het angiotensine converterend enzyme.

## DEEL I.

*Hoofdstuk I.*

Hoofdstuk I vormt een algemene introductie tot het eerste thema van dit proefschrift betreffende de acute metabole veranderingen tijdens myocardischemie. Achtereenvolgens worden in dit hoofdstuk de normale regulatiemechanismen van de stofwisseling in het hart besproken, gevolgd door de veranderingen hierin tijdens ischemie. De continue wisselwerking tussen samentrekking en verslapping van het hart maakt dat een constante toelevering van zuurstof en voedingsstoffen zoals vrije vetzuren, glucose en lactaat noodzakelijk is. Oxidatie van deze substraten vindt plaats in de citroenzuurcyclus, waarbij energie in de vorm van ATP-moleculen gevormd wordt tijdens diverse oxydatieve fosforyleringsprocessen. De laatste zijn gevoelig voor zuurstofaanbod. Tijdens ischemie en het daarmee gepaard gaande gebrek aan zuurstof en voedingsstoffen neemt de fosforylering zeer snel af. Dit heeft tot gevolg dat intermediaire stofwisselingsprodukten, zoals acyl CoA en acyl-carnitine complexen, ophopen in de hartcel. Deze kunnen leiden tot verandering van structuur en functie van diverse subcellulaire componenten, zoals de celmembraan, en tevens enzyme systemen negatief beïnvloeden, resulterend in veranderende celfunctie, verstoorde ionenuitwisseling en celzwelling. Bovendien treedt bij ischemie een daling op van de cellulaire pH door ophoping van gereduceerde coenzymen en activatie van de anaerobe glycolyse. Dit laatste heeft tot direct gevolg dat laktaat in plaats van geëxtraheerd te worden door het myocard wordt geproduceerd en reeds in een vroege fase van ischemie. Bovendien treedt bij ischemie een progressieve daling op van de cellulaire pH door accumulatie van gereduceerde coenzymen ten gevolge van activatie van de anaerobe glycolyse. Zoals blijkt uit dit proefschrift functioneert deze laatste verandering als een zeer gevoelige indicator voor het ischemische proces, althans wanneer deze kort van duur is. Bij langer durende ischemie wordt de glycolyse progressief geremd op glyceraldehyde-3-P-dehydrogenase en fosfofructokinase niveau, met als direct gevolg dat de sensiviteit van lactaatproduktie als maat voor het ischemisch proces afneemt.
Het onvermogen tot vorming van hoog-energetische fosfaten gedurende ischemie leidt tot stapeling van AMP en inorganisch fosfaat, gevolgd door defosforylering van AMP tot adenosine. Deze nucleoside beïnvloedt een aantal cardiovasculaire functies en heeft een sterke vaatverwijdende werking. Uit diagnostisch oogpunt is het van belang dat adenosine de celmembraan kan passeren, waarna het snel gedeamineerd wordt tot inosine en vervolgens tot hypoxanthine. Deze laatste nucleosiden zijn belangrijke metabole indicatoren van myocardischemie, zoals eveneens uit dit proefschrift blijkt. Tevens komt hier naar voren dat bij de mens andere metaboliten, zoals inorganisch fosfaat, kalium en glucose minder belangrijk zijn uit oogpunt van diagnostiek dan lactaat of de nucleosides, inosine en hypoxanthine. Daarentegen zijn er aanwijzingen dat een veranderde kinetiek van sommige aminozuren en van citraat gedurende het ischemisch proces gebruikt kan worden om de aanwezigheid hiervan te diagnostiseren.

*Hoofdstuk II.*

Het tweede introductiehoofdstuk betreft reguleringsmechanismen van vasomotoriek, met name die van het coronaire vaatnet. Coronaire vaatweerstand en doorbloeding worden continu gereguleerd op basis van de metabole behoefte van het myocard op dat moment. Dit autoreguleringsproces vindt plaats op microvasculair niveau en wordt bepaald door locale metabole factoren, zoals adenosine, $pO_2$ en pH. Hiernaast wordt de coronairflow gereguleerd door zowel myogene als endotheel-afhan-

kelijke controlemechanismen. Het endotheel produceert diverse krachtige vaatverwijdende stoffen, zoals de endotheel-afhankelijke relaxerende factor (EDRF), de endotheel-afhankelijke hyperpolariserende factor en prostaglandines, zoals prostacycline en prostaglandine $F_{2\alpha}$ en $-E_2$. Daarnaast produceert het endotheel krachtige vaatvernauwende stoffen, de endothelinen. Het kortwerkende EDRF wordt alleen gevormd in het intacte endotheel onder invloed van diverse circulerende agentia, zoals acetylcholine, doch ook onder invloed van de coronaire doorbloedingssnelheid, waarbij wandspanningsveranderingen en activatie van specifieke kaliumkanaaltjes een rol kunnen spelen. Het geproduceerde EDRF induceert vaatverwijding door activatie van het guanylaat cyclase systeem in de gladde spiercel. Dit systeem en het verwante adenylaat cyclase kunnen zowel direct gestimuleerd worden of na activatie van receptoren op de gladde spiercel zelf. Ten aanzien hiervan wordt in het proefschrift de nadruk gelegd op adrenergisch vasculaire controle mechanismen, gezien onze bevindingen dat neurohumorale acitivatie optreedt gedurende ischemie. Verscheidene studies suggereren dat $\alpha$-adrenergisch geïnduceerde vasoconstrictie gedurende ischemie belangrijk kan zijn. Reeds een geringe vermindering van het arteriële lumen op basis van deze of vergelijkbare mechanismen, doch gesuperponeerd op een reeds bestaande gefixeerde atherosclerotische laesie, kan leiden tot ernstige coronaire doorbloedingsstoornissen. Recente bevindingen dat endotheel-afhankelijke vaatverwijding gestoord raakt niet alleen in atherosclerotische segmenten, doch ook tijdens hypercholesterolemie in normaal ogende coronairvaten, kunnen in dit verband belangrijk zijn. Niet alleen betreft het hier een gestoorde EDRF productie, doch ook een verminderde prostaglandine synthese, factoren die naast mechanische, zoals het Bernouilly effect op de stenose-weerstand, in belangrijke mate kunnen bijdragen tot het syndroom van coronairinsufficientie. Diverse studies, inclusief eigen bevindingen vermeld in Hoofdstuk VII, suggereren dat abnormale vasomotoriek inderdaad aanwezig is in het ischemische gebied gedurende inspanning of atriaal pacen en dat deze persisteert nadat ischemie niet meer aantoonbaar is.

*Hoofdstuk III.*

In hoofdstuk III wordt de praktische toepassing van het gebruik van metabole indicatoren ter bepaling van ischemie belicht. Veranderingen in hartstofwisseling kunnen ontdekt worden op een noninvasieve wijze met gebruikmaking van diverse radio-isotopen of door middel van magnetische resonantie spectroscopie. Hoewel deze technieken duidelijke voordelen hebben ten opzichte van invasieve methodieken, wordt de toepasbaarheid hiervan beperkt door diverse factoren van technische aard. Bovendien zijn snelle metabole veranderingen, belangrijk gedurende de vroege fase van ischemie, over het algemeen niet vast te leggen met deze methoden. Dit laatste kan wel door middel van invasieve technieken, zoals daar zijn directe biochemische bepalingen van stofwisselingsproducten in arterieel en coronair veneus bloed of metingen met behulp van specifieke electroden in de bloedbaan. De laatste methode biedt de mogelijkheid tot snelle sequentiële metingen, doch is beperkt tot slechts één variabele. In tegenstelling tot het voorafgaande biedt de directe biochemische bepaling van metabolieten in het bloed, een techniek die in dit proefschrift centraal staat, de mogelijkheid om diverse stofwisselingsproducten gelijktijdig met coronairflow-veranderingen te kunnen bepalen. In hoofdstuk III wordt de gevoeligheid en specificiteit van verschillende metabolieten als indicatoren van ischemie besproken en aandacht besteed aan de optimale stress test in dit kader. Ten aanzien van dit laatste zijn twee methodes bruikbaar; die, waarbij tijdens angioplasty intermitterende coronairafsluiting wordt toegepast en de atriale pacing stress test. Bij de eerste methode kan strikt navolgen van een studieprotocol op ethische bezwaren stuiten. Een belangrijk nadeel van de tweede methode, de atrial pacing stress test, kan zijn dat deze niet altijd de condities nabootst die aanleiding geven tot ischemische aanvallen bij de patienten in het dagelijks leven. Hiertegenover staat dat de atriale pacing stress test een aantal belangrijke voordelen biedt, met name wanneer het metingen van hartstofwisseling en coronairdoorbloeding betreft. Dit proefschrift stelt het nut van lactaat en hypoxanthine als indicatoren van ischemie bij de mens centraal. Hoofdstuk III bediscussieert de in de literatuur aanwezige gegevens ten aanzien hiervan. Het is opmerkelijk en tegelijkertijd teleurstellend dat in het algemeen de gevoeligheid van lactaatproductie om myocardischemie te diagnosticeren laag is, met een incidentie variërend tussen 50% en 60% gedurende pacing. Deze matige sensitiviteit betreft waarschijnlijk een onderbelichting van de werkelijke rol van deze metaboliet ten gevolge van voornamelijk technische onvolkomenheden in de tot nu toe gepubliceerde patientenstudies. Hoe de gevoeligheid van deze metabole maat voor ischemie te verbeteren, wordt aangegeven in de verdere hoofdstukken van dit proefschrift. Andere metabolieten, zoals sommige aminozuren en citraat, blijken niet gevoeliger te zijn dan lactaat. De enige uitzondering hierop vormt mogelijk de nucleoside hypoxanthine. Daarentegen zijn adenosine en inosine bij de mens niet of nauwelijks aantoonbaar.

Indien metabole veranderingen gebruikt worden om de effectiviteit van farmacologische interventies te testen, is het een eerste noodzaak dat deze stofwisselingsstoornissen reproduceerbaar zijn tijdens intermitterende perioden van myocardischemie. Ondanks het veelvuldig gebruik van met name lactaat in dit verband, zijn er in de literatuur nauwelijks of geen gegevens ten aanzien hiervan. Ditzelfde geldt ook voor andere metabolieten. Dit aspect, de reproduceerbaarheid van cardiale stofwisselingsstoornissen tijdens ischemie, wordt aan de hand van onze bevindingen ten aanzien van nucleosiden- en lactaatveranderingen in hoofdstuk VI verder besproken.

*Hoofdstuk IV.*

Uit historische overwegingen worden in dit proefschrift eerst onze studies betreffende adenine nucleoside veranderingen tijdens ischemie besproken. De onderzoekingen hiernaar begonnen in een ischemisch varkensmodel, waarin de doorbloeding van de Ramus descendens anterior gereduceerd werd met 75% gedurende een uur. In dit model werd reeds in een vroege fase het vrijkomen van lactaat en van de nucleosiden inosine en hypoxanthine uit ischemisch gebied gemeten. Maximale veranderingen vonden plaats gedurende de eerste 10 minuten, waarbij lactaatproductie iets eerder optrad dan inosineverlies. Na de initiële 10 minuten namen de spiegels van deze metabolieten in het coronair-veneuze effluent geleidelijk af en benaderden de controle-situatie tegen het einde van het onderzoek. Daarentegen bleven de coronair-veneuze hypoxanthine spiegels relatief constant verhoogd. Veranderingen in andere metabolieten, zoals kalium en inorganisch fosfaat, waren duidelijk minder uitgesproken dan die van lactaat, inosine en hypoxanthine. Vervolgens werd het effect van myocardischemie op nucleosideverlies uit ischemisch gebied onderzocht bij patienten gedurende een atriale pacing stress test. In deze studie werden patienten onderverdeeld afhankelijk van het optreden van angina pectoris gedurende de test. Hierbij bleek dat bij angineuze patienten een significante verhoging optrad van hypoxanthine in het coronair-veneuze bloed, evenals van lactaat, doch niet van inosine, kalium, inorganisch fosfaat en glucose. Ook veranderingen in $pO_2$, $pCO_2$ en pH konden niet aangetoond worden in het coronair-veneuze effluent bij deze angineuze patienten. Hoewel bij de totale angineuze groep de coronair-veneuze hypoxanthine spiegels significant stegen en verhoogd bleven tot minstens 5 minuten na pacing, bleek toch dat slechts bij 60% van de patienten significante veranderingen plaatsvonden, in tegenstelling tot een lactaatproduktie van 80% in deze studie. Dit laatste, een relatief hoge incidentie van lactaatproduktie in deze studie, contrasteert met de getallen die in de meeste patientenstudies gerapporteerd worden en die, zoals vermeld, variëren tussen de 50 en 60%. Deze getallen berusten echter op vrij kleine patientengroepen. Van meer belang is dat deze waarnemingen gebaseerd zijn op metingen die alleen verricht werden tijdens de laatste test, in alle gevallen een atriale pacing stress test.

*Hoofdstuk V.*

Hierin wordt een onderzoek beschreven bij ruim 450 patienten, bij wie gedurende dezelfde testmetingen lactaatmetingen niet alleen werden verricht tijdens, doch met name in de directe periode na pacing. Uit deze studie blijkt heel duidelijk dat metingen verricht 15 tot 30 seconden na de atriale pacing stress test, de incidentie van geobjectiveerde lactaatproduktie significant doet toenemen van 60 tot 78%. Dit heeft tot gevolg dat lactaatproduktie een gevoeliger parameter blijkt dan standaard-variabelen zoals ST-segment depressies en stijgingen in linker ventrikel einddiastolische druk. Daarnaast blijkt lactaatproduktie sterk specifiek. In patienten zonder of met slechts geringe coronairafwijkingen werd dit slechts 9-10% gevonden in tegenstelling tot aanzienlijk hogere percentages patienten in deze groepen met abnormale electrocardiografische of hemodynamische veranderingen tijdens de atriale pacing stress test.

*Hoofdstuk VI.*

Reproduceerbaarheid van metabole veranderingen tijdens ischemie is een eerste vereiste indien deze gebruikt worden ter identificatie van de effectiviteit van interventies. Dit komt heel duidelijk naar voren uit hoofdstuk VI, waarin het effect van twee relatief ernstige perioden van ischemie, 30 minuten lang, gesepareerd door een herstelperiode van 35 minuten, op de diverse metaboliten in het varkenshart wordt besproken. Hieruit blijkt duidelijk dat gedurende de tweede periode de mate van lactaat- en adenosineproduktie beduidend minder is dan gedurende de eerste. Bovendien blijkt uit het onderzoek dat inosine-produktie persisteert gedurende de herstelperiode als uiting van blijvende ischemie, ondanks herstel van de coronairdoorbloeding. Dit laatste kan verklaren waarom de lactaatproductie minder is gedurende de tweede ischemische periode. Persisterende ischemie kan leiden tot een progressieve inhibitie van de anaerobe glycolyse. Deze biggenstudie geeft in ieder geval duidelijk aan dat het ongecontroleerd gebruik van metabolieten zoals lactaat of nucleosiden bij de beoordeling van herhaalde ischemische perioden gevaren met zich mee kan brengen. Dit

aspect werd vervolgens bestudeerd bij patienten met ischemisch hartlijden waarbij de reproduceerbaarheid van lactaatproduktie, vergeleken met electrocardiografische en hemodynamische variabelen, werd geëvalueerd tijdens herhaalde atriale pacing stress tests met wisselende intervallen, variërend van 15 tot 60 minuten. Deze studie geeft duidelijk aan dat angina en hemodymische parameters niet reproduceerbaar zijn onafhankelijk van de perioden tussen de belastingtests en dat een individuele reproduceerbaarheid van electrocardiografische veranderingen pas gevonden wordt na een uur. Daarentegen blijkt de lactaatproduktie goed reproduceerbaar vanaf 45 minuten, doch significant minder tijdens de tweede belastingtest, met kortere intervallen van 15 en 30 minuten. Gebaseerd op deze bevindingen wordt voorgesteld het effect van interventies te toetsen met een interval van ten minste 45 minuten tussen pacing tests indien lactaatproduktie als variabele wordt gebruikt.

*Hoofdstuk VII.*

Hoewel onze vroegere studies reeds suggereerden dat metabole veranderingen vroeg optreden tijdens pacing-geïnduceerde ischemie, waren er aanvankelijk geen gegevens ten aanzien van de tijdsrelatie met coronaire doorbloedingsstoornissen. Dit laatste aspect werd onderzocht door middel van een continue intracoronaire toediening van krypton-81m, een isotoop met een zeer korte halfwaarde tijd, dat de mogelijkheid biedt tot voortdurende monitoring van regionale cardiale doorbloedingsveranderingen. Bij deze techniek worden zeer vroege verminderingen in reperfusie gedetecteerd in gebieden met hooggradige coronairlaesies, enige minuten nadien gevolgd door veranderingen in gebieden met matig ernstige vernauwingen, gelijktijdig met lactaatproduktie en hypoxanthine verlies. De meeste veranderingen in krypton-81m distributie waren van lange duur, persisterend tot minstens 5 à 15 minuten na staken van de pacing stress test en nadat de meeste ischemische parameters waren genormaliseerd. Alleen hypoxanthine verlies uit het hart bleef gedurende relatief lange tijd na de test aanwezig, suggererend dat myocardischemie persisteerde, simultaan met de eveneens persisterende coronaire doorbloedingsstoornissen.

*Hoofdstuk VIII en IX.*

Zijn metabole veranderingen, zoals bijvoorbeeld lactaatproduktie gedurende ischemie, goede parameters om het effect van interventie te meten? Dit aspect wordt belicht in de hoofdstukken VIII en IX, waar het effect van verschillende vasoactieve stoffen op het ontstaan van pacing-geïnduceerde ischemie wordt besproken. In dit kader werden amiodarone, diltiazem en bepridil onderzocht bij patienten met een normale, doch ook, voor sommige agentia, bij patienten met een significant verminderde linker kamerfunctie. Ondanks het verschillend farmacologisch profiel resulteerden de drie farmaca in een duidelijk antiischemisch effect, zich uitend in een verminderde lactaatproduktie of zelfs een normalisering van het laktaatmetabolisme, in een verminderde ST-segment depressie en een verminderde stijging van de linker einddiastolische druk na pacing. De antiischemische eigenschappen werden met name duidelijk bij patienten met een gestoorde ventrikelfunctie. Ondanks het feit dat vrij hoge doseringen werden toegediend, verbeterden met name deze patienten zowel wat betreft linker kamerfunctie als wat betreft de door pacing geïnduceerde ischemie. Dit ondanks het feit dat de farmaca naast de vaatverwijdende werking tevens negatieve inotrope en chronotrope eigenschappen heben. Dit laatste werd echter overschaduwd door de sterke vaatverwijdende werking bij hoge doseringen van deze farmaca in onze studies. De drie studies zijn klinisch relevant in het licht van de vaak sterk verminderde linker kamerfunctie tijdens acute ischemie of tijdens de initiële fase van een myocardinfarct, situaties waar de acute toediening van de onderzochte farmaca belangrijk kan zijn. De antiischemische eigenschappen in deze drie studies berusten voornamelijk op een vermindering van de zuurstofbehoefte van het hart, teweeggebracht door de systemische vaatverwijdende werking van deze stoffen. Hoewel aanvankelijk een verbeterde coronairdoorbloeding optrad, was deze ten tijde van de atriale pacing stress test niet meer aanwezig. Dit kan betekenen dat de reeds aanwezige antiischemische eigenschappen kunnen worden versterkt indien deze middelen gegeven worden tijdens ischemische episoden. In dit verband kan de in dierproeven geobserveerde alpha-adrenerge vasoconstrictie van belang zijn. Farmaca zoals diltiazem en bepridil, met calciumantagonistische eigenschappen, interfereren hiermee, zoals ook wordt gesuggereerd in de huidige studies, waarbij de stijging in systeemvaatweerstand en arteriële bloeddruk, optredend tijdens ischemie, werd genivelleerd. Deze effecten ondersteunen tevens de bevindingen in deel II van dit proefschrift betreffende neurohumorale aspecten van myocardischemie en de mogelijke relatie tot vasoconstrictie.

## DEEL 2.

*Hoofdstuk X.*

Metabole veranderingen tijdens acute myocard-

ischemie is slechts één aspect van dit syndroom. Myocardischemie heeft hiernaast invloed op vrije radikalen vorming, op leucocyten, thrombocyten en daarmee verwante stoffen zoals leukotrienen en prostaglandines. Bovendien dient de mogelijkheid dat locale of circulerende neurohormonen of/en verwante systemen geactiveerd raken gedurende myocardischemie, in ernstige overweging genomen te worden. Stimulering kan een systemisch of coronaire vasoconstrictoire werking veroorzaken en daarmee het ischemisch proces verergeren.

In tegenstelling tot aantoonbare neurohumorale activatie tijdens het gevorderde stadium van hartfalen of gedurende een myocardinfarct, zijn er weinig gegevens beschikbaar ten aanzien van het effect van myocardischemie op de diverse neurohumorale systemen. Beschikbare informatie betreft uitsluitend circulerende en cardiale catecholamines. Hoewel de tendens is dat activatie niet optreedt, zijn de meningen tot nu toe niet eensluidend. Het aspect van neurohumorale activatie gedurende kortdurende perioden van myocardischemie bij patienten die alleen coronairlijden hebben zonder hypertensie of decompensatio cordis, heeft de laatste jaren onze bijzondere aandacht gehad. Met behulp van de atriale pacing stress test als model werd duidelijk aangetoond dat tijdens ischemie een significante stijging optreedt van arteriële norepinephrine en epinephrine spiegels met tegelijkertijd stimulering van het circulerend renine-angiotensine systeem, zich uitend in significante stijgingen van de arteriële angiotensine II-spiegels. Deze neurohumorale activatie is afhankelijk van de mate van ischemie en wordt niet duidelijk bepaald door de aanwezigheid van angina pectoris. Bovendien blijkt uit deze studies dat het normaal aanwezige netto verlies van norepinephrine uit het hart verandert in netto opname gedurende de test in het ischemische gedeelte van het hart. Het klinische belang van deze waarnemingen lijkt gelegen in het feit dat er tegelijkertijd een significante toename is van de systemische vaatweerstand en de arteriële drukken. Een causaal verband kon, bij afwezigheid van specifieke antagonisten, in deze studies niet met zekerheid aangetoond worden, doch lijkt aannemelijk in het licht van de aanwezige tijdscorrelatie; de neurohumorale- en vaatweerstands-veranderingen treden gelijktijdig op en persisteren tot 5 minuten na de test. Eveneens geldt voor beide een duidelijke relatie met de mate van ischemie.
De toegenomen vaatweerstand beïnvloedt ongetwijfeld de wandspanning van de linker kamer en daarmee de zuurstofbehoefte van het myocard. Dit heeft tot gevolg dat de reeds aanwezige ischemie alleen maar versterkt wordt. Een verergering van ischemie kan eveneens optreden secundair aan coronair-vasoconstrictoire werkingen ten gevolge van de toegenomen catecholamine-opname in het ischemisch gedeelte van het hart. Dat adrenerge modulatie van de coronaire vaatweerstand kan optreden gedurende door pacing-geïnduceerde ischemie wordt gesuggereerd door onze waarnemingen dat een hoge dosering labetolol de normaal optredende afname in coronaire vaatweerstand tegengaat, in tegenstelling tot een lage dosering. Dit effect berust waarschijnlijk op de relatief meer uitgesproken betablokkerende eigenschappen van de hogere dosering labetalol en resulteert in een vermindering van het antiischemische effect van deze stof in deze dosering, vergeleken met de lage dosering. Dit ondanks het feit dat met de hoge dosering labetalol de zuurstofbehoefte van het hart sterker wordt verminderd dan met een lage dosering. Deze bevindingen, enerzijds de versterkte neurohumorale activatie tijdens ischemie en anderzijds de gelijktijdige systemische en mogelijk coronaire vasoconstrictie, suggereren dat interventies, gericht op specifieke modulering van deze neurohumorale veranderingen, van therapeutisch belang kunnen zijn bij de behandeling van myocardischemie.

**Hoofdstuk XI.**

In het laatste hoofdstuk van dit proefschrift wordt deze suggestie nader onderzocht. In het licht van de verhoogde arteriële angiotensine II-spiegels naast de catecholaminen-activatie tijdens acute myocardischemie werd een onderzoek uitgevoerd naar de mogelijke antiischemische eigenschappen van een angiotensine-converterende enzyme remmer, enalaprilat. Behoudens een modulerend effect op neurohumorale activatie gedurende ischemie, zijn een aantal theoretische overwegingen aanwezig, die het concept dat dergelijke middelen antiischemische eigenschappen kunnen bezitten, ondersteunen. Behoudens remming van circulerend en locaal angiotensine II-vorming beïnvloeden deze middelen bradykinine afbraak, prostaglandine-synthese en sympathische adrenerge activiteit. Op basis van intrinsiek hemodynamische eigenschappen zoals negatief inotrope effecten en systemische vaatverwijding, naast potentieel coronair vaatverwijdende effecten is een gunstige beïnvloeding van de verhouding myocardiale zuurstofbehoefte versus -voorziening te verwachten. Desondanks zijn de bevindingen in patientenstudies ten aanzien van antiangineuze eigenschappen niet hoopgevend. Deze betreffen alle vrij kleine, merendeels ongecontroleerde studies in ambulante patienten met grotendeels geringe tot matige inspanningsgebonden angina pectoris. Hoewel sommige van deze studies een zekere mate van antiischemische activiteit claimen van de

betreffende converterende enzymremmer, is het totaalbeeld in deze niet hoopgevend. Onze eigen studie daarentegen laat een zeer duidelijk effect zien op pacing-geïnduceerde ischemie. De studie werd uitgevoerd tijdens twee identieke atriale pacing stress tests met een interval van een uur, waarbij enalaprilat werd toegediend 15 minuten voor de tweede stress test. In tegenstelling tot duidelijk aanwezige neurohumorale activatie en verhoogde arteriële drukken gedurende de eerste, onbehandelde test, waren deze totaal afwezig bij de tweede belastingstest na enalaprilat. Bovendien bleek enalaprilat een significante vermindering van de myocardischemie teweeg te brengen. De basis voor deze antiischemische eigenschappen, bij afwezigheid van veranderingen zowel in het zuurstofaanbod als de zuurstofbehoefte van het hart, kan alleen verklaard worden door de afwezige neurohumorale activatie gedurende de tweede pacing stress test en de daarmee gepaard gaande normalisering van de arteriële drukken. Op deze manier werd indirect een vermindering van de zuurstofbehoefte van het myocard bereikt. Bovendien bestaat de mogelijkheid, dat een locaal modulerend effect op coronaire vaten in het ischemische gebied bijdroeg aan de antiischemische werking van deze ACE-remmer, daar enalaprilat de versterkte norepinephrine-opname in het ischemische gedeelte van het hart eveneens tegenging. De resultaten van deze studie benadrukken het belang van systemische en cardiale neurohumorale activatie gedurende ischemie en het potentiële belang van specifiek neurohumoraal modulerende agentia, zoals ACE-remmers en alpha-adrenerge antagonisten bij de behandeling van myocardischemie.

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