Aspects of Myocardial Infarction-induced Remodeling relevant to the Development of Heart Failure

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Aspects of Myocardial Infarction-induced Remodeling relevant to the Development of Heart Failure

Aspecten van remodeling na een myocardinfarct relevant voor het ontwikkelen van hartfalen

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CONTENTS

ABBREVIATIONS

ACE:	Angiotensin I converting enzyme
Ad:	Adenosine
Asp:	Aspirin
AT:	Angiotensine II
ATP:	Adenosine Triphosphate
AVP:	Arginine Vasopressine
BL:	Baseline
BW:	Body Weight
Cap:	Captopril
CF:	Coronary Flow
CO:	Cardiac Output
CSA:	Cross-Sectional Area
CVP:	Central Venous Pressure
dP/dt:	Pressure Change per unit time
ECG:	Electrocardiogram
ECT:	Early Captopril Treatment
ET:	Ejection Time
HP:	Heart Period
HR:	Heart Rate
HWW:	Heart Wet Weight
ip:	Intraperitoneal
IP:	Isoproterenol
LV:	Left Ventricle
LVFW:	Left Ventricular Free Wall
MAP:	Mean Arterial Pressure
MI:	Myocardial Infarction
MP:	Methylprednisolone
mRNA:	Messenger Ribonucleic Acid
NPR:	Nitroprusside
NS:	Not Significant
NSAID:	Non-steroid Anti-inflammatory Drug
PG:	Prostaglandin
RV:	Right Ventricle
SR:	(Picro) Sirius Red
TPR:	Total Peripheral Resistance
Tx:	Thromboxane
W/L:	Wall to Lumen Ratio
WT:	Wall Thickness

CHAPTER 1

GENERAL INTRODUCTION

THE STRUCTURAL RESPONSE OF THE HEART TO MYOCARDIAL INFARCTION AND ITS RELATION WITH HEART FAILURE

EPIDEMIOLOGY OF HEART FAILURE

Heart failure can be defined as the pathophysiological state in which the pump function of the heart is insufficient to meet the metabolic demands of the body (Guyton, 1986; Ruggie, 1986). Thus, heart failure is a pathophysiological condition (rather than a disease per se), and can occur in the course of a number of cardiovascular diseases. In Western countries, hypertension and coronary artery disease or a combination of both account for the majority of cases of heart failure (McKee & Castelli, 1971; Kannel & Castelli, 1972; Eriksson & Svardsudd, 1989). Cardiomyopathies (of genetic, viral, toxic or idiopathic origin) and congenital heart disease are other important etiological factors (Eriksson & Svardsudd, 1989). In developing countries, acquired abnormalities of heart valves due to the sequelae of streptococcal infection are a common cause of heart failure (Killip, 1985).

The incidence of heart failure gradually increases with age, resulting in an incidence rate of 10/1000 per year for men, and 8/1000 per year for women (McKee & Castelli, 1971; Kannel & Castelli, 1972; Ho *et al.*, 1993) in the over-65 age group. A prevalence of 10% has been reported for Sweden in the same age group (Eriksson & Svardsudd, 1988, 1989). The incidence of heart failure has increased 3-fold over the last 15 years (Garg *et al.*, 1993), which can probably be attributed to increased life expectancy in general, and to improved management of coronary heart disease and hypertensive heart disease, which are both associated with an increased risk of sudden cardiac death (Kannel & Schatzkin, 1985). Pharmacological management of hypertension (Natsume, 1993) and coronary artery disease (Carbajal & Deedwania, 1995) has been greatly improved, and the introduction of coronary care units (CCUs) (Hildebrandt *et al.*, 1994) and thrombolytic agents (Stevenson *et al.*, 1993; Simoons, 1995) have rapidly reduced early mortality after myocardial infarction.

Patients with heart failure can present the following symptoms: i) dyspnoea on exertion, which can progress to dyspnoea after moderate exercise to finally dyspnoea even in rest, ii) sudden onset or rapidly progressive dyspnoea, with orthopnoea and paroxysmal nocturnal dyspnoea, iii) general symptoms like fatigue, anorexia or cachexia, iv) ankle oedema or abdominal distension due to fluid retention (Davies & Bayliss, 1994). Pharmacological management of congestive heart failure includes the use of diuretics,

digitalis glycosides, nitrates, ACE inhibitors, and intravenous inotropic therapy (catecholamines, dopamine receptor agonists and phosphodiesterase inhibitors (Davies & Bayliss, 1994). Heart failure is a severe condition, with 50% mortality within 5 years of onset of symptoms, and even 50% mortality at 1 year for patients in the most severe symptomatology group (NYHA class III/IV) (Matoba & Matsui, 1990; Franciosa & Wilen, 1983; Cohn & Rector, 1988; Aronow & Ahn, 1990). Despite the successful introduction of angiotensin I converting enzyme (ACE)-inhibitors (Sigurdsson & Swedberg, 1995), prognosis of cardiac failure remains poor. Therefore, better understanding of the pathophysiological mechanisms in heart failure and the consequences of therapeutical intervention is needed.

MYOCARDIAL INFARCTION AND HEART FAILURE

Coronary artery disease, often resulting in myocardial infarction and thus loss of contractile myocardium, is a common cause of heart failure (McKee & Castelli, 1971; Kannel & Castelli, 1972). In the SOLVD (Studies on Left Ventricular Dysfunction) trials, ischemic heart disease was even identified as the most frequent cause (Bangdiwala *et al.*, 1992; Bourassa *et al.*, 1993). Loss of contractile tissue after myocardial infarction (MI) can severely affect pump function of the left chamber of the heart, mainly depending on the exact magnitude of myocardial necrosis (Mathey *et al.*, 1974). Infarcts affecting 40% or more of the left ventricle lead to severe cardiac dysfunction, resulting in sudden cardiac death or acute congestive heart failure (Page *et al.*, 1971). In contrast, smaller infarctions may be initially associated with only minor alterations of hemodynamic parameters because of compensatory mechanisms.

Acute myocardial infarction evokes activation of compensatory mechanisms including: **i) Stretch-modulated enhancement of cardiac performance** at increased preload, the Frank-Starling phenomenon (Gordon *et al.*, 1966). **(ii) Increased sympathetic nervous system activity**, leading to increased contractility of the heart and arteriolar vasoconstriction in most vascular beds (Remme, 1986). Plasma catecholamine levels are increased early after MI (McAlpine *et al.*, 1988), indicating sustained sympathetic nervous system activation (Esler *et al.*, 1990). High local concentrations of catecholamines in the

cardiac interstitium may occur through release from adrenergic nerve terminals, which can result in myocardial necrosis even in the non-infarcted segments of infarcted hearts (Schömig, 1990). After the acute phase, high catecholamine levels are associated with adverse prognosis (Swedberg *et al.*, 1990). **iii)** Stimulation of the **renin-angiotensin-aldosterone system** (RAAS) after MI (Vaughan *et al.*, 1990; Rouleau *et al.*, 1993) results in increased levels of angiotensin II (Vaughan *et al.*, 1990; Rouleau *et al.*, 1993), which in turn acts as a potent vasoconstrictor. Secondly, it facilitates noradrenaline release during sympathetic stimulation. Thirdly, it leads to increased release of aldosterone (Vaughan *et al.*, 1990; Rouleau *et al.*, 1993), which promotes retention of sodium and water (Francis, 1985; Parmley, 1985; Laragh, 1986). **iv)** Increased production of **arginine-vasopressin** (AVP, or anti-diuretic hormone) by the pituitary gland (Rouleau *et al.*, 1994). Vasopressin inhibits diuresis and is a potent vasoconstrictor (Heyndrickx *et al.*, 1976). **v) Endothelin.** Hypoxia can trigger release of endothelin from vascular endothelium, which results in increased plasma levels in the first day after MI (Lechleitner *et al.*, 1993). Endothelin acts as a strong coronary vasoconstrictor and as a mitogen for vascular smooth muscle cells. In addition, it exerts a positive inotropic effect and can inhibit renin release from the juxta-glomerular cells (Lechleitner *et al.*, 1993). **vi) Natriuretic peptides.** In response to atrial stretching, atrial natriuretic peptide (ANP) is released from granules in atrial myocytes (Lerman *et al.*, 1993), and exerts diuretic and natriuretic effects in the kidneys. Hence, ANP is a functional antagonist of vasopressin and angiotensin II.

Immediately activated mechanisms (increased sympathetic outflow and the Frank-Starling phenomenon) are sustained by neurohumoral activation, which is followed by a structural response of the heart. All structural alterations in cardiac architecture, at organ as well as at tissue and cellular level, are referred to as myocardial 'remodeling'. Although cardiac remodeling occurs as a structural response to the increased hemodynamic load on the surviving myocardium, it often fails to prevent the development of heart failure.

In the rat MI model, in which large transmural infarctions are induced by surgical permanent occlusion of the left coronary descending artery (Selye *et al.*, 1960), gradual deterioration of cardiac pump capacity has been described by DeFelice and collaborators (1989): Cardiac output index was depressed but stable in the period from 7 to 20 weeks

after MI, whereas this parameter became markedly depressed in the period from 20 to 35 weeks. The underlying mechanisms which are involved in the transition from left ventricular dysfunction to heart failure are poorly understood. Different aspects of post MI remodeling will now be discussed, as well as their possible role in the transition from post myocardial infarction LV dysfunction towards heart failure.

MYOCARDIAL INFARCTION-INDUCED CARDIAC REMODELING

Gross morphological changes

Large myocardial infarctions result in alterations in the left ventricular architecture, at organ as well as at tissue and cellular level. These alterations are referred to as myocardial 'remodeling'. The shape and size of the injured left ventricle is changed as a result of remodeling of the ischemic area as well as the normally perfused region (Figure 1.1).

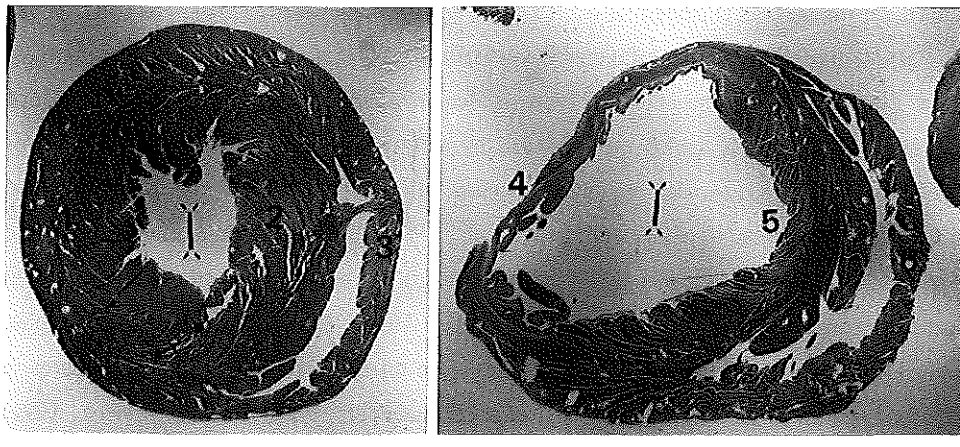

Figure 1.1: Gross morphological changes due to coronary artery ligation in the rat. View at low magnification of haematoxylin-eosin stained sections cut perpendicular to the longitudinal axis, halfway between the base and the apex of the heart. The bar represents 1 mm. Left: sham-operated heart, 1=left ventricular free wall, 2=interventricular septum, 3=right ventricle. Right: ligated heart, 4=infarcted area, 5=interventricular septum, 6=right ventricle. The infarcted heart shows thinning of the infarct, eccentric hypertrophy and dilation of the left chamber.

i) Remodeling of the occluded region. During the first hours after coronary artery occlusion, loss of cellular integrity as well as signs of acute inflammation including interstitial edema can be observed in the infarcted region (Fishbein *et al.*, 1978a, 1978b). After an initial phase of collagen degradation (Takahashi *et al.*, 1990; Cleutjens *et al.*, 1995), fibroblast proliferation and collagen deposition occur to form scar tissue as the eventual replacement of cardiomyocytes. Thinning and elongation of the infarcted segment has been described by Hutchins and Bulkley (1978), and is called 'infarct expansion' and may contribute to LV dilation in the early phase after MI. Infarct expansion is a consequence of slippage between cardiac muscle bundles, resulting in a reduced number of myocytes across the infarcted wall, in the period before resorption of necrotic myocardium (Weisman *et al.*, 1988). Cell stretch and loss of interstitial space contribute less to infarct expansion. Reduction of myocyte cell number across the infarcted wall could also be attributed to myocyte programmed cell death, apoptosis. The group of Anversa has investigated this phenomenon in detail, and concluded that apoptosis, rather than necrotic cell death, was the major form of cell death after myocardial infarction (Kajstura *et al.*, 1996). The entire process is completed within weeks to months depending, amongst other factors, on the species (Fishbein *et al.*, 1978a, 1978b).

ii) Remodeling of non-infarcted myocardium. Another process which contributes to acute post MI left ventricular dilation is lengthening of non-infarcted segments of contractile myocardium. In MI rats (induced by surgical coronary artery ligation), side-to-side slippage of ventricular myocytes occurs in the first days after infarction and contributes to LV enlargement (Olivetti *et al.*, 1990). After the acute phase, eccentric hypertrophy of myocytes (increase of myocyte length by addition of sarcomeres in series) further expands the left ventricle (Olivetti *et al.*, 1991). Besides eccentric hypertrophy, concentric growth of myocytes (increase of myocyte thickness by addition of sarcomeres in parallel) occurs in the non-infarcted part of the injured left ventricle (Olivetti *et al.*, 1991) (Figure 1.2). Although hypertrophy is regarded to be a compensatory response of the non-infarcted myocardium to an increased hemodynamic load, it is still unclear why it often postpones rather than prevents the development of heart failure. Olivetti *et al.* (1991) postulated that cardiac remodeling is an inadequate response to the increased hemodynamic

load on the spared myocardium. In their perspective, eccentric hypertrophy would only further increase diastolic wall stress by increasing chamber radius (law of Laplace, T=P*r/2h: wall stress equals intracavitary pressure times radius, divided by 2 times the wall thickness; Meerson, 1983). The remaining high wall stress would subsequently lead to further eccentric hypertrophy, and a vicious circle leading to heart failure would follow. In an enlarged left chamber, however, diastolic wall stress would only remain elevated if diastolic filling pressure would not be substantially reduced by the larger intraventricular volume (law of Laplace). In contrast to the idea of Olivetti's group, reactive hypertrophy can be considered as an adequate response, though not sufficient to normalise cardiac load. Elevated diastolic pressure despite the enlarged LV cavity could indicate functional defects of the hypertrophied remodeled myocardium leading to maintained neurohumoral activation and thus increased filling of the cardiovascular system (Watkins *et al.*, 1976). Thus, progressive LV dilation may be the victim rather than the cause of persistent pump failure of the heart. Therefore, it is useful to further study cellular and biochemical features of remodeled non-infarcted myocardium.

In MI rats, progressive LV dilation has been documented as increasing rightward shift of volume-pressure relations of diastolically arrested hearts, from 2-106 days after infarction (J. Pfeffer, 1991). Similar to experimental MI in rats, in explanted hearts from patients undergoing heart transplantation because of end-stage heart failure as a result of chronic coronary artery disease, eccentric hypertrophy became evident from a 4.6-fold expansion of LV cavity volume concomitant with a decreased mass-to-chamber volume ratio (Beltrami *et al.*, 1994). In MI patients, progressive ventricular dilation has been described, especially after infarction of the anterior LV wall (Warren *et al.*, 1988; Gadsbøll *et al.*, 1989), and has already early been recognized to be related to adverse clinical outcome (Shanoff *et al.*, 1969; Kostuk *et al.*, 1973).

Remodeling after myocardial infarction: the myocytes

Muscle cells constitute approximately 85% of ventricular tissue volume, and myocyte growth is therefore a major determinant of changes in ventricular dimensions resulting from an increased volume, pressure or combined volume/pressure load on the myocardium often in combination with hormonal stimulation (Anversa *et al.*, 1986b). Myocytes lose the

ability to divide, shortly after birth (Zak, 1974; Ueno *et al.*, 1988), although myocyte polyploidy through nuclear division has been reported in hypertrophied hearts (Anversa *et al.*, 1990; Olivetti *et al.*, 1994; Quaini *et al.*, 1994; Reiss *et al.*, 1994; Liu *et al.*, 1995), and even the possibility of myocyte hyperplasia has been suggested (Olivetti *et al.*, 1994). After myocardial infarction, compensatory growth of spared myocardium therefore occurs through an increase in size of the remaining viable myocytes (Anversa *et al.*, 1985a, 1986b). In rats with large infarctions, at 1 month after coronary artery ligation, myocytes in the region bordering the infarct had enlarged by 81% (33% increase in length and 17% in transverse diameter), and myocytes in spared LV myocardium remote from the infarct had hypertrophied by 32% (19% increase in length and 5% in transverse diameter). Similarly, in explanted hearts from patients with end-stage ischemic cardiomyopathy, there was a 2-fold increase of myocyte cell volume per nucleus, through a 16% increase in myocyte diameter and a 51% increase in length (Beltrami *et al.*, 1994).

Myocyte cell growth can be directly induced by stretch of the cell membrane (Komuro *et al.*, 1991; Yazaki & Komuro, 1992), and be further stimulated by different growth-promoting molecules like angiotensin II (Schunkert *et al.*, 1995), noradrenaline (Simpson & McGrath, 1983), endothelin (Sugden *et al.*, 1993), and different peptide growth factors (Bogoyevitch *et al.*, 1995; Decker *et al.*, 1995; Florini & Ewton, 1995; Palmer *et al.*, 1995).

Although cardiac growth after MI has the potential to restore the amount of contractile myocardium, the increased volume per cell is parallelled by a number of mechanical and biochemical alterations of myocytes and surrounding tissue that may be involved in the ultimate hemodynamic deterioration towards cardiac failure (Figure 1.2): i) The mean **oxygen diffusion distance** is increased due to reduced cardiomyocyte surface to intracellular volume ratio, which is further aggravated by increased interstitial tissue volume and decreased capillary density (Meerson, 1983; Anversa *et al.*, 1984, 1985b, 1986a; Olivetti *et al.*, 1991), which is most pronounced in the area bordering the infarct zone (Olivetti *et al.*, 1986). Cellular hypoxia may be detrimental, by limiting the ATP production by mitochondrial oxidative phosphorylation in already functionally stressed myocytes. In addition, like tissue stretching (Cheng *et al.*, 1995), cellular hypoxia might

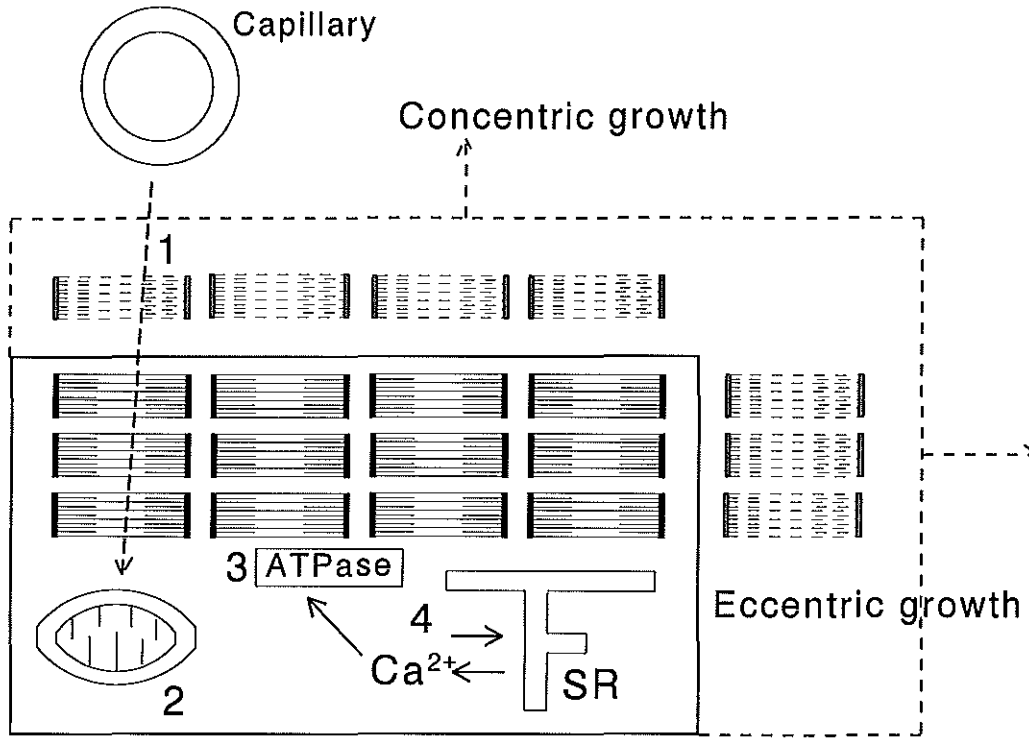

Figure 1.2: Increase of cardiomyocyte volume through addition of sarcomeres in series (eccentric growth) or in parallel (concentric growth), increasing cellular length and width, respectively. Increased cell volume can be accompanied by: 1) increased oxygen diffusion distance for oxygen from the capillaries to the mitochondria, 2) decreased ratio of mitochondria and myofibril number, 3) decreased myofibrillar ATPase activity, 4) impaired calcium handling.

trigger apoptosis (Tanaka *et al.*, 1994), which would further reduce the number of viable myocytes. Cellular loss would then demand further hypertrophy of remaining myocytes, resulting in a vicious circle of events leading to heart failure. **ii)** The ratio of **mitochondria to myofibril number** is decreased (Anversa *et al.*, 1986a), indicating imbalance between ATP generating and ATP consuming processes. Indeed, in spared hypertrophied myocardium of MI hearts, energy reserve (intracellular levels of high-energy phosphates) is

reduced (Neubauer *et al.*, 1995), and mitochondrial oxygen consumption rate is decreased (Sanbe *et al.*, 1995). In remodeled hearts at 2 months after MI, enhanced anaerobic metabolism was suggested by increased lactate dehydrogenase (LDH) activity and impaired creatine kinase (CK) activity in the residual contractile tissue bordering the infarct (Laser *et al.*, 1996). **iii) Ca^{2+} handling** in hypertrophied myocytes is impaired (Litwin & Morgan, 1992). Recent work in non-infarcted hypertrophied rat myocardium from MI hearts showed a significant reduction of ATP-dependent Ca^{2+} uptake activity of sarcoplasmic reticulum membrane fractions, which was most pronounced in the surviving myocardium bordering the infarcted area (van Heugten *et al.*, 1996). In the latter study, switch to the hypertrophic (fetal) phenotype was illustrated by increased mRNA levels of atrial natriuretic factor (ANF) in ventricular myocardium, in parallel with the regional degree of reactive hypertrophy. In addition, mRNA for the sarcoplasmic reticulum membrane calcium pump and phopholamban (a protein regulating the activity of the sarcoplasmic Ca^{2+} pump) mRNA levels were slightly reduced. In addition, Ca^{2+} sensitivity of myofibrils from hypertrophied myocardium have been reported to be reduced (Cheung *et al.*, 1994). These biochemical alterations of ventricular myocytes are reflected by impaired contraction (Litwin *et al.*, 1991a, 1991b, 1995; Litwin & Morgan, 1992; Cheung *et al.*, 1994; Kramer *et al.*, 1996; Melillo *et al.*, 1996) and relaxation (Litwin *et al.*, 1991a) of hypertrophied, non-infarcted myocardium. **iv) ATPase activity of the contractile apparatus** within the cardiomyocyte is decreased. In rat myocardium, ATPase activity is lower due to a switch to the slower myosin isoform (V_3 myosin isoenzyme) (Geenen *et al.*, 1989), which was especially pronounced in the viable part of the infarcted LV free wall. In human failing left ventricle, a switch to troponin T isoform TnT_2 was inversely related to myofibrillar ATPase activity (Anderson *et al.*, 1992). **v) Responsiveness to sympathetic nervous system drive** may be impaired due to down-regulation of ß-adrenergic receptor number and reduced intracellular transmission of the signal, although this remains an area of controversy: Both down-regulation (Warner *et al.*, 1992; Kozlovskis *et al.*, 1990) and up-regulation (Clozel *et al.*, 1987) as well as unchanged number (van Veldhuisen *et al.*, 1995) of ß-adrenoceptors in non-infarcted myocardium have been reported. Similarly, adenylate cyclase activity of the hypertrophied, spared myocytes has been observed to be impaired (Warner *et al.*, 1992) or

enhanced (Kozlovskis *et al.*, 1990). Despite these conflicting reports, there is consensus about the concept of a reduced ß-adrenergic receptor-mediated inotropic response, once left ventricular dysfunction has deteriorated to heart failure (Bristow *et al.*, 1990). A complication of the use of human myocardial tissue obtained at cardiac transplantation would be the confounding effect of pharmacological inotropic support in the period before transplantation.

It is still unclear how the different changes at the cellular level within the myocyte are interrelated. The increased diffusion distance for oxygen may be the initiating stimulus for the aforementioned biochemical changes, since it can result directly from an increase of myocyte volume. Relative oxygen deficit of the myocyte, in combination with a relative impaired oxidative phosphorylation capacity (decreased number of mitochondria per myofibril, Anversa *et al.*, 1986a) could be related to the decreased potential of generating high-energy phosphates and result in a number of changes affecting the contractile apparatus of the cardiomyocyte, such as lower ATPase activity of myofibrils and other energy-requiring processes.

Remodeling after myocardial infarction: the cardiac interstitium

About 15% of the myocardium volume is occupied by tissue space between the cardiomyocytes (Anversa *et al.*, 1986b). Despite the relatively small volume, about two third of the cells of the heart are found in the cardiac interstitium: mainly fibroblasts and vascular cells (endothelial and smooth muscle cells), and to a lesser extent mast cells and macrophages. In the extracellular compartment of the cardiac interstitium a network of small fibers consisting of type I and III collagens connects cardiomyocytes to other cardiomyocytes, cardiomyocytes to small blood vessels, and cardiomyocytes to large collagen fibers. Fibrillar collagens have a high rigidity and are extremely resistant to proteolytic digestion (Werb, 1982). The collagen network helps to maintain cellular architecture of the myocardium, under the wall stress generated by a much higher intracardiac than extracardiac pressure. Collagen types I and III are produced by interstitial fibroblasts, which also produce other extracellular matrix proteins, such as collagenase (Bashey *et al.*, 1992; Brilla *et al.*, 1992; Eghbali, 1992), as well as glycosaminoglycans, glycoproteins and microfibrillar proteins and elastin.

Fibroblasts produce procollagen molecules, which can form collagen triple helices, through a process of maturation and formation of (intra- and intermolecular) cross-links (Stryer, 1981). Hormonal and mechanical factors that can induce fibroblast proliferation and/or increased fibroblast synthesis of extracellular matrix proteins have been reviewed by Booz & Baker (1995), and include: Angiotensin II, aldosterone, endothelin, peptide growth factors (transforming growth factor-$ß_1$, TGF-$ß_1$; platelet-derived growth factor, PDGF; basic fibroblast growth factor, bFGF), certain prostaglandins (PGE_1, PGE_2, $PGF_{2\alpha}$; Otto *et al.*, 1982) and mechanical stretch (Sadoshima *et al.*, 1992). Fibroblast recruitment can reflect a reactive process to hormonal stimuli and mechanical stress (without cardiomyocyte necrosis: 'interstitial fibrosis') or cardiac reparation after cardiomyocyte necrosis ('replacement fibrosis') (Weber *et al.*, 1989).

Degradation of extracellular matrix may be required under certain normal and pathological conditions to allow growth and development, angiogenesis and wound healing (Murphy & Reynolds, 1993). Fibrillar collagen can be cleaved by specific matrix metalloproteinases (Woessner, 1991; Murphy & Reynolds, 1993), and further degraded by other extracellular matrix enzymes (Woessner, 1991), or by intracellular collagen degradation in fibroblasts and macrophages (Everts *et al.*, 1985; Beertsen, 1987). In summary, the state of the collagenous network is the result of a slow, but continuous turn-over, consisting of formation, maturation and cross-linking, and extra- and intracellular degradation. In different forms of cardiac remodeling, the equilibrium between anabolic and catabolic processes can alter in either direction. Substantial increases of fibrillar collagen content, which have been described in pressure overload-induced hypertrophy, may result in decreased myocardial compliance (Jalil *et al.*, 1989; Conrad *et al.*, 1995). On the other hand, enhanced collagen degradation has been associated with side-to-side slippage of cardiomyocytes and consequently with chamber dilation (Whittaker *et al.*, 1991). Indeed, pharmacological inhibition of collagen cross-linking (ß-aminopropionitrile) resulted in a larger left ventricle and decreased myocardial stiffness (Kato *et al.*, 1995).

Myocardial ischemia, such as occurs after coronary artery occlusion, results in enhanced activities of collagenases and other proteinases, associated with rapid degradation of the extracellular matrix (Takahashi *et al.*, 1990; Cleutjens *et al.*, 1995). Compromised

tissue integrity following damage to the collagenous framework leads to side-to-side slippage of myocytes in the infarcted wall and contributes to wall thinning and infarct expansion (Hutchins & Bulkley, 1978; Weisman *et al.*, 1988; Olivetti *et al.*, 1990; Whittaker *et al.*, 1991). Exaggerated lengthening of infarcted segments can result in aneurysm formation (occurring in 10-15% of MI patients, and associated with 50% mortality at 5 years, Meizlish *et al.*, 1984; Keenan *et al.*, 1985) or increased risk of rupture of the infarcted wall, and is increased at higher afterload (Connelly *et al.*, 1991; Jugdutt *et al.*, 1996).

Inflammatory response to cell death after coronary artery occlusion in rats becomes evident by cellular infiltration (neutrophils followed by lymphocytes and plasma cells) progressing from the border zone into the more central areas of the infarct (Fishbein *et al.*, 1978a). Other signs of inflammation are present, such as interstitial oedema and vascular congestion. Simultaneous to the inflammation, increasing numbers of fibroblasts occur around, and later in the infarcted myocardium, representing the initial phase of tissue repair. Fishbein and collaborators observed similar histopathological changes in autopsy material from patients died of MI, although the evolution of the response appears to be slower in man than in rats (Fishbein *et al.*, 1978b). In the granulation tissue that surrounds the infarcted tissue, myofibroblasts (fibroblast-like cells which are positive for α-smooth muscle actin) appear (Sun & Weber, 1996) which express mRNA for angiotensin I converting enzyme (Sun & Weber, 1996). These cells may have a role in giving extra tensile strength to the infarcted area (Willems *et al.*, 1994) or even scar contraction. Collagen deposition and gradual resorption of necrotic cells (Fishbein *et al.*, 1978a, 1978b) result in the formation of scar tissue which is less compliant than non-infarcted myocardium (Connelly *et al.*, 1991).

In addition to infarct expansion, side-to-side slippage of cardiomyocytes has been described in the non-infarcted part of the left ventricle (Olivetti *et al.*, 1990), which would contribute to acute post MI chamber dilation. However, in contrast to the infarcted area, no changes in collagenolytic activity were reported in surviving myocardium (Cleutjens *et al.*, 1995). On the other hand, recently it has been reported that the inflammation (with a potential increase in collagenolytic activity, Everts *et al.*, 1985) that follows coronary artery

occlusion is not limited to the infarcted area, but involves infiltration by inflammatory cells of viable myocardium as well (Sulpice *et al.*, 1994). Therefore, it remains unclear whether the reported decreased myocyte cell number across the non-infarcted wall is associated with damage to the collagen network or with cell loss. After the acute phase, proliferation of fibroblasts in the interstitium of non-infarcted myocardium has been reported (Smits *et al.*, 1992), associated with 2 to 3-fold increase in interstitial collagen at 2 weeks (Smits *et al.*, 1992) and at 13 weeks (McCormick *et al.*, 1994) after MI. The interstitial collagen accumulation was strongest in the viable myocardium bordering the scar, where collagen cross-linking was enhanced as well (McCormick *et al.*, 1994). Similar to these studies in rats, in autopsy material from MI patients (with 6 weeks- to 13 year-old infarctions), interstitial collagen volume fraction in viable myocardium was increased from 4% to 10% (Volders *et al.*, 1993). Photomicrographs in the latter publication suggest a combination of interstitial and replacement fibrosis in the non-infarcted tissue, implying additional cardiomyocyte loss in the course of the post MI remodeling process. Microinfarcts can result from atherosclerotic occlusion of small coronary arteries, but also as remote cardiomyocyte death early after MI in myocardium which is not perfused by the occluded coronary artery (as demonstrated in a dog model of occlusion-reperfusion induced MI, Corday *et al.*, 1975), or result from catecholamine toxicity (Schömig, 1990) or from the metabolic consequences of pronounced cellular hypertrophy later in the course of post MI remodeling.

Increased collagen content in non-infarcted tissue is associated with increased stiffness, as was demonstrated by Litwin *et al.* (1991b) in viable papillary muscles from rat MI hearts. Interestingly, in the latter study chronic treatment with the angiotensin I converting enzyme (ACE)-inhibitor captopril (0-21 days after coronary artery ligation) prevented reactive hypertrophy but not collagen accumulation in the papillary muscles, which was associated with a lack of effect on papillary muscle stiffness. Thus, these experimental data support the concept that myocardial stiffness is determined by the mechanical characteristics of the collagen weave, rather than by cardiomyocyte hypertrophy. In contrast to the decreased compliance of isolated fibers of non-infarcted myocardium, compliance of the whole left chamber, distorted and heterogenous due to post

MI remodeling, is increased (Raya *et al.*, 1988). Mirsky *et al.* (1983) proposed that left ventricular chamber stiffness was not only determined by myocardial stiffness but also by LV geometry, especially the ratio of ventricular cavity to wall volume.

Beltrami and coworkers (1994) have investigated the histopathology of hearts obtained from patients who had to undergo cardiac transplantation because of chronic coronary artery disease in its terminal stage, also referred to as 'ischemic cardiomyopathy'. The authors describe extensive fibrosis in all patients, and define 3 types of fibrosis: Segmental fibrosis (a healed myocardial infarct (>1 cm^2 of myocardium), replacement fibrosis (discrete areas of myocardial scarring developed as a result of focal myocyte loss), and interstitial fibrosis (widening of the interstitial space with collagen accumulation in the absence of apparent myocytolytic necrosis or focal cell death. Ischemic cardiomyopathy in these patients was characterized by healed LV myocardial infarction(s), and by multiple foci of replacement fibrosis and diffuse interstitial fibrosis, which affected the non-infarcted myocardium of both ventricles. This resulted in an increased volume fraction of myocardium that was occupied by interstitium, even in the presence of pronounced cardiomyocyte hypertrophy. It is clear that additional cardiomyocyte loss in non-infarcted myocardium in combination with increased oxygen diffusion pathway length through myocyte hypertrophy and increased interstitial volume are threatening factors for heart function in the course of post MI remodeling.

Remodeling after myocardial infarction: the coronary circulation

Vascular adaptation in the vascular beds which are perfused by the remaining patent coronary arteries after MI is required. The amount of contractile tissue which the patent coronary arteries feed has increased (due to compensatory hypertrophy), and this myocardium has to operate at increased wall stress (due to increased preload) (Olivetti *et al.*, 1991). Therefore, a greater amount of nutrients and oxygen is needed by the spared part of the heart. Increased oxygen delivery to hypertrophied viable tissue can be achieved by increasing resting coronary blood flow through dilation of resistance arteries (Chilian *et al.*, 1986). During further increased oxygen demand, such as during exercise, resistance arteries will quickly reach their fully dilated state. If peak oxygen demand of the hypertrophied viable myocardium exceeds the supply by the fully dilated vascular bed,

ischemic stress will threaten function and viability of myocytes (Hearse, 1990). In addition to this potentially inadequate oxygen supply by the coronary arteries, oxygen availability may also be hampered at a cellular level by increased pathway length for oxygen to diffuse from interstitial capillaries to mitochondria within the cardiomyocyte (Anversa *et al.*, 1985b). Therefore, structural adaptations of the vascular bed perfused by the remaining patent coronary arteries are needed to prevent cellular hypoxia during peak oxygen demand. In addition to meeting the demands of non-infarcted hypertrophied myocardium, angiogenesis is needed to accomplish healing of the infarcted area. Inadequate structural adaptation of the vascular bed to hypertrophy of the surrounding myocytes may play a role in the decompensation from LV dysfunction to heart failure (Vatner & Hittinger, 1993).

Maximal flow through a vascular bed is thought to be determined by the total cross-sectional area (CSA) of the resistance arteries (Gordon, 1974). Therefore, maximal flow through the remaining patent coronary arteries could be achieved by either an increase in size or an increase in number of the resistance arteries of these vascular beds. An increased number of arterioles was concluded from studies on hypertrophied myocardium after experimental pressure-overload in dogs (Tomanek *et al.*, 1989), since arteriolar density in these experiments was unchanged despite prominent myocyte hypertrophy. Arterioles could be formed from preexisting capillaries (Mikawa & Fischman, 1992), and could account for the higher number of arterioles reported by Tomanek and coworkers in pressure overload-induced hypertrophy (1989).

In addition to structural adaptations at the level of resistance arteries, hampered cardiomyocyte oxygenation could also be counteracted at the cellular level by capillary growth. Following myocardial infarction in rats, a marked increase in capillary number has been demonstrated in the margins of the infarcted zone (Fishbein *et al.*, 1978a; Nelissen-Vrancken *et al.*, 1996), which is associated with pronounced ACE expression by endothelial cells (Passier *et al.*, 1995). In autopsy material of patients who died at various time intervals after MI, similar proliferation of small vessels at the margins of the infarct have been demonstrated up to a month after MI (Fishbein *et al.*, 1978b). Tissue extracts of human myocardial infarcts exert pronounced angiogenic activity when applied to the chick chorioallantoic membrane assay (Kumar *et al.*, 1983; Shahabuddin *et al.*, 1985).

The current knowledge about angiogenesis has recently been reviewed by Battegay (1995). In this paper, angiogenesis is defined as the formation of new capillaries from existing microvessels by sprouting (cellular outgrowth). Hypoxia is assumed to drive angiogenesis (Shweiki *et al.*, 1992; Plate *et al.*, 1993; Michenko *et al.*, 1994). In hypoxic conditions, various cell types including inflammatory cells, vascular wall cells and fibroblasts release angiogenic factors. In response to angiogenic stimuli, endothelial cells penetrate the basement membrane, migrate into the direction of the stimulus, and start rapid proliferation. Proteolytic activity in the cardiac interstitium enables migration of endothelial cells (Folkman & Shing, 1992). Finally, endothelial cells organise to form a new capillary tube. Increased number of arterioles have been demonstrated in different experimental models of cardiac hypertrophy (Tomanek *et al.*, 1989; White *et al.*, 1992; Chen *et al.*, 1994), as well as chronic myocardial ischemia (White & Bloor, 1992). Formation of extra arterioles supposedly involves proliferation and migration of vascular smooth muscle cells along old or newly formed capillaries to transform them into arterioles (Mikawa & Fischman, 1992).

In remodeled myocardium, vessel density (number of vessels/mm^2 of myocardium) is the result of 2 processes: angiogenesis (increasing vessel number) and myocyte hypertrophy. In the non-infarcted myocardium of rat MI hearts, capillary angiogenesis is inadequate in maintaining normal tissue capillarization: As soon as 3 days after MI, capillary density (number of capillaries/mm^2 of myocardium) was reduced, and oxygen diffusion pathway lengthened (Anversa *et al.*, 1985b). Both after 4.5 and 6 weeks, similar limitation of capillarization was found in spared, hypertrophied myocardium (Turek *et al.*, 1978; Anversa *et al.*, 1986a, 1986b). It appears that despite the increased cardiomyocyte cell volume, capillary number per myocyte remains unaltered (approximately 1) and, hence, probably insufficient for adequate oxygenation of the hypertrophied cardiomyocytes. Decreased capillary density is especially pronounced in the non-infarcted tissue near the infarct region (Olivetti *et al.*, 1986; Sladek *et al.*, 1996). Data documenting vessel density in viable human myocardium after MI are still scarce. Small studies using autopsy material suggest that both capillary density (Yarom *et al.*, 1992) and arteriolar density (Jantunen & Collan, 1989) are maintained, and even tend to be increased, in the presence of MI-induced

compensatory hypertrophy. Hence, angiogenesis in the vascular beds which supply the hypertrophied myocardium must have occurred. However, it remains unclear if besides MI-induced remodeling, additional ischemia due to coronary artery atherosclerosis of the arteries supplying non-infarcted tissue increases the angiogenic response (White & Bloor, 1992). Moreover, detailed studies about regional arteriolar and capillary density after human MI are not available to date.

In addition to vascular growth, MI-related myocardial remodeling of the spared part of rat hearts is associated with collagen accumulation around small intramural coronary arteries (Sun *et al.*, 1994; Sun & Weber, 1996), which was preceded by collagen type I mRNA expression in fibroblasts or fibroblasts-like cells at these fibrous sites. Studies in spontaneously hypertensive rats treated with the ACE inhibitor lisinopril (Brilla *et al.*, 1991) have demonstrated that regression of perivascular fibrosis does not result in normalization of maximal coronary flow unless it is combined with normalization of thickness of the vascular smooth muscle cell layer. The medial thickening of resistance arteries is thought to be induced by increased perfusion pressure of these vessels, such as occurs during hypertension, and is thus unlikely to play a role in experimental MI, in which blood pressure is not increased (Schoemaker *et al.*, 1991).

SUMMARY

Large myocardial infarction (MI) leads to scar formation, distorted left ventricular (LV) geometry, as well as cellular and biochemical changes in cardiac muscle and interstitium of the viable part of the heart. Structural response to loss of contractile tissue is called cardiac remodeling, and is considered to represent initial adaptation of the heart, but is also thought to be involved in the later deterioration towards heart failure. Thinning and expansion of the infarcted area, in combination with side-to-side slippage of cardiomyocytes in spared tissue result in chamber dilation during the first days after MI. Thereafter, increased hemodynamic load on surviving myocardium leads to compensatory growth of myocytes. Myocytes grow by addition of sarcomeres in series (eccentric growth) and in parallel (concentric growth). Eccentric hypertrophy results in further LV dilation. The interstitium of viable myocardium gains in volume, and after an initial phase of

collagen degradation, collagen synthesis is exaggerated to yield an increased content of collagen fibers, limiting the compliance of the interstitial collagen network. In rat MI hearts, vascular growth is inadequate to maintain normal capillary density of hypertrophied myocardium. Reduced capillary density, greater cardiomyocyte volume and increased interstitial space lead to hampered oxygenation of the cardiomyocytes. The relative limitation to ATP production (less oxygen and less mitochondria) may lead to down-regulation of myofibrillar ATP consumption (lower ATPase), at the expense of the velocity of contraction and relaxation.

AIMS OF THIS THESIS

The studies in this thesis were carried out to identify aspects of myocardial infarction-induced remodeling of non-infarcted tissue that may be involved in the transition from LV dysfunction to heart failure. Our research focused on two areas:

i) The first part of this thesis (Chapters 2-5) sets out to define determinants of tissue perfusion of hypertrophied non-infarcted myocardium, as well as to describe regional variation of this parameter. The consequences for cell metabolism during an additional ischemic period were determined. Finally, a widely used therapy in MI patients, chronic treatment with an ACE inhibitor, was evaluated in MI rats regarding effects on regional tissue perfusion and metabolic response to an additional ischemic period.

ii) The second part of this thesis (Chapters 6-8) deals with interstitial and perivascular collagen accumulation in the spared part of MI hearts. An alternative approach was used: The effects of chronic treatment with low-dose aspirin on collagen accumulation are described. Subsequently, we investigated if altered collagen accumulation could beneficially effect *in vitro* LV compliance and function. Finally, effects of this treatment on *in vivo* cardiac function of MI rats were evaluated.

CHAPTER 2

DETERMINANTS OF CORONARY RESERVE IN RATS SUBJECTED TO CORONARY ARTERY LIGATION OR AORTIC BANDING

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ABSTRACT

We investigated if decreased coronary reserve in rat hearts after coronary artery ligation or aortic banding is related to remodeling of resistance arteries. Maximal coronary flow (absolute flow) and tissue perfusion (flow corrected for heart weight) were determined in isolated perfused rat hearts after intracoronary adenosine or nitroprusside injection, at 3 and 8 weeks after coronary artery ligation or 4-5 weeks after aortic banding. Perivascular collagen and media thickness of resistance arteries were determined by morphometry. Maximal coronary flow of infarcted hearts had been restored to sham values at 3 weeks. Growth of cardiac muscle mass from 3 to 8 weeks exceeded the increase in maximal coronary flow, leading to decreased tissue perfusion at 8 weeks. A slight, transient increase in perivascular collagen, but no media hypertrophy, was found after infarction. After aortic banding, perivascular fibrosis and media hypertrophy led to a decreased maximal coronary flow in both the hypertrophied left and the non-hypertrophied right ventricle. Consequently, perfusion of the left ventricle was most severely reduced. Reduced maximal perfusion after aortic banding is determined by both cardiac hypertrophy and vascular remodeling. In contrast, during infarction-induced remodeling, reduction of perfusion is mainly determined by disproportional cardiac hypertrophy relative to vascular growth rather than by vascular remodeling.

INTRODUCTION

A decreased coronary vasodilator reserve has been described in different animal models of pressure overload-induced cardiac hypertrophy (Mueller *et al.*, 1978; O'Keefe *et al.*, 1978; Tomanek *et al.*, 1985; Canby & Tomanek, 1989; Tomanek *et al.*, 1989; Brilla *et al.*, 1991), and it is recognized to play a role in the transition from left ventricular hypertrophy to heart failure (Vatner & Hittinger, 1993). The flow capacity of the coronary vascular bed (absolute flow) is thought to depend on the total cross-sectional area (CSA) of the resistance vasculature, which can be changed by: i) arteriolar growth during remodeling, increasing CSA (Tomanek *et al.*, 1989), ii) medial layer hypertrophy of resistance arteries, decreasing CSA (Tomanek *et al.*, 1985; Brilla *et al.*, 1991), or iii) loss of functional arterioles, arteriolar rarefaction, decreasing CSA (Prewitt *et al.*, 1982). The resultant change of flow capacity relative to the increase in cardiac mass determines cardiac perfusion (flow per g of muscle mass).

In contrast to pressure overload-induced hypertrophy, data concerning coronary reserve in reactive hypertrophy following myocardial infarction (MI) are relatively scarce (Karam *et al.*, 1990; Drexler *et al.*, 1992; Nelissen-Vrancken *et al.*, 1996). Moreover, it is still unclear if remodeling of resistance arteries, including accumulation of collagen in the adventitia of resistance arteries (Sun *et al.*, 1994), contributes to a decreased maximal cardiac perfusion.

The present study was carried out to investigate whether post MI remodeling can be associated with a decreased coronary reserve, and, if coronary reserve would be impaired, whether this can be related to vascular remodeling of resistance arteries and/or to the stage of cardiac remodeling. Studies were performed using the rat MI model, at 3 weeks, shortly after completion of scar formation (Fishbein *et al.*, 1978a) at the compensated stage of cardiac remodeling, and at 8 weeks when progression into decompensation occurs (J. Pfeffer *et al.*, 1985, 1991). For comparison, hearts from rats with experimental renovascular hypertension were studied (interrenal aortic banding, IRAB). In this model, cardiac hypertrophy was expected in the left but not the right ventricle, whereas pronounced vascular pathology was anticipated in both ventricles (Tomanek *et al.*, 1985).

MATERIALS AND METHODS

Male Wistar rats (270-320 g, Harlan Zeist, The Netherlands) were used in this study. Rats were housed at a 12 h light/dark cycle with standard rat chow and water available ad libitum. The experiments were carried out after approval of the University ethics committee for the use of experimental animals.

Myocardial infarction and interrenal aortic banding models Under pentobarbital (60 mg/kg, i.p.) anesthesia, **left anterior descending coronary artery ligation** was performed (Selye *et al.*, 1960). Briefly, after the trachea was intubated, an incision was made in the skin overlying the 4th intercostal space, with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65/min, tidal volume 3 ml), and the thoracic cavity was opened by cutting the intercostal muscles. The heart was left in situ and a 6-0 silk suture was looped under the left anterior descending coronary artery approximately 2 mm from its origin. The suture was tied except in sham operation (thoracotomy-sham group). Ribs were pulled together with 3-0 silk. Subsequently, the muscles were returned to their normal position, and the skin was sutured. In rats that were randomised for **interrenal aortic banding (IRAB)**, a midline laparotomy was performed under pentobarbital (60 mg/kg, i.p.) anesthesia. The intestines were kept aside with gauzes, and the abdominal aorta was exposed. In the segment between the left and right renal artery, a 23 Gauge needle was positioned alongside the aorta. To make a fixed stenosis, the aorta was tied off together with the needle, except in sham operation (laparotomy-sham group). The needle was then removed, and the abdomen was sutured. Before isolation of the heart, at 4-5 weeks after surgery, polyethylene catheters were inserted into the carotid (PE-50) and femoral (PE-10) artery under pentobarbital anesthesia, and connected to a pressure transducer (Viggo-Spectramed, Oxnard, USA), in order to measure the pressure gradient over the banded aorta segment. To evaluate unilateral renal atrophy due to chronic ischemia, the ratio of left to right kidney weight was determined. Only animals with a left to right kidney weight ratio of <0.9, indicative of left kidney atrophy, were included in analysis.

Coronary vasodilation and distribution of coronary flow Under pentobarbital anesthesia, the heart was rapidly excised and mounted for perfusion with an oxygenated Krebs-Henseleit buffer (composition in mM: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, D-glucose 10; pH=7.4; 37°C) at a constant pressure of 85 mmHg, using the Langendorff technique. Heart rate was kept constant at 350 beats/min by pacing with a Grass stimulator (Viggo-Spectramed, Oxnard, USA). Left ventricular (LV) end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume. Coronary flow was measured by a flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the ostia of the coronary arteries. After a stabilization period of 15 minutes, baseline values were obtained and maximal coronary flow during vasodilation was determined. Adenosine (0.1 ml of a 10^{-2} M solution, Janssen Chimica, Geel, Belgium) was injected into the perfusing buffer just before it entered the coronary arteries, as a fixed dose since baseline coronary flows were comparable for all groups, and maximal coronary flow was measured. After a re-stabilization period, similarly 0.1 ml of a 10^{-2} M sodium nitroprusside solution (University

Hospital Dijkzigt's pharmacy) was injected into the perfusing buffer. These doses of vasodilators were found to induce maximal effect in complete dose-response curves obtained in pilot experiments. Ventricles were weighed after removal of atria and large vessels. In order to investigate the contribution of cardiac hypertrophy, in a separate group of rats subjected to IRAB as described above, regional distribution of coronary flow was determined. The distribution of blood flow was determined with 15 ± 1 (S.D.) μm diameter microspheres labelled with either ^{113}Sn or ^{46}Sc (N.E.N. Dupont, Boston, USA). For each measurement (baseline and nitroprusside-induced maximal vasodilation), a suspension of about 8,000 microspheres, labelled with one of the isotopes, was injected into the perfusing buffer just before it entered the coronary arteries. In pilot experiments, coronary flow after injection of about 25,000 microspheres (10.5 ± 2.4 ml/min, n=5), did not differ from baseline coronary flow (11.4 ± 2.7 ml/min). Radioactivity was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using a suitable window for discriminating the different isotopes. All data were processed by a set of specially designed computer programs (Saxena *et al.*, 1980).

Measurement of perivascular collagen The amount of perivascular collagen was measured in 6 hearts randomly selected from each experimental group (Smits *et al.*, 1992; Brilla *et al.*, 1993). Briefly, the hearts were fixated by perfusion with 3.6% phosphate-buffered formaldehyde. The ventricles were cut into 4 slices from apex to base, after removal of the atria and the large vessels. The slices were kept in formaldehyde for at least 24 hours. After fixation, the slices were dehydrated and paraffin embedded. Deparaffinized 5 μm thick sections were incubated for 5 min with 0.2% (wt/vol) aqueous phosphomolybdic acid, and subsequently incubated for 45 min with 0.1% Sirius Red F3BA (C.I. 35780, Polysciences Inc., Northampton, UK) in saturated aqueous picric acid, washed for 2 min with 0.01M HCl, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany). In each heart, perivascular collagen around 3-5 different resistance arteries (luminal diameter <150 μm) in the right ventricle, as well as 6-10 resistance arteries in the interventricular septum was measured. In infarcted hearts, arteries selected for measurement were located in vital myocardium and did not approximate the border zone of the infarction. The perivascular picrosirius red positive area was corrected for luminal area of the vessel (Brilla *et al.*, 1993).

Measurement of media thickness Deparaffinized sections were incubated for 90 minutes with a resorcine-fuchsine solution at 60°C, and subsequently for 2 minutes with a Van Giesson solution, flushed with alcohol, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany). The tunica media areas of 8-10 resistance arteries (luminal diameter < 150 μm) in interventricular septum and right ventricle were measured and corrected for luminal area. In infarcted hearts, arteries selected for measurement were located in vital myocardium and did not approximate the border zone of the infarction.

Data analysis Results comprise data from 6 to 12 animals per group. Data are expressed as group means ± S.E.M, unless indicated otherwise. Only data from MI hearts with an infarcted area comprising the major part of the LV free wall were included in the study, since smaller infarctions

Table 2.1 Characterization of experimental groups

	SHAM	MI (3 wks)	SHAM	MI (8 wks)	SHAM	IRAB
n	10	10	7	9	12	9
BW (g)	388 ± 11	371 ± 7	417 ± 13	401 ± 11	394 ± 11	363 ± 18
HWW (mg)	985 ± 37	928 ± 22	1080 ± 41	1336 ± 78*	1066 ± 50	1261 ± 71*
HWW/BW ($x10^{-3}$)	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	3.3 ± 0.2*	2.7 ± 0.1	3.5 ± 0.1*
L/R kidney weight					0.99 ± 0.02	0.45 ± 0.11*

MI 3 wks, hearts 3 weeks after myocardial infarction; MI 8 wks, hearts 8 weeks after myocardial infarction; IRAB, hearts from interrenal aortic banded rats at 4-5 weeks after surgery; BW, body weight at end of protocol; HWW, heart wet weight; HWW/BW, heart wet weight to body weight ratio; L/R kidney weight, left to right kidney weight ratio. *: $P<0.05$ versus sham values.

Table 2.2 Ratios of maximal to baseline coronary flow

	SHAM	MI (3 wks)	SHAM	MI (8 wks)	SHAM	IRAB
Adenosine	2.0 ± 0.1	2.0 ± 0.2	2.3 ± 0.4	2.4 ± 0.2	2.5 ± 0.2	2.1 ± 0.1
Nitroprusside	1.7 ± 0.1	1.7 ± 0.2	2.4 ± 0.5	2.6 ± 0.2	2.4 ± 0.2	2.1 ± 0.2

Ratios of maximal coronary flow after intracoronary injection of vasodilators to baseline coronary flow. MI 3 wks, hearts 3 weeks after myocardial infarction; MI 8 wks, hearts 8 weeks after myocardial infarction; IRAB, hearts from interrenal aortic banded rats.

are known to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Effects of MI-induced remodeling were evaluated by comparing data from MI hearts with data from contemporary thoracotomy-sham hearts. Effects of hypertension-induced remodeling were evaluated by comparing data from IRAB-hearts with data from laparotomy-sham hearts. Effects of time in MI-induced remodeling were assessed by comparison of deviation from sham values at 3 weeks and at 8 weeks after surgery. Finally, difference between effects of MI-induced and hypertension-induced remodeling were studied comparing deviation from sham values caused by coronary artery ligation and IRAB, respectively. Differences were tested for statistical significance using Student's *t*-test for independent groups, and were considered statistically significant if $P<0.05$.

RESULTS

Evaluation of MI and IRAB models

Coronary artery ligated hearts showed large transmural infarctions, which were located in the lateral (free) wall of the left ventricle. A total of 6 out of 30 MI hearts (3 weeks and 8 weeks combined) were excluded from analysis because the infarcted area comprised only a minor part of the left ventricular free wall. Despite the replacement of a considerable part of the myocardium by lighter scar tissue, wet weight of the entire heart was not decreased in MI hearts at 3 weeks. At 8 weeks after infarction, cardiac mass was even higher in MI hearts, indicative of progressive hypertrophy of surviving myocardium. This was reflected in a significantly increased heart wet weight to body weight ratio in MI hearts at 8 weeks (Table 2.1). In rats after IRAB, both carotid and femoral artery blood pressure were measured to evaluate the pressure gradient over the banded aorta segment. Hypertension after IRAB was demonstrated by a significantly raised mean arterial blood pressure, as measured in the carotid artery (140 ± 8 versus 115 ± 4 mmHg). With a decreased blood pressure distal to the banded segment (95 ± 6 versus 115 ± 5 mmHg), a substantial pressure gradient was present after IRAB. After exclusion of 4 out of 13 banded rats, because of absence of unilateral kidney atrophy (left to right kidney weight ratio of <0.9), IRAB significantly decreased left to right kidney weight ratio at 4 weeks. Cardiac hypertrophy was indicated by a significantly increased heart wet weight and heart wet weight to body weight ratio (Table 2.1).

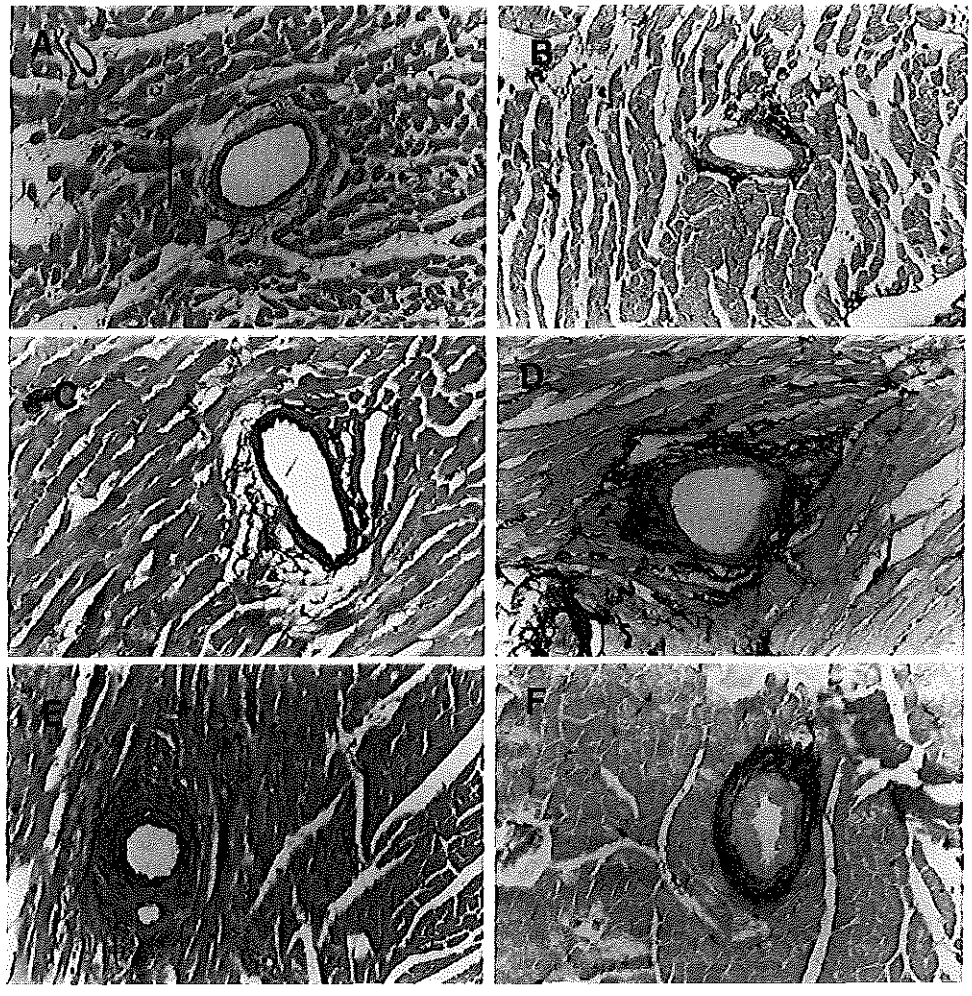

Figure 2.1: Photomicrographs of resorcine-fuchsine stained sections (A/C/E) and picrosirius red stained sections (B/D/F) showing resistance arteries in A/B: normal myocardium after sham operation, C/D: non-infarcted myocardium 3 weeks after MI (normal media thickness, increased perivascular collagen), E/F: heart from aortic banded rat (increased media thickness, increased perivascular collagen). In photomicrograph E, the stained line within the tunica media (arrowheads) suggests growth of the tunica media outside its normal boundaries. The bar in photomicrograph A indicates 100 μm, and accounts for all photomicrographs.

Vascular remodeling in MI hearts and hearts after IRAB

MI-induced remodeling was associated with a significantly increased collagen/lumen area ratio of resistance arteries (indicating perivascular fibrosis) at 3 weeks, but not at 8 weeks after surgery. At neither 3 nor 8 weeks, tunica media/lumen area ratio of the resistance vasculature within non-infarcted myocardium in MI hearts was different from sham values (Figure 2.1, panels C and D, Figure 2.2).

After IRAB, a striking change from the normal microscopic appearance of the myocardium was present (Figure 2.1, panels E and F). There were areas of focal necrosis as well as pronounced perivascular fibrosis of resistance arteries, in both the non-hypertrophied right ventricle and the hypertrophied left ventricle. The observed perivascular fibrosis was reflected in a distinct increase in the collagen/lumen area ratio. Media/lumen area ratio was significantly increased, indicating growth of the vascular smooth muscle medial layer of resistance arteries (Figure 2.2). Both collagen/lumen and media/lumen area ratios were equally increased in resistance vessels in the hypertrophied left and the non-hypertrophied right ventricle. Both in MI hearts and hearts after IRAB, lumen diameters of measured vessels were similar to those analyzed in sham-operated controls, and ranged from 35 to 140 μm.

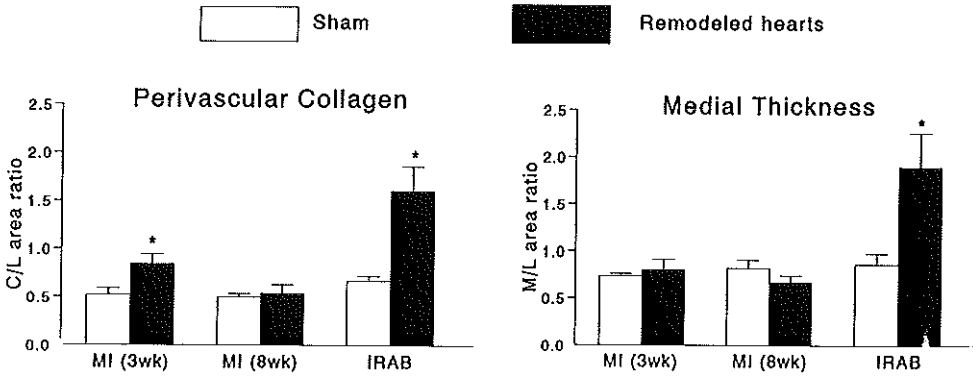

Figure 2.2: Remodeling of resistance arteries in non-infarcted myocardium of MI hearts at 3 and 8 weeks, or hearts from rats after aortic banding (black bars) and hearts from sham-operated rats (white bars). Left panel: perivascular collagen as measured by collagen to lumen (C/L) area ratio, right panel: tunica media thickness as measured by media to lumen (M/L) area ratio. *, $P<0.05$ versus sham values.

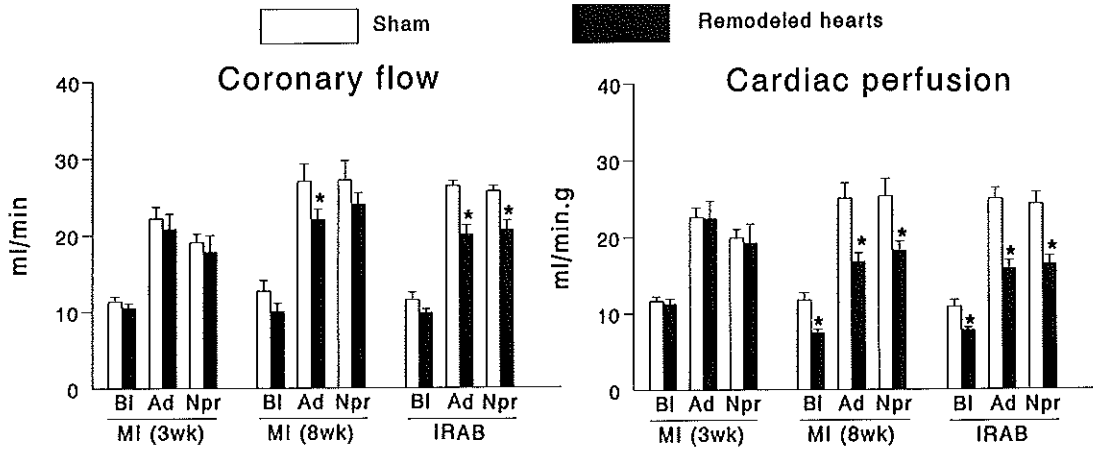

Figure 2.3: Coronary flow (left panel, absolute values, ml/min) and cardiac perfusion (right panel, values corrected for heart weight, ml/min.g) in hearts after MI or aortic banding (black bars) and sham operation (white bars). Bl: baseline, Ad: adenosine, Npr: nitroprusside. *, $P<0.05$ versus sham values.

Coronary flow and tissue perfusion of remodeled hearts

In hearts at 3 weeks after MI, both baseline and maximal coronary flow (ml/min), as well as tissue perfusion (ml/min.g) were not decreased compared to normal control hearts (Figure 2.3). At 8 weeks, baseline coronary flow was not different from sham values, whereas maximal flow was reduced with adenosine but not with nitroprusside. Corrected for mass of perfused tissue, baseline and maximal perfusion (with either vasodilator) were decreased.

In hearts remodeled after IRAB, coronary flow at baseline did not differ from values in sham-operated control hearts, but peak flow to both adenosine and nitroprusside was reduced. Myocardial perfusion was decreased both at baseline and during maximal vasodilation, compared to control hearts.

Ratios of maximal/baseline coronary flow (or maximal/baseline perfusion) did not differ between remodeled hearts and sham-operated control hearts, though tended to be lower in hearts after IRAB (Table 2.2).

Distribution of coronary flow after IRAB

In IRAB rats used to study regional distribution of coronary flow, there was only a modest increase in heart wet weight (987 ± 51 vs 922 ± 60 mg, NS). This was totally attributable to the left ventricle, since right ventricular weight did not differ from sham values (163 ± 4 vs 188 ± 12 mg, NS). Left ventricular hypertrophy was indicated by a significantly increased left ventricle/body weight ratio (2.7 ± 0.2 vs 2.2 ± 0.1 mg/g). Baseline coronary flow nor myocardial perfusion were affected in these hearts, neither in the right nor in the left ventricle (Figure 2.4). During nitroprusside-induced maximal vasodilation, coronary flow was restricted equally in left and right ventricles of banded rats (29 ± 7 vs 33 ± 4% decrease versus sham values). However, corrected for tissue mass, left ventricular perfusion (hypertrophy present) was more affected than perfusion of the right ventricle (no hypertrophy) (37 ± 5% vs 22 ± 5% decrease compared to sham values).

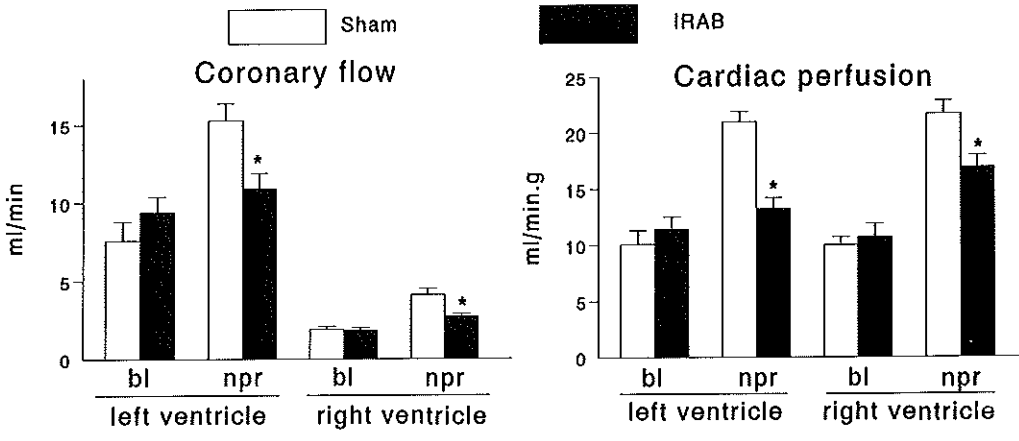

Figure 2.4: Coronary flow (left panel, absolute values, ml/min) and cardiac perfusion (right panel, values corrected for heart weight, ml/min.g) in left and right ventricles of hearts 4-5 weeks after aortic banding (black bars) and sham operation (white bars). Bl: baseline, Npr: nitroprusside. *, $P<0.05$ versus sham values.

DISCUSSION

In pressure overload-induced cardiac hypertrophy, a decreased coronary reserve has been recognized as a potential mechanism for the eventual development of heart failure (Vatner & Hittinger, 1993). Relative to the amount of information about coronary reserve in pressure-overload induced hypertrophy (Mueller *et al.*, 1978; O'Keefe *et al.*, 1978; Tomanek *et al.*, 1985; Canby & Tomanek, 1989; Tomanek *et al.*, 1989; Brilla *et al.*, 1991), studies on flow reserve in MI-induced reactive hypertrophy are scarce. The aim of the present study was to measure flow capacity of the coronary vascular bed as well as tissue perfusion, and to identify determinants of a decreased coronary reserve in MI hearts. The main findings were: i) Flow capacity of the coronary vascular bed in MI hearts had been restored to sham values at 3 weeks, ii) progression of post MI cardiac hypertrophy from 3 to 8 weeks exceeded the increase in flow capacity, causing a decreased myocardial perfusion at 8 weeks, and iii) perivascular collagen accumulation in MI hearts appeared to be transient and comparatively mild to the perivascular fibrosis seen in hearts after renovascular hypertension, and was not related to the decreased coronary reserve.

Flow capacity of the coronary vascular bed in MI hearts

In the rat MI model, permanent occlusion of one of the 3 coronary arteries will acutely lead to a substantial reduction of the coronary vascular bed. Maximal coronary flow is assumed to be determined by total cross-sectional area (CSA) of the resistance vasculature. The restored flow capacity at 3 weeks after coronary artery ligation, despite the initially considerably reduced total CSA, implies angiogenesis in the vascular beds perfused by the two remaining coronary arteries. Post MI angiogenesis involves growth of capillaries (Anversa *et al.*, 1986a), but would also include arteriolar growth, as observed in pressure overload-induced hypertrophy (Tomanek *et al.*, 1989). An increase in size and/or number of arterioles would increase the total CSA of the resistance vasculature perfused by the remaining 2 patent coronary arteries.

Progression of hypertrophy versus increase of flow capacity in MI hearts

Maximal flow capacity was 15% higher at 8 weeks compared to 3 weeks after MI, indicating an equivalent increase in CSA. However, this was exceeded by the increase of

myocyte mass; MI hearts weighed 44% more at 8 weeks compared to 3 weeks. Therefore, the decreased baseline and maximal cardiac perfusion at 8 weeks can be explained by an inadequate growth of the vasculature relative to the growth of cardiac muscle. Our observation of an impaired maximal cardiac perfusion, associated with a disproportional growth of muscle mass relative to growth of the vasculature, is in agreement with data of Karam and coworkers (1990), who found a 43% decrease of maximal tissue perfusion in left ventricles, and a 33% decrease in right ventricles of MI hearts, while myocyte size was significantly increased in both left and right ventricles.

Similar to our findings, Nelissen-Vrancken *et al.* (1996) found a time-related normalization of maximal coronary flow, but a disproportional increase in tissue mass compared to maximal coronary flow. However, the latter was found in right ventricles and interventricular septa of MI hearts, but not in the region where the most pronounced hypertrophy would be expected (surviving part of the left ventricular free wall) (Olivetti *et al.*, 1986). This may be explained by the fact that effects of time in MI-induced remodeling were evaluated by comparing the timepoints of 1 week and 3 weeks after MI, and substantial increase of regional tissue mass may already occur in the first week after MI (Anversa *et al.*, 1985a).

Comparison of maximal flow and perfusion of isolated hearts with *in vivo* data

Comparing our *in vitro* data with *in vivo* measurements in the rat MI model (Drexler *et al.*, 1992) revealed that at 8 weeks coronary reserve determined with radioactive microspheres as ratio of maximal to baseline flow was reduced. However, *in vivo* coronary flow at baseline was slightly higher in MI than in normal hearts and was based on measurements using only the non-infarcted myocardium. In chapter 3, we will show that buffer flow to infarcted tissue of MI hearts averages about 8% of total coronary flow. Thus, total baseline coronary flow of MI hearts *in vivo* may have been even higher. Therefore, a higher coronary flow at baseline, possibly due to the hemodynamic state, rather than a decreased flow capacity would be responsible for the reduced coronary reserve. Both baseline and maximal coronary flow of buffer-perfused hearts were higher than of the blood-perfused hearts from the aforementioned studies (Karam *et al.*, 1990;

Drexler *et al.*, 1992), probably due the higher viscosity of blood than buffer. However, the *ratios* of maximal to baseline coronary flow of sham hearts in our experiments and in the aforementioned studies were similar. Moreover, in chapter 4 we will show that *in vitro* maximal coronary flow with either nitroprusside or adenosine is comparable to the values during post-ischemic vasodilation, indicating that the decreased maximal tissue perfusion *in vitro* in fact represents an actual limitation of this parameter *in vivo*.

Vascular remodeling and coronary reserve in MI hearts

A decreased maximal coronary flow in hypertensive rats has been attributed to media hypertrophy of resistance arteries (Brilla *et al.*, 1991), which was confirmed by the present study. Remodeling of resistance arteries in MI hearts, however, appeared to be confined to accumulation of perivascular collagen without media hypertrophy. Furthermore, the collagen accumulation was transient and relatively mild compared to the perivascular fibrosis observed in hearts from hypertensive rats. Moreover, the perivascular collagen accumulation in MI hearts at 3 weeks was not associated with a reduction in maximal coronary flow capacity or myocardial perfusion, whereas the depressed maximal tissue perfusion at 8 weeks occurred in the absence of perivascular fibrosis. Therefore, the reduced maximal tissue perfusion in MI hearts is probably related to a reduced density rather than to remodeling of resistance arteries.

Vascular remodeling and coronary reserve in hearts after pressure overload

In contrast to MI hearts, morphometry of resistance arteries in hearts from rats with renovascular hypertension revealed severe perivascular fibrosis and a prominent hypertrophy of the tunica media. Media hypertrophy has been attributed to hypertension-induced increase of perfusion pressure (Brilla *et al.*, 1991). Thus, hypertension probably contributed in the development of the vascular pathology after aortic banding. Media hypertrophy of resistance arteries may account for a decreased maximal CSA, although the perivascular collagen accumulation could have contributed to the reduced maximal flow capacity and perfusion as well. However, Brilla and coworkers (1991) showed that in spontaneously hypertensive rats, the reduced coronary reserve was only normalized with regression of both perivascular fibrosis and the media hypertrophy of resistance arteries

(with high-dose lisinopril), and not by regression of perivascular fibrosis alone (with low-dose lisinopril). In the present study, vascular remodeling of resistance vessels was comparable in the hypertrophied left ventricle and in the non-hypertrophied right ventricle, resulting in an equal reduction of maximal coronary flow. Consequently, peak myocardial perfusion of the left ventricle was more affected than right ventricular perfusion. Apparently, vascular remodeling in renovascular hypertension controls maximal flow capacity, while hypertrophy of the surrounding myocardium determines the severity of maximal perfusion reduction.

Conclusion

Prominent vascular remodeling after aortic banding, including severe perivascular fibrosis and tunica media hypertrophy of resistance arteries, limits flow capacity of the coronary vascular bed. Thus, in hypertension-induced hypertrophy, the reduction of maximal tissue perfusion is determined by both vascular remodeling and the degree of cardiac hypertrophy. In MI-induced cardiac remodeling, a mild and transient perivascular fibrosis was observed, without media hypertrophy. Coronary flow capacity had been restored, whereas maximal tissue perfusion was decreased at 8 weeks, when vascularization was lagging behind cardiac hypertrophy. In MI-induced cardiac remodeling, reduction of cardiac perfusion is not determined by vascular remodeling, but mainly by disproportional cardiac hypertrophy relative to vascular growth. Regression of cardiac hypertrophy or stimulation of vascular growth may therefore be appropriate pharmacotherapeutic strategies to restore the reduced coronary reserve after MI.

CHAPTER 3

REGIONALLY DIFFERENT VASCULAR RESPONSE TO NITROPRUSSIDE AND TO VASOPRESSIN IN THE REMODELED INFARCTED RAT HEART

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ABSTRACT

Remodeling after myocardial infarction (MI) is associated with vascular adaptation, increasing vascular capacity in non-infarcted myocardium, and angiogenesis in the infarcted part during wound healing and scarring. We investigated regional vascular reactivity in the infarcted rat heart. Transmural infarction of the left ventricular free wall was induced by coronary artery ligation. After 3 weeks, regional coronary flow during maximal vasodilation (nitroprusside, NPR) and submaximal vasoconstriction (arginine-vasopressin, AVP) was studied in buffer-perfused hearts. The main findings were: i) A reduced vasodilator response (to NPR) in the viable part of the left ventricular free wall, where hypertrophy was most pronounced, resulting in reduced maximal tissue perfusion of the myocardium bordering the scar (19.7 ± 0.6 versus 25.7 ± 1.2 ml/min.g), whereas perfusion of other non-infarcted regions was preserved. ii) A 54% lower vasodilator response (to NPR) and a 25% stronger vasoconstriction (to AVP) in scar tissue compared to viable parts of MI hearts. Microscopy showed that resistance arteries had thicker walls in scar tissue than in contractile myocardium, morphometrically substantiated by 2 to 3-fold greater wall/lumen ratios. These data indicate a deviant response of scar vessels of MI hearts, and in the non-infarcted part a reduced coronary reserve in the most hypertrophied region. Whereas the former may be caused by different vessel structure, the reduced vasodilator reserve of the spared part of the left ventricular free wall may indicate vasodilation at rest due to insufficient vascular growth. The most hypertrophied region would be most at risk of further ischemic damage.

INTRODUCTION

Myocardial infarction (MI) induces compensatory hypertrophy and remodeling of the non-infarcted myocardium (Anversa *et al.*, 1985a, 1985b, 1986a). Remodeling of the infarcted heart is accompanied by vascular adaptation, leading to increased capacity of the vascular bed in the non-infarcted part (Chapter 2; Nelissen-Vrancken *et al.*, 1996) and to angiogenesis in the infarcted tissue (Kumar *et al.*, 1983; Battler *et al.*, 1993), associated with wound healing and scar formation.

A decreased vasodilator reserve in the infarcted heart has recently been reported (Karam *et al.*, 1990; Drexler *et al.*, 1992). However, information about vascular reactivity in the different regions of the heterogeneous infarcted heart is still incomplete. Regional differences in vascular reactivity could result from regional variation in: i) The degree of vessel growth relative to myocyte growth. In regions with decreased arteriolar density, compensatory vasodilation to meet tissue oxygen demand could lead to decreased vasodilator reserve. ii) The contribution of newly formed vessels, with different pharmacological properties (Andrade *et al.*, 1992a, 1992b), to the vascular adaptation process. iii) Wall to lumen ratio of resistance vessels, mechanically changing effects of vasoactive substances (Mulvany *et al.*, 1978; Folkow & Karlström, 1984; Korner *et al.*, 1989).

The aim of the present study was to investigate regional differences in vascular reactivity in the post-MI remodeled rat heart. Studies were performed at 3 weeks, just after completion of scar formation (Fishbein *et al.*, 1978a) at the compensated stage of cardiac remodeling (Schoemaker *et al.*, 1991). Regional vasoreactivity was assessed by measurement of regional flow changes in the isolated heart, during maximal vasodilation and submaximal vasoconstriction, as compared to baseline perfusion. Geometry of resistance arteries in the different parts of the heart was studied by morphometric assessment of wall to lumen ratio of these vessels, as this appeared to be a determinant of coronary reserve in hypertrophied hearts (Chapter 2).

MATERIALS AND METHODS

Male, Wistar rats (270-320 g, Harlan Zeist, The Netherlands) were used in this study. Rats were housed at a 12 h light/dark cycle with standard rat chow and water available ad libitum. The experiments were carried out after approval of the University Ethics Committee for the use of experimental animals. Under pentobarbital (60 mg/kg, i.p.) anesthesia, left anterior descending coronary artery ligation was performed as described in detail in chapter 2 (page 32).

Regional vasoreactivity At 3 weeks after surgery, when infarct healing is considered to be completed (Fishbein *et al.*, 1978a), hearts were isolated under pentobarbital anesthesia and mounted for Langendorff perfusion and instrumented for functional measurements (Chapter 2, page 32). Left ventricular end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume and hearts were allowed to stabilize for at least 20 min before baseline measurements were obtained. The

distribution of coronary blood flow was determined with 15 ± 1 (S.D.) μm diameter microspheres labelled with either ^{113}Sn, ^{95}Nb, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, USA). After the stabilization period, microspheres were injected to obtain baseline values for regional coronary flow. Subsequently, maximal coronary flow was determined using a 0.1 ml bolus injection of a 10^{-2}M sodium nitroprusside solution (Dijkzigt University Hospital's pharmacy, Rotterdam, The Netherlands) (Chapter 2, page 32). Microspheres were injected when maximal vasodilation was reached. After re-stabilization, submaximal vasoconstriction was induced by a 0.1 ml bolus injection of a 10^{-2}M arginine-vasopressin solution (Sigma, Deisendorf, Germany). At the peak of vasoconstrictor response, as monitored by coronary flow decrease, a third injection of microspheres was given. Doses of both sodium nitroprusside and arginine-vasopressin were based upon complete dose-response curves obtained in pilot experiments. For each measurement a suspension of 0.1 ml containing about 8,000 microspheres, labelled with one of the isotopes, was mixed and injected into the perfusing buffer just before it entered the coronary arteries. In pilot experiments, coronary flow after injection of about 25,000 microspheres (10.5 ± 2.4 ml/min, n=5) did not differ from baseline coronary flow (11.4 ± 2.7 ml/min). At each measurement, coronary effluent was collected to measure microsphere by-pass of capillaries through leakage or arteriovenous anastomotic flow. After the experiment the ventricles were separated from atria and large vessels, and subsequently divided into right ventricle, interventricular septum and left ventricle free wall. Left ventricular free walls of MI hearts were further divided into viable tissue and scar tissue, by macroscopic appearance (Figure 3.1). Tissues were weighed and radioactivity in tissues and coronary effluent was counted for 10 min in a γ-scintillation counter (Packard, Miniaxi autogamma 5000), using suitable windows for discriminating the different isotopes. All data were processed by specially designed computer programs (Saxena *et al.*, 1980).

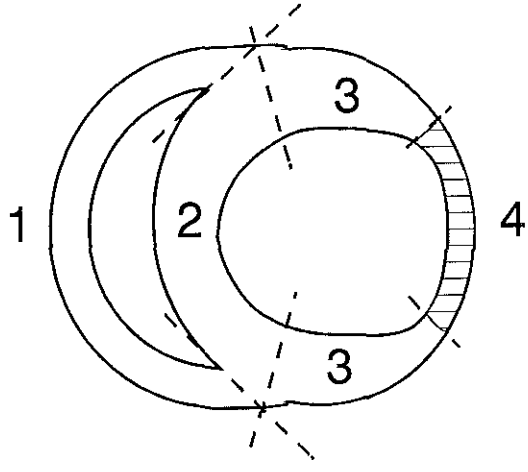

Figure 3.1: Dissection of the heart after regional flow experiment. 1: right ventricle, 2: interventricular septum, 3: viable part of the left ventricular free wall, 4: infarct scar.

Histology of resistance arteries In a separate group of rats, resistance arteries (luminal diameter <150 μm) were studied in sham hearts (n=6), and in non-infarcted myocardium as well as in scar tissue of MI hearts (n=6). Briefly, the hearts were fixated by perfusion with 3.6% phosphate-buffered formaldehyde. The ventricles were cut into 4 slices from apex to base, after removal of atria and large vessels. The slices were dehydrated and paraffin embedded. Deparaffinized 5 μm thick sections were incubated for 90 min with a resorcin-fuchsin solution at 60°C, and subsequently for 2 min with a Van Gieson solution, flushed with alcohol, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany) (see also Chapter 2, page 33). Vascular wall thickness was indexed by the ratio of wall area to luminal area, in about 20 vessels in each heart. To avoid measurement of components of the venous side of the coronary vascular bed, only vessels with an internal elastic membrane were measured (Simionescu & Simionescu, 1988).

Data analysis Results comprise regional flow data from 9 hearts of sham-operated rats and 10 MI hearts, and morphometric data from 6 sham hearts and 6 MI hearts. Data are expressed as group means ± S.E.M. unless indicated otherwise. Data from MI hearts were only included if the infarcted area comprised the major part of the left ventricular free wall, since smaller infarctions are known to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Differences between the sham and MI groups were tested with Student's t-test for independent groups. Differences between the different regions of MI or sham hearts were tested with ANOVA, followed by post-hoc t-tests. Differences were considered statistically significant if $P<0.05$.

RESULTS

Left descending coronary artery ligation resulted in transmural infarction of the LV free wall. Data of 2 animals were excluded from analysis, because of infarctions comprising only a minor part of the LV free wall.

Regional tissue weight

MI hearts weighed significantly more than sham-operated control hearts (Table 3.1). Since body weight did not differ between MI and sham-operated rats, heart weight to body weight ratio was also increased. MI-induced compensatory hypertrophy was most prominent in the free wall of the LV. Tissue mass of this region was significantly increased (+20%), despite replacement of most of the myocyte mass by relatively light scar tissue (185 ± 27 mg).

Coronary flow and tissue perfusion; response to nitroprusside and vasopressin

At baseline, coronary flow to the ventricles was distributed as follows (in sham and

Table 3.1 Body weight and regional heart weight

	SHAM	MI
BW (g)	342 ± 10	332 ± 10
Total ventricular		
weight (mg)	888 ± 26	1046 ± 35*
weight/BW (mg/g)	2.6 ± 0.1	3.2 ± 0.1*
1) LV free wall		
weight (mg)	441 ± 31	528 ± 41*
weight/BW (mg/g)	1.3 ± 0.1	1.6 ± 0.1*
2) Interventricular septum		
weight (mg)	251 ± 9	277 ± 17
weight/BW (mg/g)	0.7 ± 0.1	0.8 ± 0.1
3) Right ventricle		
weight (mg)	196 ± 14	241 ± 18
weight/BW (mg/g)	0.6 ± 0.1	0.7 ± 0.1

SHAM: hearts from sham-operated rats, MI: myocardial infarction, BW: body weight; LV free wall, left ventricular free wall (including scar tissue in infarcted hearts); *: $P<0.05$ versus sham hearts.

MI hearts, respectively): RV, 20.3 ± 2.4% and 24.7 ± 1.9%; interventricular septum, 28.6 ± 1.5% and 30.6 ± 2.0%; LV free wall, 53.4 ± 2.9% and 44.7 ± 2.1%, divided into 35.7 ± 2.2% to viable myocardium and 9.0 ± 1.1% to scar tissue (Table 3.2). A small flow of about 0.5 ml/min by-passed the coronary capillary bed, through leakage or arteriovenous anastomotic flow, as measured by radioactivity count of coronary effluent. Total coronary baseline flow in sham and MI hearts did not differ (8.5 ± 0.9 and 9.0 ± 0.4 ml/min, respectively). In sham hearts, there was a strong correlation between regional coronary flow and tissue weight (r=0.808, $P<0.0001$; Figure 3.2, left panel). A similar relationship between weight and flow was found for contractile parts of MI hearts, at baseline conditions. However, scar tissue received less coronary flow per weight than contractile parts of MI hearts, resulting in a relatively low perfusion of scar tissue (Table 3.2).

During nitroprusside-induced maximal vasodilation, ventricular coronary flow was similar in MI and sham hearts (19.6 ± 0.8 *vs* 21.0 ± 0.6 ml/min). After correction for ventricular mass, maximal perfusion was reduced in MI hearts. Maximal perfusion of ventricular myocardium (viable and scar tissue together) averaged 18.8 ± 0.6 ml/min.g, and perfusion of viable myocardium alone 21.2 ± 0.7 ml/min.g, whereas maximal perfusion of myocardium in sham hearts averaged 23.7 ± 0.8 ml/min.g. The amount of flow that by-

Table 3.2 Distribution of coronary flow and regional tissue perfusion

	Coronary flow (ml/min)		Tissue perfusion (ml/min.g)	
	SHAM	MI	SHAM	MI
Baseline				
Viable LV free wall	4.7 ± 0.7	3.2 ± 0.2	10.4 ± 1.1	9.6 ± 0.6
Scar tissue	...	0.8 ± 0.1	...	4.6 ± 0.5
Interventricular septum	2.5 ± 0.3	2.8 ± 0.2	10.0 ± 1.4	10.1 ± 0.7
Right ventricle	1.4 ± 0.2	2.2 ± 0.2*	7.0 ± 1.1	9.4 ± 0.8
Atria and large vessels	0.3 ± 0.1	0.5 ± 0.1	1.2 ± 0.4	1.4 ± 0.2
By-pass flow	0.5 ± 0.2	0.6 ± 0.2	...	...
Nitroprusside				
Viable LV free wall	11.2 ± 0.8	6.7 ± 0.6*	25.7 ± 1.2	19.7 ± 0.6*
Scar tissue	...	1.4 ± 0.2	...	7.8 ± 0.8
Interventricular septum	5.6 ± 0.3	6.0 ± 0.3	22.3 ± 1.1	22.1 ± 1.1
Right ventricle	4.1 ± 0.4	5.5 ± 0.5*	21.2 ± 1.3	22.9 ± 1.4
Atria and large vessels	0.8 ± 0.2	1.1 ± 0.2	2.5 ± 0.7	2.7 ± 0.5
By-pass flow	2.5 ± 0.5	2.0 ± 0.5	...	...
Arginine-vasopressin				
Viable LV free wall	1.9 ± 0.4	1.2 ± 0.3	4.5 ± 1.0	3.9 ± 0.9
Scar tissue	...	0.1 ± 0.1	...	0.5 ± 0.3
Interventricular septum	0.8 ± 0.2	1.0 ± 0.3	3.4 ± 0.9	3.9 ± 1.3
Right ventricle	0.6 ± 0.2	0.9 ± 0.1	3.1 ± 1.0	3.6 ± 0.5
Atria and large vessels	0.2 ± 0.1	0.2 ± 0.2	0.7 ± 0.5	0.5 ± 0.4
By-pass flow	0.3 ± 0.2	0.6 ± 0.5	...	...

Distribution of coronary flow and regional tissue perfusion at baseline, during nitroprusside-induced maximal vasodilation and during arginine-vasopressin-induced submaximal vasoconstriction in hearts from sham-operated rats (SHAM) and infarcted hearts (MI); viable LV free wall, viable left ventricular free wall (scar tissue excluded); by-pass flow, buffer flow by-passing coronary capillaries through leakage or arteriovenous anastomotic flow; *: $P<0.05$ versus values in sham hearts.

passed the capillary bed increased after nitroprusside injection, suggesting arteriovenous anastomotic flow rather than leakage. The relationship between regional coronary flow and tissue weight in sham hearts was even stronger after nitroprusside than at baseline conditions (r=0.951, $P<0.0001$; Figure 3.2, right panel). Right ventricles and interventricular septa of MI hearts showed a similar weight-flow relationship. However, LV free walls of MI hearts deviated from the normal weight-flow relationship. Thus, spared LV free wall myocardium of MI hearts was hypoperfused compared to the corresponding region in sham hearts (Table 3.2), during the vasodilator response of vessels in this area (Figure 3.3). For infarcted tissue, the deviation from the normal weight-flow relationship,

already present at baseline, increased during vasodilation (Figure 3.2), due to a decreased vasodilator response of vessels in scar tissue compared to contractile tissue (Figure 3.3).

Arginine-vasopressin reduced total coronary flow similarly in MI hearts and sham hearts. Flows to the different regions of sham hearts were equally reduced, as expressed by percentage decrease from baseline coronary flow (Figure 3.3). Within MI hearts, coronary flow to contractile tissue was equally reduced as in sham hearts, whereas coronary flow to scar tissue was more reduced than the flow to contractile myocardium. Coronary flow to scar tissue of MI hearts during vasoconstriction with vasopressin was reduced to almost zero.

Resistance artery morphology in scar tissue and viable tissue of MI hearts

The scar showed a heterogenous tissue and vascular structure, as is illustrated in Figure 3.4. Resistance arteries with a thick wall, as well as large venous structures (lacking a lamina elastica interna), sometimes surrounded by surviving myocytes, and completely occluded vessel structures could be seen. Figure 3.5 shows resistance arteries in normal hearts from sham-operated animals, as well as in non-infarcted myocardium (normal vessel wall thickness) and in scar tissue (thick vessel wall) of MI hearts. Some vessels in the scar tissue showed a fragmented elastin membrane within the vessel wall, together with an intact internal elastic membrane at the luminal surface of the vessel. The material between the fragmented membrane and the actual internal elastic membrane proved to be cellular, since nuclei were seen in haematoxylin-eosin stained sections. Thick vessel walls in the infarcted area were substantiated by morphometric measurements of vessel wall area and lumen diameter, as shown in Figure 3.6. Lumen diameter was inversely related to wall/lumen ratio, in control hearts as well as in viable myocardium in MI hearts (shams: $r=-0.478$, $P<0.0001$; MI hearts: $r=-0.494$, $P<0.0001$). The increased wall to lumen ratio of resistance vasculature in MI hearts was limited to the infarcted area. Between the different contractile regions of MI hearts, wall to lumen ratios of resistance vessels did not differ, nor did these vessels differ from resistance arteries in corresponding regions of hearts from sham-operated rats.

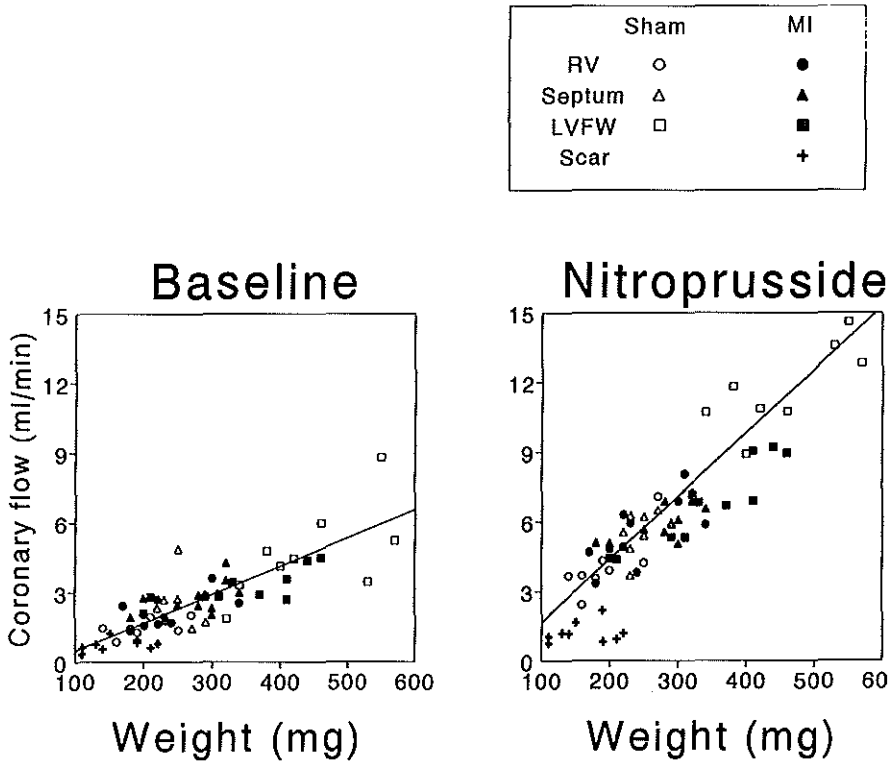

Figure 3.2 Regional weight and coronary flow
Relationship between regional weight and coronary flow in different parts of hearts from sham-operated rats (open symbols) or MI hearts (black symbols). Left panel: at baseline conditions; Right panel: during nitroprusside-induced maximal vasodilation. The regression lines for regions of hearts from sham-operated rats are shown: At baseline, coronary flow (ml/min)=0.012 x weight (mg) - 0.746; during maximal vasodilation, coronary flow (ml/min)=0.027 x weight (mg) - 0.936.

DISCUSSION

The present study was carried out to investigate regional differences in vascular reactivity in the post-MI remodeled rat heart. The main findings were: i) Vasodilator response was reduced in the viable part of the left ventricular free wall of MI hearts, where reactive hypertrophy was most pronounced. This resulted in impaired peak perfusion of the viable myocardium bordering the scar tissue, and ii) resistance arteries in scar tissue showed thick vessel walls, associated with a reduced vasodilator and an enhanced vasoconstrictor response.

Vasodilator response in non-infarcted myocardium of MI hearts

To induce maximal vasodilation, an endothelium- and receptor-independent vasodilator (sodium nitroprusside) was used, in order to ensure peak tissue perfusion by maximal vasodilation independent of decreased endothelial function after MI (Drexler *et al.*, 1992; Drexler & Lu, 1992) and altered pharmacological properties of newly formed vessels (Andrade *et al.*, 1992a, 1992b). Reduced maximal cardiac perfusion has been described during MI-induced remodeling (Karam *et al.*, 1990; Drexler *et al.*, 1992; Nelissen-Vrancken *et al.*, 1996; Chapter 2). Impaired global myocardial perfusion has been explained by a disproportional degree of hypertrophy relative to vascular growth, rather than by vascular remodeling (Chapter 2). In the present study, maximal coronary flow in MI hearts and sham hearts was similar, despite permanent occlusion of one of the 3 coronary arteries in MI hearts, which acutely reduces coronary flow by approximately 30%

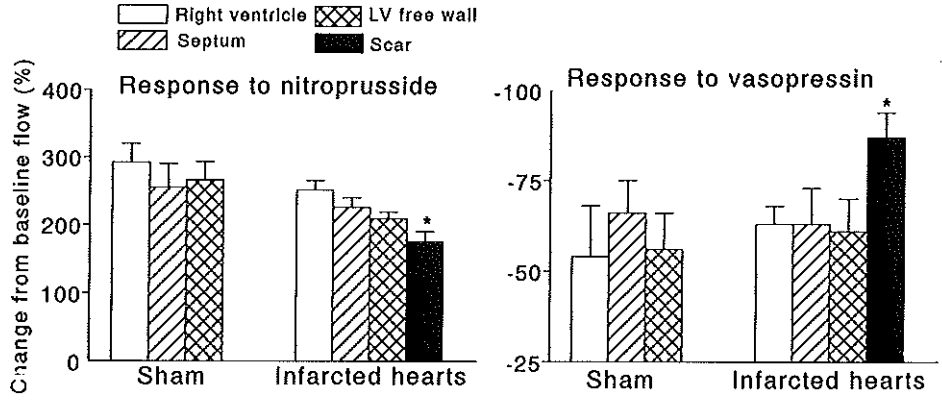

Figure 3.3 Regional coronary flow response
Coronary flow response of different regions of sham and MI hearts, during nitroprusside-induced maximal vasodilation (Left panel, expressed as % increase from baseline flow), and vasopressin-induced submaximal vasoconstriction (Right panel, expressed as % decrease from baseline flow).
*: $P<0.05$ versus other regions within MI hearts.

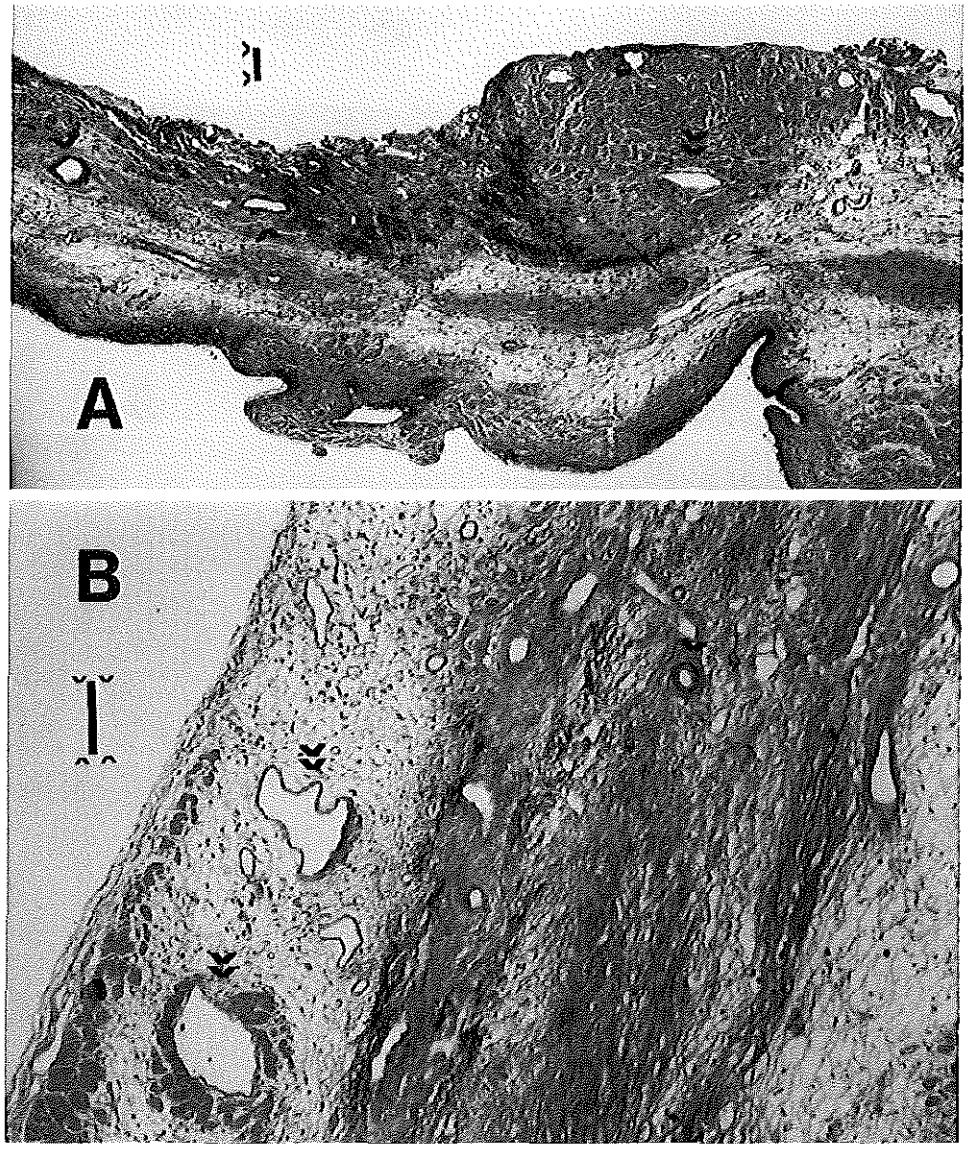

Figure 3.4: Photomicrographs of resorcin-fuchsin stained sections, showing the infarct scar. The bars represent 100 μm. Arrowheads: resistance arteries (<150 μm luminal diameter) (lamina elastica interna present). Double arrowheads: large venules (lamina elastica interna absent). In panel A, the arrow indicates an obliterated vascular structure. In panel B, a large venule is surrounded by surviving cardiomyocytes.

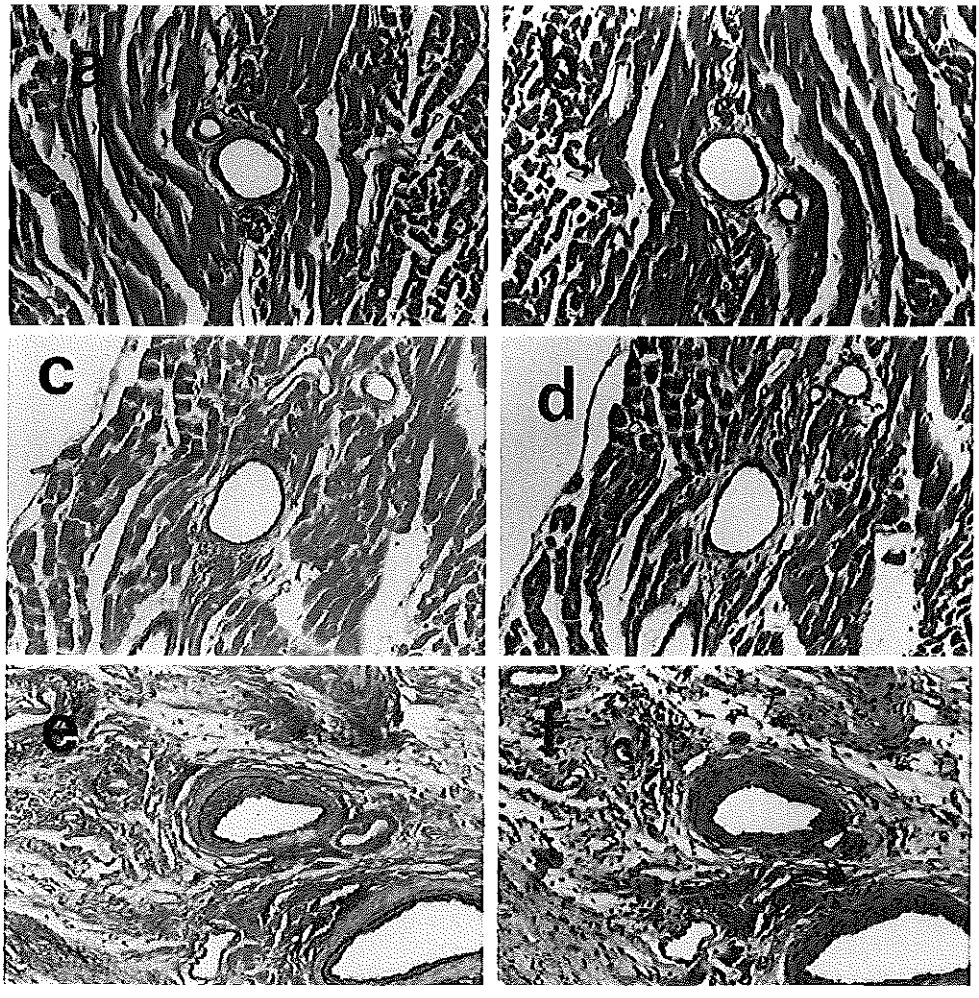

Figure 3.5 Photomicrographs of resorcin-fuchsin stained sections (A/C/E) and haematoxylin-eosin stained sections (B/D/F) showing resistance arteries in A/B: normal myocardium after sham operation, C/D: non-infarcted myocardium 3 weeks after MI (normal medial thickness), E/F: scar tissue of MI hearts (thick vessel wall). The stained line within the vessel wall (arrowhead) suggests growth of the vessel wall outside its original boundaries. After haematoxylin-eosin staining, the material at the inside of the vessels shows the presence of cellular nuclei. The bar in photomicrograph A indicates 100 μm, and accounts for all photomicrographs.

(unpublished data). The restored flow capacity at 3 weeks after coronary artery ligation implies angiogenesis in the vascular beds perfused by the two remaining coronary arteries. This is in agreement with previous studies by us and by other authors (Chapter 2; Nelissen-Vrancken *et al*, 1996). MI hearts showed only a moderate increase in right ventricular and interventricular septum weight, which was accompanied by a preserved maximal myocardial perfusion. Hypoperfusion in MI hearts during maximal vasodilation was limited to the spared part of the left ventricular free wall, where marked hypertrophy was found. Despite replacement of the major part of myocyte mass by lighter scar tissue, left ventricular free wall mass was increased after MI. This was associated with a noticeably decreased maximal myocardial perfusion. Similar findings can be obtained from a study of Nelissen-Vrancken *et al.* (1996); at 5 weeks, only peak perfusion of the viable part of the left ventricular free wall was reduced compared to sham-operated control hearts. Based on these data, the perfusion deficit was already present at 1 week after MI, followed by an equal increase in vascular capacity relative to weight increase in sham and MI hearts, from 1 to 5 weeks after surgery. This suggests a substantial hypertrophic response of this region in the first week after MI, with vascular growth seriously lagging behind. Insufficient vascular growth relative to increased muscle mass would decrease vascular density. The decreased density of resistance arteries can be compensated by vasodilation at baseline conditions. In accordance with the data in the present study, this compensatory vasodilation would be unmasked during maximal tissue metabolic demands as decreased vasodilator reserve and impaired peak tissue perfusion.

Morphology of resistance arteries in scar tissue in relation to local vasoreactivity

In the present study, resistance vessels within the infarcted zone were found to have greater wall to lumen ratios compared to resistance arteries in normal hearts or in the non-infarcted myocardium of MI hearts, which could explain the higher increase of vascular resistance with vasoconstriction. Dilated vessels like those reported by Nelissen-Vrancken *et al.* (1996) were confined to the border zone between infarct scar and viable myocardium, and were mainly veins, since they did not have a lamina elastica interna. Increased wall thickness of resistance arteries in hypertension has been associated with an increased vasoconstrictor response, especially if the wall hypertrophy causes encroachment

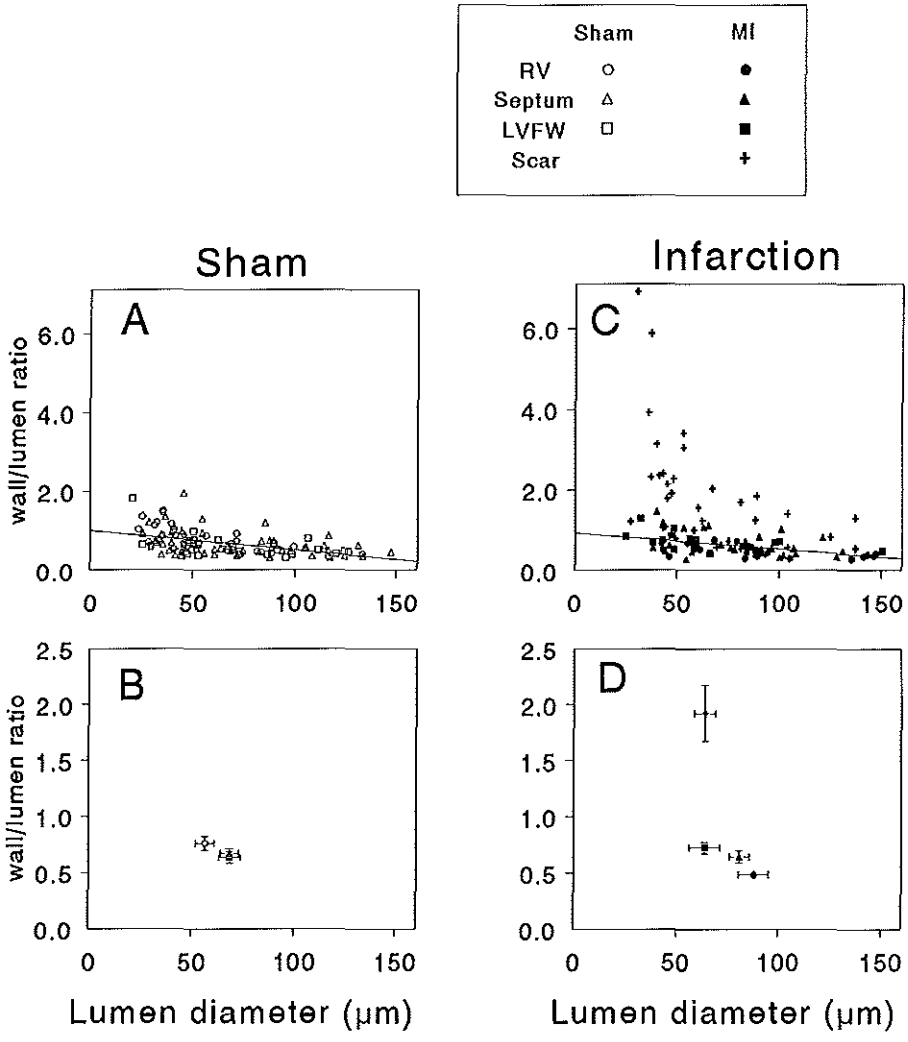

Figure 3.6 Resistance artery wall thickness
Vessel wall thickness as indexed by vessel wall to lumen (W/L) area ratio. Panel A: relationship between vessel lumen diameter and wall/lumen ratio in different regions of sham-operated hearts. Panel B: mean vascular dimension and wall/lumen ratio in different regions of sham-operated hearts. Panel C: relationship between vessel lumen diameter and wall/lumen ratio in different regions of MI hearts. The regression line is derived from all measurements in non-infarcted myocardium, which was not different from the regression line in sham-operated hearts. Wall/lumen ratio of vessels in scar tissue is clearly greater. Panel D: mean vascular dimension and wall/lumen ratio in different regions of MI hearts.

on the inner lumen (Mulvany *et al.*, 1978; Folkow & Karlström, 1984; Korner *et al.*, 1989). The finding of much thicker walls of resistance arteries in the scar than vessels in contractile myocardium with the same luminal cross-sectional area can offer a feasible explanation for the increased vasoconstrictor response. Other factors may also be involved in the increased vasoconstrictor response, such as i) increased length of the resistance vasculature, ii) altered fibrous matrix of the vessel wall (Folkow & Karlström, 1984), iii) isoform shift of actin in vascular smooth muscle (Owens & Thompson, 1986), iv) altered pharmacological profile of neovasculature (Andrade *et al.*, 1992a, 1992b) and coronary collaterals (Harrison *et al.*, 1986; Peters *et al.*, 1989). However, decreased vasodilation to the receptor- and endothelium-independent vasodilator nitroprusside suggests a structural rather than a pharmacological factor in the different vasoreactivity.

Vascularization of scar tissue in rat MI hearts would mainly depend on angiogenesis during the healing phase, as an intense inflammatory reaction precedes scar formation (Fishbein *et al.*, 1978a; Kumar *et al.*, 1983). Inflammatory modulation of the structure of newly forming vessels is possible, like the arteriosclerotic changes of resistance arteries seen in cardiac allografts (Paul *et al.*, 1994; Furukawa *et al.*, 1996). However, at present it cannot be excluded that the original vascular bed is reperfused by ingrowing new vessels from the vascular beds of non-infarcted myocardium. Therefore, greater wall thickness in scar tissue might arise if reflow is established in partly obliterated vessels.

Conclusion

In MI-induced remodeling, decreased maximal myocardial perfusion is limited to the area with the most pronounced cardiomyocyte hypertrophy, the spared part of the left ventricular free wall. This region, bordering the infarcted area, is at risk during increased metabolic demands, such as during exercise and stress. Eventually, ischemic cell death in this region will contribute to the development of heart failure. In resting conditions, there is a small but substantial coronary flow to the scar tissue. Resistance arteries within the scar area have a greater wall to lumen ratio than vessels in normal hearts or than in other regions of MI hearts. Different morphology of resistance vessels in scar tissue may explain the increased vasoconstrictor response.

CHAPTER 4

SENSITIVITY TO ISCHEMIA OF CHRONICALLY INFARCTED RAT HEARTS; EFFECTS OF LONG-TERM DELAYED CAPTOPRIL TREATMENT

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ABSTRACT

Myocardial infarction (MI)-induced hypertrophy of non-infarcted myocardium, in parallel with interstitial and perivascular fibrosis and a decreased capillary density, could increase sensitivity to ischemia. The structural cardiac changes can be regressed by long-term captopril treatment. In the present study, ischemic sensitivity in relation to cardiac perfusion was studied in isolated perfused hearts of untreated and captopril-treated MI rats. In chronically (8 weeks) infarcted hearts, maximal vasodilation to administered adenosine and nitroprusside, as well as to endogenously released vasodilators during reperfusion, was decreased, suggesting impaired cardiac perfusion. Ischemic release of purines and lactate was reduced in MI hearts, indicating decreased sensitivity to ischemia of the remodeled myocardium. Captopril treatment (3-8 weeks post MI), regressing hypertrophy without affecting flow capacity of the coronary vascular bed, restored maximal cardiac perfusion. Ischemic ATP breakdown was not affected by captopril, whereas lactate release was even further reduced, suggesting alterations towards a more aerobic ATP production. These data indicate that despite the reduced maximal cardiac perfusion, remodeled myocardium of MI hearts was less sensitive to ischemia. Regression of hypertrophy by chronic captopril restored maximal cardiac perfusion and led to a better preservation of aerobic ATP production during ischemia.

INTRODUCTION

Cardiac hypertrophy is regarded an independent risk factor for cardiovascular mortality (Levy *et al.*, 1990). One of the proposed mechanisms is an increased sensitivity to ischemia of the hypertrophied myocardium. In rats, concentric but not eccentric hypertrophy is associated with enhanced ischemic vulnerability, which could be attributed to differences in cardiac perfusion (Harmsen *et al.*, 1994). Myocardial infarction (MI) evokes compensatory hypertrophy of the non-infarcted myocardium of a mixed eccentric/concentric type, since myocyte dimensions increase both in width and in length (Anversa *et al.*, 1985a). The reactive hypertrophy after MI is accompanied by interstitial (van Krimpen *et al.*, 1991) and perivascular (Sun *et al.*, 1994) fibrosis, and a reduction in

capillary density (Anversa *et al.*, 1985b, 1986a). These changes also occur in concentric hypertrophy, in which an increased sensitivity to ischemia has been reported (Canby & Tomanek, 1990). However, information about ischemic vulnerability of the remodeled myocardium post MI is not available yet.

Treatment with angiotensin converting enzyme (ACE) inhibitors has now become a common therapy after MI, since it improves heart function and prognosis in patients (M. Pfeffer *et al.*, 1992), as well as in MI rats (J. Pfeffer *et al.*, 1987; Schoemaker *et al.*, 1991). An important mode of action of ACE inhibitor therapy is its effect on the structural changes in surviving myocardium post MI. Captopril treatment prevents or regresses cardiac hypertrophy (J. Pfeffer *et al.*, 1985) and interstitial fibrosis (van Krimpen *et al.*, 1991) of spared myocardium. However, it is still unknown whether these effects on remodeling also increase tolerance to ischemia. Promising results were obtained with enalapril treatment in spontaneously hypertensive rats (Schoemaker *et al.*, 1994).

In the present study, sensitivity to ischemia in relation to cardiac hypertrophy and remodeling, as well as baseline and maximal cardiac perfusion, were investigated in chronically infarcted rats. In parallel, the effects of long-term captopril treatment were studied.

MATERIALS AND METHODS

Male, Wistar rats (270-320 g, Harlan Zeist, The Netherlands) were used in this study. Rats were housed at a 12 h light/dark cycle with standard rat chow and water available ad lib. Captopril (Squibb, Princeton, NJ, USA) treatment (2 g/l of drinking water; J. Pfeffer *et al.*, 1985, 1987) was started 3 weeks after infarction and was continued until the end of the experiment, 8 weeks after surgery. The experiments were approved by the University ethics committee for the use of experimental animals. Myocardial infarction was induced by coronary artery ligation as described in detail in chapter 2 (page 32).

Response to coronary vasodilators: At 8 weeks after surgery, the heart was rapidly excised under pentobarbital anesthesia and mounted for Langendorff perfusion and instrumented for functional measurements as described in detail in chapter 2 (page 32). Left ventricular end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume. After a stabilization period of 15 min, maximal coronary flow during vasodilation was determined. 0.1 ml of a 10^{-2} M adenosine solution (Janssen Chimica, Geel, Belgium) was injected into the perfusing buffer just before entering the coronary arteries, followed by a re-stabilization period, and subsequently 0.1 ml of a 10^{-2} M sodium

nitroprusside solution (Department of Pharmacy, University Hospital Dijkzigt, Rotterdam) was injected into the perfusing buffer. These doses were found to induce maximal effect in dose-response curves, obtained in pilot experiments. Coronary flow is expressed as absolute values of ml/min, as index for flow capacity of the coronary vascular bed, as well as values corrected for heart weight (mainly myocytes), representing cardiac perfusion.

Ischemia and reperfusion: Perfusion pressure was abruptly lowered to 15 mmHg. In pilot experiments, continued pacing during ischemia was often found to induce severe arrhythmias. Therefore, during ischemia, hearts were allowed to beat spontaneously. Because of the very low values, coronary flow during ischemia was measured by timed collection of coronary effluent. During the last minute of ischemia and the first minute of reperfusion, coronary effluent was sampled on ice and stored at -80°C until assayed for lactate and purines. After 30 min of low-flow ischemia, perfusion pressure was reset to 85 mmHg. Maximal coronary flow during reperfusion (reactive vasodilation) was determined.

Determination of purines and lactate: Release of purines into the coronary effluent, calculated as concentration times flow per heart weight, was used to investigate loss of ATP catabolites from the myocytes (Schrader *et al.*, 1977; Achterberg *et al.*, 1984). Cardiac loss of ATP catabolites during ischemia correlates well with myocardial ATP breakdown, as measured with [^{31}P]-nuclear magnetic resonance (Harmsen & Seymour, 1988). Concentration of purines was determined as described in detail by Smolenski *et al.* (1990). Briefly, the ATP catabolites uric acid, uracil, cytidine, adenosine, inosine, hypoxanthine, xanthine and uridine were determined by high-performance liquid chromatography on a C_{18}-μBondapak column (Millipore Waters Co., Milford, Mass, USA). Coronary effluent (100 μl) was injected directly into the system, eluted with a 15% (v/v) solution of acetonitrile in 150 mM potassium dihydrogen orthophosphate, containing 150 mM potassium chloride adjusted to pH 6.0 with potassium hydroxide. Peaks were monitored by absorption at 254 nm or 280 nm for uric acid. The release of lactate into the coronary effluent was used as an indicator of the activity of anaerobic glycolysis in the cardiomyocyte (Vrobel *et al.*, 1982). Lactate concentration in coronary effluent was determined as described in detail by Marbach & Weil (1967) (reagents: Sigma Diagnostics, Deisenhofen, Germany). Briefly, lactic acid was converted by lactate oxidase to pyruvate and H_2O_2. In the presence of the formed H_2O_2, peroxidase catalyzed the oxidative condensation of chromogen precursors to produce a coloured dye with an absorption maximum at 540 nm. Lactate concentration could be determined, being directly proportional to the increase of absorption at 540 nm.

Data analysis: Data are expressed as group means ± S.E.M., unless indicated otherwise. Only data from infarcted hearts with an infarcted area comprising the major part of the left ventricular free wall were included in the study, since smaller infarctions are known to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Data were analyzed using one-way analysis of variance (ANOVA), followed by a post-hoc *t*-test (Wallenstein *et al.*, 1980). Differences were considered statistically significant if $P<0.05$.

RESULTS

All infarctions were transmural and were located in the lateral (free) wall of the left ventricle. Four hearts (2 in the untreated MI group and 2 in the captopril-treated MI group) were excluded from analysis because only a minor part of the left ventricular free wall was infarcted. Results comprise data from 7 sham hearts, 10 untreated MI hearts, and 7 captopril-treated MI hearts. Heart weight was significantly increased after MI compared to sham-operated controls. Captopril treatment reduced weight of MI hearts to sham values. Because body weight was reduced as well in captopril treated rats, the ratio of heart weight to body weight was not influenced by captopril treatment (Table 4.1).

Coronary vasodilation

Baseline coronary flow was lower in MI hearts, although this did not reach statistical significance (Figure 4.1). However, cardiac perfusion (coronary flow corrected for heart weight) was found to be significantly depressed after MI. Captopril treatment did not alter baseline coronary flow or cardiac perfusion.

Maximal *absolute* values for coronary flow, indicating maximal flow capacity of the coronary vascular bed, were decreased in MI hearts with adenosine but not with nitroprusside. After correction for heart weight, maximal cardiac perfusion with both vasodilators was reduced. Chronic captopril treatment restored maximal cardiac perfusion in MI hearts.

Reactive vasodilation during reperfusion resulted in similar maximal values for cardiac perfusion as obtained after exogenous vasodilator administration for all groups (Table 4.2). Maximal reactive vasodilation was depressed in MI hearts, but was restored by captopril treatment.

Table 4.1 Body weight and cardiac weight

	SHAM	MI	MI+CAP
BW (g)	417 ± 13	402 ± 10	341 ± 11*#
HWW (g)	1.08 ± 0.04	1.34 ± 0.07*	1.07 ± 0.06#
HWW/BW ($x10^{-3}$)	2.6 ± 0.1	3.3 ± 0.2*	3.2 ± 0.2*

MI: untreated infarcted hearts; MI+CAP: captopril-treated infarcted hearts; BW: body weight; HWW: heart wet weight; HWW/BW: heart wet weight to body weight ratio. *: Significantly different from shams, #: Significantly different from untreated infarction.

Table 4.2 Peak cardiac perfusion after vasodilators and during reperfusion

	SHAM	MI	MI+CAP
Adenosine	25.0 ± 2.0	16.7 ± 1.2*	24.3 ± 1.5#
Nitroprusside	25.3 ± 2.3	18.2 ± 1.2*	23.5 ± 1.2#
Reperfusion	23.5 ± 1.2	15.2 ± 1.1*	20.8 ± 1.0#

Maximal cardiac perfusion (ml/min.g) after intracoronary injection of adenosine, of nitroprusside, and during reperfusion after 30 min of low-flow ischemia. MI: untreated infarcted hearts; MI+CAP: captopril-treated infarcted hearts. *: Significantly different from shams; #: Significantly different from untreated infarcted hearts.

Left ventricular function during ischemia and reperfusion

At baseline conditions, left ventricular systolic pressure was significantly decreased after MI (53 ± 7 vs 79 ± 12 mmHg in shams). Furthermore, contractility and relaxation were depressed in MI hearts, indicated by a decreased peak velocity of pressure change ($+(dP/dt)_{max}$ and $-(dP/dt)_{max}$). Chronic captopril treatment did not significantly alter these parameters (Figure 4.2).

Lowering the perfusion pressure to 15 mmHg resulted in low-flow ischemia (0.86 ± 0.05, 0.78 ± 0.07 and 0.78 ± 0.07 ml/min.g, in shams, untreated MI and captopril-treated MI hearts, respectively, at the beginning of the ischemic period). Resetting the perfusion pressure to 85 mmHg induced reactive vasodilation with a maximum after approximately 1 min. Captopril restored the reduced maximal postischemic perfusion in MI hearts (Table 4.2), but did not influence perfusion at other timepoints.

Ischemia caused a marked bradycardia (heart rate 65 ± 19, 45 ± 5 and 53 ± 13 min^{-1} in shams, untreated MI and captopril-treated MI hearts, respectively, after 30 min of ischemia). Reperfusion quickly restored heart rate to baseline values (281 ± 29, 298 ± 17

Table 4.3 Concentrations (μM) of purines and lactate in coronary effluent

		SHAM	MI	MI+CAP
Purines	Ischemia	13.1 ± 1.1	10.4 ± 1.1*	7.3 ± 0.6*
	Reperfusion	3.1 ± 0.3	3.1 ± 0.5	2.2 ± 0.2
Lactate	Ischemia	981 ± 134	941 ± 68	444 ± 56*#
	Reperfusion	111 ± 28	175 ± 42	103 ± 11

MI, untreated infarcted hearts; MI+CAP, captopril treated infarcted hearts; Ischemia, last minute of 30 minutes of ischemia; Reperfusion, first minute of reperfusion. * Significantly different from shams, # significantly different from untreated infarcted hearts.

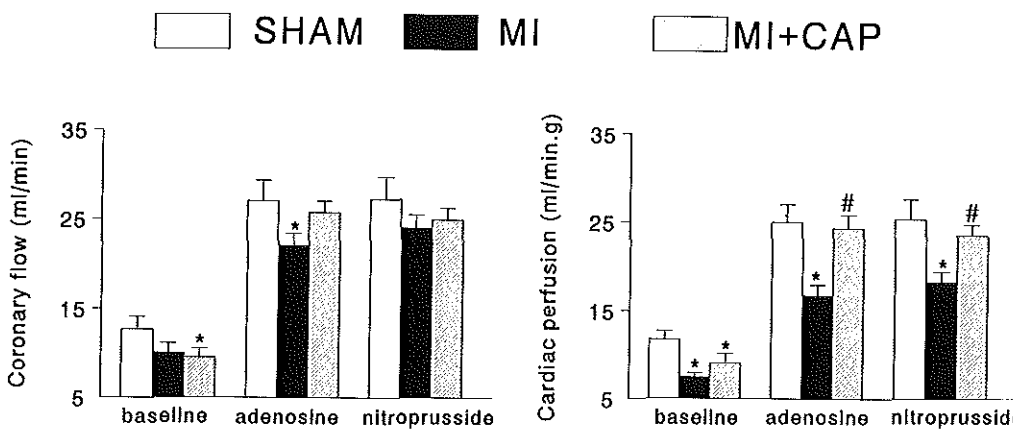

Figure 4.1: Coronary flow (absolute values, left panel) and coronary flow corrected for cardiac wet weight (cardiac perfusion, right panel), at baseline and after intracoronary bolus injection of adenosine and nitroprusside. Open bars: sham hearts, black bars: untreated infarcted hearts (MI), hatched bars: captopril treated infarcted hearts (MI + CAP). * Significantly different from sham values, # significantly different from untreated infarcted hearts.

and 277 ± 27 min^{-1} in shams, untreated MI and captopril-treated MI hearts, respectively, after 1 min of reperfusion). Furthermore, a profound depression of left ventricular function was present during ischemia. Pressure-rate product (left ventricular systolic pressure times heart rate), as index for cardiac work, averaged 1710 ± 166 $mmHg.sec^{-1}$ in sham hearts during ischemia, and was significantly lower in MI hearts (953 ± 171 and 825 ± 218 $mmHg.sec^{-1}$ in untreated and captopril-treated hearts, respectively). During reperfusion, parameters for left ventricular function were restored to baseline values. $+(dP/dt)_{max}$ was significantly lower in MI hearts compared to shams during the entire experiment, while these differences for $-(dP/dt)_{max}$ and systolic pressure only reached statistical significance at baseline and during reperfusion. Captopril therapy did not significantly alter *in vitro* functional parameters in MI hearts at any timepoint.

Cardiac release of purines and lactate

During the last minute of ischemia, the concentration of purines in coronary effluent was significantly lower in MI hearts compared to shams, and even further reduced after captopril treatment (Table 4.3). Since coronary flows were not similar for the experimental groups, purine release was calculated as concentration times flow per heart weight.

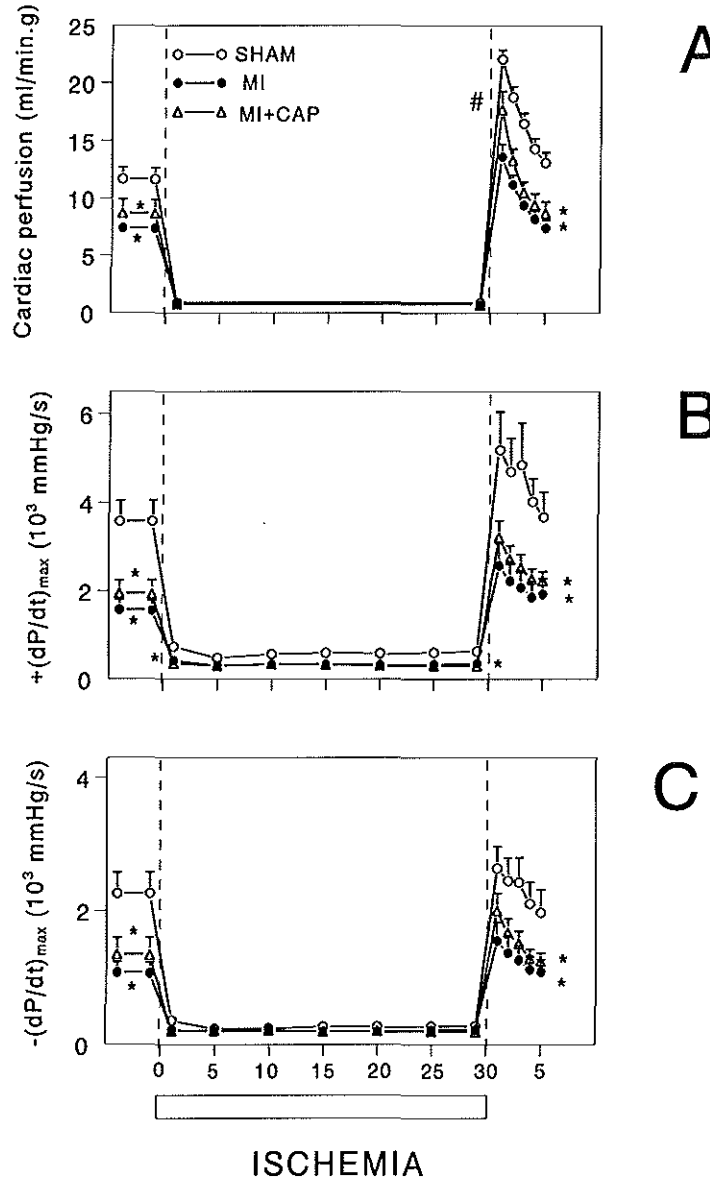

Figure 4.2: Physiological parameters during Langendorff perfusion, at baseline, during ischemia and reperfusion. Open circles: sham hearts, black circles: untreated infarcted hearts (MI), triangles: captopril-treated infarcted hearts (MI + CAP). Panel A: cardiac perfusion, Panel B: peak velocity of left ventricle pressure rise during contraction ($+(dP/dt)_{max}$), Panel C: peak velocity of left ventricle pressure decline during relaxation ($-(dP/dt)_{max}$). * Significantly different from sham values, # significantly different from untreated infarcted hearts.

Purine release was also lower in MI hearts than in shams, which was not affected by captopril treatment. Similarly, during the first minute of reperfusion, release of purines was significantly lower in untreated MI hearts compared to shams, and not altered by captopril treatment (Figure 4.3).

Lactate concentrations in coronary effluent were comparable in untreated MI hearts and shams, but significantly reduced in captopril-treated MI hearts (Table 4.3). Cardiac release of lactate appeared to be significantly lower in MI hearts, and even further reduced after captopril treatment (Figure 4.3).

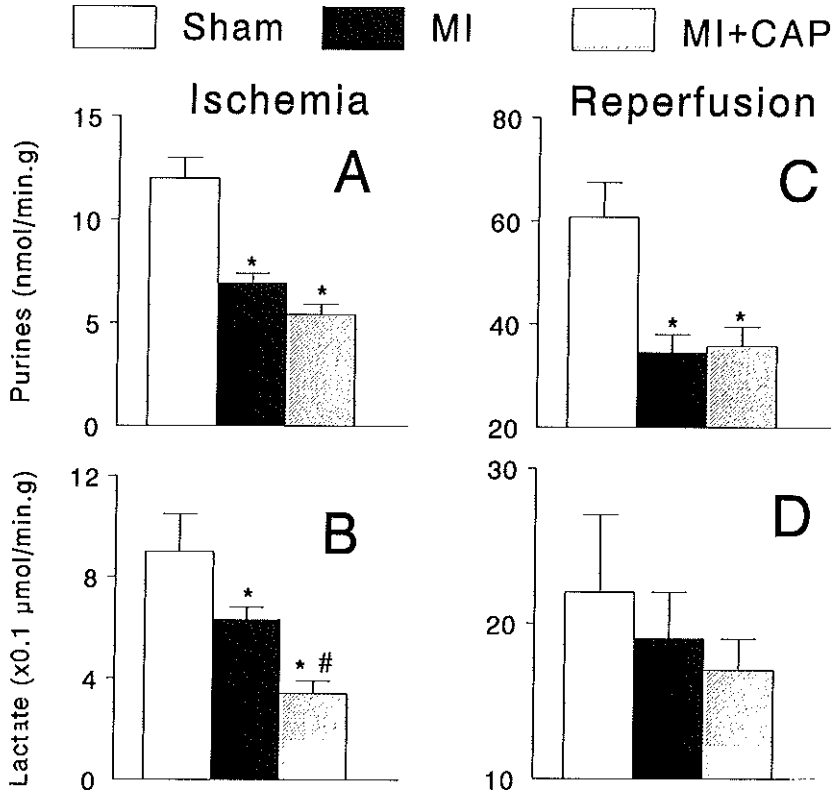

Figure 4.3: Cardiac release of purines (Panels A and C) and lactate (Panels B and D) into coronary effluent, during ischemia (Panels A and B) and reperfusion (Panels C and D). Open bars: sham hearts, black bars: untreated infarcted hearts (MI), hatched bars: captopril treated infarcted hearts (MI + CAP). * Significantly different from sham values, # significantly different from untreated infarcted hearts.

DISCUSSION

Hypertrophy and remodeling of the spared myocardium after MI may increase the sensitivity to ischemia of this tissue, and hence the risk of additional morbidity, and mortality. In the present study, the sensitivity of remodeled, chronically infarcted hearts to acute ischemia in relation to cardiac perfusion, and the effects of long-term captopril treatment were studied.

Maximal vasodilation with adenosine and nitroprusside

Ischemia evokes release of vasodilatory substances like adenosine (Saito *et al.*, 1981) and nitric oxide (Kostic & Schrader, 1992). Vascular changes associated with postinfarction remodeling, such as perivascular fibrosis (Sun *et al.*, 1994), could mechanically restrict vasodilation. To investigate this possibility, maximal coronary flow to exogenous adenosine, as well as to nitroprusside, was measured.

Maximal coronary flow to adenosine, but not to nitroprusside, was found to be reduced in MI hearts. These *absolute* values, not corrected for heart weight, represent maximal flow through the coronary vascular bed. The observation that vasodilation induced by nitroprusside did not differ between MI hearts and sham hearts, implies that the maximal flow capacity of the coronary vascular bed is not decreased in MI hearts. In the presence of a permanently occluded left descending coronary artery (one of the 3 major coronary arteries), a maximal flow that is not different from maximal flow in sham hearts, indicates angiogenesis or growth of native vessels of the vascular bed of the remaining 2 patent coronary arteries. In the present study, only measurements of total coronary flow, without evaluation of regional distribution, were obtained. Therefore, the flow that is actually directed to the non-infarcted part of the left ventricle, with increased metabolic demands to compensate for the loss of contractile tissue in the infarcted area, is unknown. Nevertheless, it is clear from our observations that the remodeling induced by MI does not cause a mechanical limitation to vasodilation. This is in agreement with results from studies in experimental hypertension, showing that coronary medial thickening (not occurring in MI hearts), rather than perivascular fibrosis is associated with decreased maximal vasodilation (Brilla *et al.*, 1991). The reduced maximal flow with adenosine

would then be related to changed pharmacological rather than to structural properties of the coronary vascular bed.

Maximal cardiac perfusion, *i.e.* coronary flow per heart weight, however, was decreased for both vasodilators. This is in agreement with results from *in vivo* studies of coronary reserve in the MI rat model (Karam *et al.*, 1990). Insufficient vascular growth relative to the increase of cardiomyocyte volume in postinfarction cardiac remodeling (Anversa *et al.*, 1986a; Chapter 2) could be responsible for this phenomenon.

In the present study, captopril treatment reduced heart weight without affecting the maximal flow through the coronary vascular bed, and hence restored maximal cardiac perfusion. Similar findings have been reported with spontaneously hypertensive rats (Canby & Tomanek, 1989). The observation that regression of hypertrophy by captopril is associated with normalization of maximal cardiac perfusion supports the hypothesis that a disproportionate growth of myocyte volume relative to cardiac vascularization is responsible for the reduced maximal tissue perfusion in MI hearts.

Vasodilation during reperfusion

The heart responds to transient ischemia with reactive vasodilation due to release of vasodilatory substances, including nitric oxide (Kostic & Schrader, 1992) and adenosine (Saito *et al.*, 1981). Maximal coronary flow during reperfusion, in the present study, reached similar values as obtained with administered vasodilators, in each experimental group. These data indicate that maximal coronary vasodilation may indeed occur *in vivo* as well after an ischemic episode. Similar to the maximal values obtained after administered vasodilators, peak cardiac perfusion during reperfusion was depressed in MI compared to sham hearts, and was restored after captopril treatment. The explanation for these findings may therefore be similar to that for the maximal vasodilation with administered vasodilators.

Sensitivity to acute ischemia

Cardiac remodeling due to chronic hypertension can increase sensitivity to ischemia (Canby & Tomanek, 1990; Harmsen *et al.*, 1994). The pressure overload-induced cardiac remodeling shows many similarities with post MI remodeling, including myocyte

hypertrophy, interstitial and perivascular fibrosis, and reduced capillary density. One of the explanations for enhanced ischemic vulnerability is the increased oxygen diffusion distance, caused by myocyte hypertrophy with a relatively inadequate increase of capillary surface. This is supported by metabolic adaptations within the myocytes, including a shift from V_1 to V_3 isomyosin (Geenen *et al.*, 1989), with a lower ATPase activity, and thus a lower oxygen consumption for the same force of contraction. In MI hearts, the marked depression of peak velocity of left ventricular pressure rise rather than the left ventricular pressure per se, indicates a more economical ATP usage.

Acute ischemia results in cardiomyocyte hypoxia, which in turn impairs oxidative phosphorylation (Wilson *et al.*, 1977), the primary means of ATP production under normal conditions (high ATP yield). During ischemia, decreased ATP regeneration can finally result in the loss of ATP catabolites, purines, from the cell (Schrader *et al.*, 1977). In order to preserve intracellular ATP levels during ischemia, anaerobic glycolysis is activated. However, anaerobic ATP production (low ATP yield) is insufficient to meet the cellular ATP demand (Hearse, 1979). Moreover, during anaerobic glycolysis intracellular pH falls, which hampers cell function (Katz, 1973).

In the present experiments, the ischemic ATP release from MI hearts was lower than from sham hearts, which could not be explained by differences in coronary flow or heart weight. Decreased ATP loss was also not attributable to a greater ATP production by anaerobic glycolysis in MI hearts compared to shams, since lactate release from MI hearts was lower as well. Therefore, the explanation for this phenomenon may rather lie in the lower ATP consumption in MI hearts, which would be in concordance with the slower contraction in the present study and the isomyosin shift reported by Geenen *et al.* (1989).

Although captopril treatment started after 3 weeks in MI rats has been found to restore *in vivo* heart function (Schoemaker *et al.*, 1991) already after 2 weeks of treatment, and remained restored up to 3 months of treatment (M. Pfeffer *et al.*, 1985), *in vitro* left ventricular function was not significantly improved. The discrepancy can probably be explained by the measurement of *in vitro* left ventricular function as mechanical parameters of isovolumic contraction at a fixed preload and heart rate, which may not correlate well with *in vivo* pump capacity of the heart. Captopril treatment did not alter the reduction in

ATP-breakdown in MI hearts compared to sham hearts, but further reduced lactate release. Although ACE inhibitor treatment has been found to reverse the V_1 to V_3 isomyosin shift in MI hearts (Michel *et al.*, 1988), *in vitro* cardiac dynamics were not changed accordingly by captopril. Thus, like untreated MI hearts, captopril-treated MI hearts probably had a lower ATP turnover than sham hearts. Moreover, since lactate release in MI hearts was even further reduced by captopril, aerobic ATP production during acute ischemia in MI hearts may be longer preserved after captopril treatment. It is feasible that the captopril-induced regression of hypertrophy could importantly contribute to a more aerobic metabolism during ischemia. By regressing hypertrophy but not vessel growth, captopril therapy would reduce the oxygen diffusion distance. This is supported by the restoration of maximal cardiac perfusion by captopril treatment. In hypertrophied rat hearts due to pressure overload, chronic captopril therapy restored the capillary density (Canby & Tomanek, 1989) and decreased myocyte sensitivity to hypoxia (Canby & Tomanek, 1990).

In conclusion, MI-related cardiac remodeling was associated with a decreased maximal cardiac perfusion to administered as well as to endogenous vasodilators. However, MI hearts were less sensitive to an additional acute ischemic period, as indicated by a lower cardiac ATP breakdown. Captopril treatment resulted in a restoration of maximal cardiac perfusion. The decreased ischemic ATP breakdown in MI hearts was unaffected by captopril treatment, but was accompanied by a lower lactate release, suggesting an alteration towards a more aerobic ATP production.

Clinical implications

Since MI commonly occurs as a complication of diffuse coronary artery disease, MI patients will be at risk for additional ischemic attacks in the remaining myocardium. The reduced coronary reserve in MI hearts, as indicated by reduced maximal cardiac perfusion, may limit cardiac function and lead to ischemia during periods of increased coronary flow demand, such as occurs during stress and exercise. Restoration of maximal perfusion with captopril may therefore reduce the number of ischemic episodes in MI patients, and this would be, additional to the hemodynamic improvement, another mechanism for increased exercise tolerance and improved clinical outcome.

Lactate production causes proton accumulation within the myocyte, which hampers

cellular function in an already stressed hemodynamic situation. Similar to MI rats, a reduced ischemic lactate production in captopril treated MI patients may save myocytes for acidosis induced damage (Armiger *et al.*, 1977).

CHAPTER 5

EARLY CAPTOPRIL TREATMENT PREVENTS HYPERTROPHY BUT NOT VASCULAR GROWTH AFTER MYOCARDIAL INFARCTION IN RATS

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Submitted for publication

ABSTRACT

Delayed captopril treatment, started after the healing phase of myocardial infarction (MI), improves perfusion by reducing tissue weight without affecting vascular capacity of the heart. This results in a preservation of aerobic metabolism when remodeled MI hearts are subjected to an additional period of low-flow ischemia. Early captopril treatment (ECT), during the healing phase, prevents reactive hypertrophy. However, if angiotensin II-induced vascular growth is also prevented, perfusion would not be improved. The present study evaluated the effects of ECT on regional perfusion and ischemic sensitivity. Wistar rats, subjected to coronary artery ligation, received normal drinking water or captopril (2 g/l drinking water, started 1 day after MI). After 3 weeks, regional coronary flow was measured in isolated hearts, using radioactive microspheres. Maximal vascular capacity was measured during nitroprusside-induced vasodilation. In other MI hearts, release of purines and lactate in response to low-flow ischemia was determined. Despite regional differences in the degree of compensatory hypertrophy in MI hearts, ECT caused a similar weight reduction in all parts. However, vascular capacity was not affected by ECT and even tended to be higher in the viable part of LV free walls. Preventing compensatory hypertrophy but not angiogenesis resulted in improved peak tissue perfusion. ECT reduced ischemic lactate release during an additional period of low-flow ischemia. These data indicate that ECT beneficially influences the vascularization/tissue mass ratio, which is reflected in a better preservation of aerobic metabolism during an additional ischemic event.

INTRODUCTION

Following myocardial infarction (MI), treatment with angiotensin I converting enzyme (ACE) inhibitors has been shown to decrease reactive hypertrophy and attenuate progressive ventricular dilatation in experimental animals as well as in patients (J. Pfeffer *et al.*, 1985, 1987, 1988; Pfeffer & Pfeffer, 1988; Raya *et al.*, 1989; Sharpe *et al.*, 1990; Litwin *et al.*, 1991a, 1991b; Oldroyd *et al.*, 1991; J. Pfeffer, 1991; Sharpe *et al.*, 1991; Bonaduce *et al.*, 1992; Jugdutt *et al.*, 1992; Galcera-Tomas *et al.*, 1993; Ray *et al.*, 1993;

Jugdutt *et al.*, 1995; Jugdutt, 1995). Moreover, captopril treatment has been proved to reduce morbidity and mortality (Pfeffer *et al.*, 1985; Rutherford *et al.*, 1994; ISIS-4, 1995).

In chapter 4, delayed captopril treatment (started after completion of scar formation, at 3 weeks) in the rat MI model was found to induce regression of hypertrophy without effects on flow capacity of the coronary vascular bed. Consequently, cardiac perfusion and metabolic response to an additional ischemic period were improved. Delayed captopril treatment in this model has been shown to improve cardiac function (Schoemaker *et al.*, 1991). However, early captopril treatment (started at 1 day after infarction) failed to improve pump capacity of the heart. Since besides in hypertrophy and remodeling, angiotensin II is an important growth factor in angiogenesis (Le Noble *et al.*, 1993; Munzenmaier & Greene, 1996), we hypothesized that failure of early captopril treatment to improve cardiac function in MI rats is caused by the impairment of adaptive vascular growth associated with early reactive hypertrophy and remodeling. In order to test this hypothesis, regional cardiac mass and regional capacity of the coronary vasculature were measured in isolated perfused hearts of MI rats, treated with captopril from 1 day to 3 weeks after MI. In addition, metabolic response to an additional low-flow ischemic period were studied in a separate group of rats.

MATERIALS AND METHODS

Male, Wistar rats (270-320 g, Harlan, Zeist, The Netherlands) were used in this study. Rats were housed under a 12 h light/dark cycle with standard rat chow and water available at libitum. Rats were subjected to coronary artery ligation or sham operation as described in detail in chapter 2 (page 32). MI rats were randomised to receive either normal drinking water or captopril (Squibb, Princeton, NJ, USA) treatment (2 g/l of drinking water; J. Pfeffer *et al.*, 1985, 1987). Captopril treatment was started 24 h after infarction and continued until the end of the experiment, at 3 weeks after surgery. Since in previous studies no effects of long-term captopril therapy on hypertrophy or remodeling parameters could be found in sham-operated rats (van Krimpen *et al.*, 1991), we only studied effects of treatment on MI rats. The experiments were approved by the University Ethics Committee for the use of experimental animals.

At 3 weeks after surgery, the heart was excised under pentobarbital anesthesia, and mounted for Langendorff perfusion, and instrumented for functional measurements as described in detail in chapter 2 (page 32).

Regional coronary flow protocol The distribution of coronary flow was determined with 15 ± 1

(S.D.) μm diameter microspheres labelled with either ^{113}Sn, ^{95}Nb, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, USA). After a stabilisation period of 20 min, microspheres were injected to obtain baseline values for regional coronary flow. Subsequently, maximal coronary flow was determined using a 0.1 ml bolus injection of a 10^{-2}M sodium nitroprusside solution (Dijkzigt University Hospital's pharmacy, Rotterdam, The Netherlands). Microspheres were injected when maximal coronary flow was reached. The dose of sodium nitroprusside was based upon complete dose-response curves obtained in pilot experiments. For each measurement a suspension of 0.1 ml containing about 8,000 microspheres, labelled with one of the isotopes, was mixed and injected into the perfusing buffer just before it entered the coronary arteries. In pilot experiments, coronary flow after injection of about 25,000 microspheres (10.5 ± 2.4 ml/min, n=5), did not differ from baseline coronary flow (11.4 ± 2.7 ml/min, n=5). Coronary effluent was collected in the first min after injection of the microspheres in order to quantify microsphere by-pass of the capillary bed through leakage or arteriovenous anastomotic flow. After the experiment the ventricles were separated from atria and large vessels, and subsequently divided into right ventricle, interventricular septum and left ventricle free wall. Left ventricular free walls of MI hearts were further divided into viable tissue and scar tissue, by macroscopic appearance (see Figure 3.1, page 48). Tissues were weighed and radioactivity in tissues was counted for 10 min in a γ-scintillation counter (Packard, Miniaxi autogamma 5000), using suitable windows for discriminating the different isotopes. All data were processed by specially designed computer programs (Saxena *et al.*, 1980).

Ischemia and reperfusion protocol Perfusion pressure was abruptly lowered from 85 to 15 mmHg. In pilot experiments, continued pacing during ischemia was often found to induce severe arrhythmias. Therefore, during ischemia, hearts were allowed to beat spontaneously. Coronary effluent was sampled at 3 timepoints: after stabilization during baseline perfusion, during the last minute of low-flow ischemia, and during the first minute of reperfusion. Collected samples were kept on ice and stored at -80°C until assayed for purines and lactate.

Determination of purines and lactate Myocardial release of purines into the coronary effluent, calculated as concentration x flow per heart weight, was used to investigate loss of ATP catabolites from the myocytes (Schrader *et al.*, 1977; Achterberg *et al.*, 1984). The loss of ATP catabolites from myocytes during ischemia correlates well with myocardial ATP breakdown, as measured with [^{31}P]nuclear magnetic resonance (Harmsen & Seymour, 1988). The concentration of purines was determined as described in detail by Smolenski *et al.* (1990). Briefly, the ATP catabolites uric acid, uracil, cytidine, adenosine, inosine, hypoxanthine, xanthine and uridine were determined by high-performance liquid chromatography on a C18-μBondapak column (Millipore Waters CO., Milford, MA, USA). Coronary effluent (100 μl) was injected directly into the system, eluted with a 15% (v/v) solution of acetonitrile in 150 mM potassium dihydrogenorthophosphate, containing 150 mM potassium chloride adjusted to pH 6.0 with potassium hydroxide. Peaks were monitored by absorption at 254 nm and 280 nm for uric acid.

The release of lactate into the coronary effluent was used as an indicator of the activity of anaerobic glycolysis in the cardiomyocyte (Vrobel *et al.*, 1982). Lactate concentration in coronary

effluent was determined as described in detail by Marbach & Weil (1967) (reagents, Sigma Diagnostics, Deisenhofen, Germany). Briefly, lactic acid was converted by lactate oxidase to pyruvate and H_2O_2. In the presence of H_2O_2 formed, peroxidase catalyzed the oxidative condensation of chromogen precursors to produce a coloured dye with an absorption maximum at 540 nm. Lactate concentration could be determined, being directly proportional to the increase of absorption at 540 nm.

Data analysis Data are expressed as group means ±S.E.M., unless indicated otherwise. Only data from MI hearts with an infarcted area comprising the major part of the LV free wall were included in the study, since smaller infarctions are known to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Data were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc *t*-tests. Data from functional measurements during the low-flow ischemia protocol were regarded as repeated measurements and therefore analyzed using a two-way ANOVA. Differences were considered statistically significant if $P<0.05$.

RESULTS

Regional tissue perfusion

MI hearts weighed significantly more than sham-operated control hearts. Since body weight did not differ between MI and sham-operated rats, heart weight to body weight ratio was also increased (Table 5.1). MI-induced hypertrophy was most prominent in the LV free wall. Tissue mass of this region was significantly increased (+12%), despite replacement of most of the myocyte mass by relatively light scar tissue (180 ± 19 mg). Captopril treatment caused a reduction in body weight of MI rats, as well as prevention of reactive hypertrophy. The reduction of cardiac mass by captopril treatment was not confined to the most hypertrophied region, the LV free wall, but resulted from a reduced weight of all parts of MI hearts, including the infarct scar (105 ± 13 mg in MI hearts after captopril therapy).

Baseline coronary flow to the ventricles was similar in the different experimental groups (12.4 ± 0.9, 11.4 ± 0.5 and 11.8 ± 0.5 ml/min in sham, untreated MI and captopril-treated MI hearts, respectively). Similarly, coronary flow during nitroprusside-induced maximal vasodilation, indicating total coronary vascular capacity, was not different in sham and MI hearts (20.7 ± 0.7 and 19.9 ± 0.9 ml/min, respectively), neither was it different in hearts from captopril-treated MI rats (20.1 ± 0.8 ml/min). Baseline as well as maximal

Table 5.1 Body weight and regional myocardial tissue weight

	SHAM	MI	MI+CAP
n	16	16	20
BW (g)	368 ± 5	359 ± 4	334 ± 4*#
Total ventricular			
weight (mg)	1071 ± 21	1232 ± 56*	1033 ± 41#
weight/BW (mg/g)	2.92 ± 0.07	3.45 ± 0.18*	3.11 ± 0.14
n	9	9	13
LV free wall			
weight (mg)	572 ± 18	638 ± 17*	516 ± 14*#
weight/BW (mg/g)	1.54 ± 0.04	1.74 ± 0.05	1.52 ± 0.04#
Interventricular septum			
weight (mg)	237 ± 20	257 ± 17	209 ± 10#
weight/BW (mg/g)	0.64 ± 0.05	0.70 ± 0.05	0.62 ± 0.03
Right ventricle			
weight (mg)	220 ± 6	253 ± 28	190 ± 8#
weight/BW (mg/g)	0.60 ± 0.02	0.69 ± 0.08	0.56 ± 0.02

SHAM, hearts from sham-operated rats; MI, myocardial infarction; CAP, captopril; BW, body weight; LV free wall, left ventricular free wall (including scar tissue in infarcted hearts).
In the upper part of the table, data from ischemic sensitivity studies and regional flow studies are combined. In the lower part of the table, only data from the regional flow studies (in which regional tissue weight was determined) are presented. *: $P<0.05$ versus sham values; #: $P<0.05$ versus values of untreated infarcted hearts.

coronary flow to the different regions of MI hearts was not influenced by captopril treatment (Table 5.2).

In hearts from sham-operated animals, there was a highly significant correlation between regional coronary flow and tissue weight (at baseline: r=0.941, $P<0.0001$; during maximal vasodilation: r=0.954, $P<0.0001$; Figure 5.1, Panels A and B). Right ventricles and interventricular septa of MI hearts showed a similar weight-flow relationship as cardiac regions from sham-operated rats, but LV free walls deviated from the weight-flow relationship of control hearts. However, in MI hearts from captopril-treated rats, all contractile parts, including the LV free walls, showed a similar weight-flow relationship as that of tissues from sham-operated hearts. Global ventricular myocardial perfusion during peak vasodilation was decreased in MI hearts compared to shams (17.1 ± 0.8 versus 20.2 ± 0.8 ml/min.g), which was restored after captopril treatment (21.9 ± 1.0 ml/min.g). This restored peak perfusion was explained by an improved maximal perfusion of LV free walls and interventricular septa of MI hearts with captopril treatment (Table 5.3). The

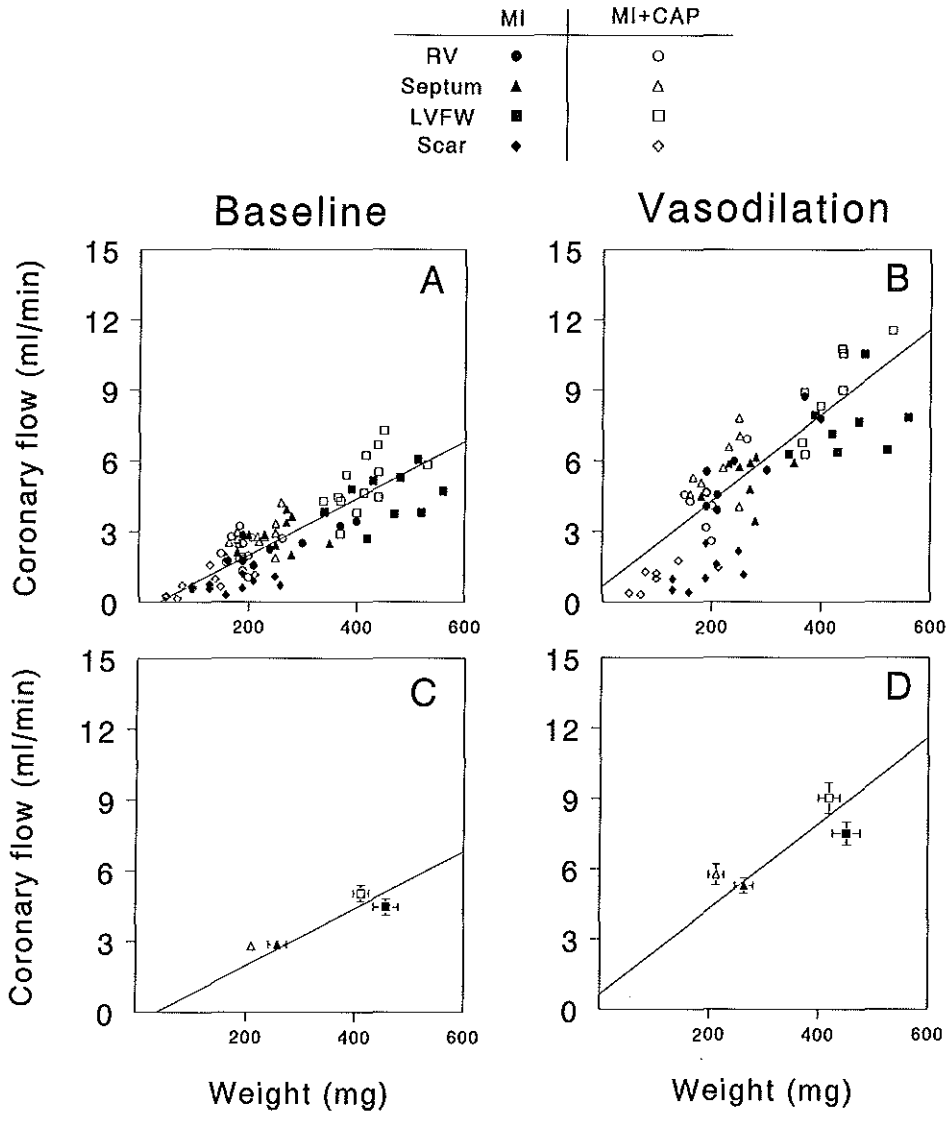

Figure 5.1: Relationship between regional coronary flow and tissue weight of different parts of hearts from untreated MI rats (black symbols) and captopril-treated MI rats (MI+CAP, open symbols). Panel A: During baseline perfusion. Panel B: During nitroprusside-induced maximal vasodilation. The line represents the relationship between coronary flow and weight of different parts of hearts from sham-operated rats, at baseline: Coronary flow (ml/min) = 0.012*Weight (mg) - 0.446; During maximal vasodilation: Coronary flow (ml/min) = 0.018*Weight (mg) + 0.648.
Panel C and D show the relationship of mean coronary flow and mean tissue weight of interventricular septa (triangles) and left ventricular free walls (squares) of MI hearts to the regression line of normal hearts.

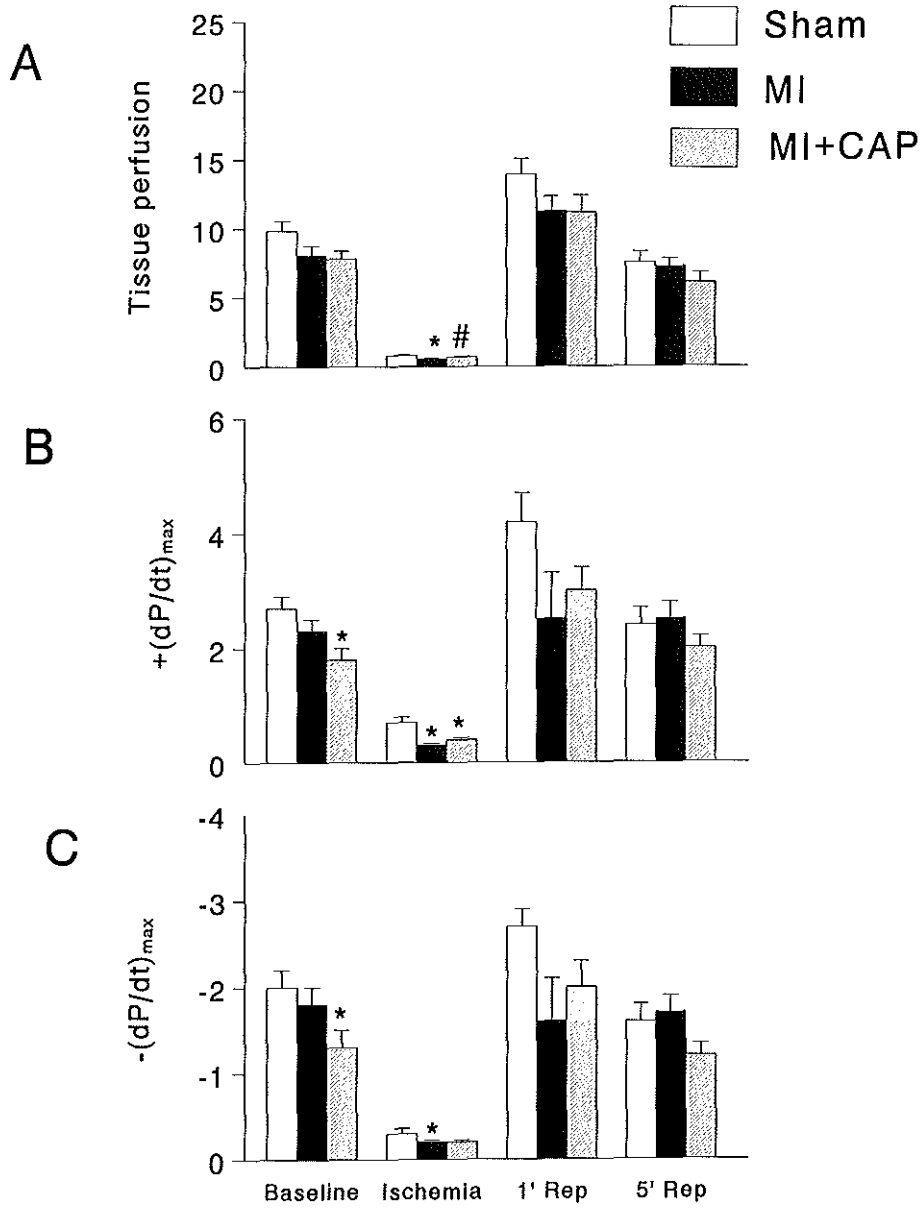

Figure 5.2: Functional parameters at baseline perfusion, after 30 min of ischemia, and at 1 min and 5 min of reperfusion (1' Rep and 5' Rep). Panel A: cardiac perfusion (ml/min.g). Panel B: Peak velocity of LV isovolumic contraction, $+(dP/dt)_{max}$ (10^3 mmHg/s). Panel C: Peak velocity of LV isovolumic relaxation, $-(dP/dt)_{max}$ (10^3 mmHg/s).
*: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI hearts.

improvement of perfusion of interventricular septa was mainly due to weight reduction, whereas increased perfusion of LV free walls appeared to be rather caused by a combination of weight reduction and an increased vascular capacity in this region (as indicated by an upward and leftward shift: Figure 5.1, Panels C and D). Scar tissue of MI hearts was hypoperfused compared to contractile parts, both in untreated and captopril-treated MI hearts, especially during maximal vasodilation of the coronary vasculature (Figure 5.1, Panels A and B).

Functional response to low-flow ischemia

Under baseline conditions, LV developed pressure was similar in untreated MI hearts and shams (78 ± 7 versus 84 ± 5 mmHg, respectively), but tended to be lower in MI hearts after captopril (65 ± 7 mmHg). Peak velocity of contraction and relaxation were lower in MI hearts, being significantly depressed in the MI group with captopril (Figure 5.2).

Lowering the perfusion pressure to 15 mmHg resulted in low-flow ischemia (1.43 ± 0.26, 0.66 ± 0.11, 1.02 ± 0.06 ml/min.g, in sham, untreated MI and captopril-treated MI hearts, respectively, at 1 min after onset of ischemia). Compared to values from

Table 5.2 Distribution of coronary flow (ml/min)

	SHAM	MI	MI+CAP
Baseline			
Viable LV free wall	6.4 ± 0.5	4.5 ± 0.3	5.1 ± 0.3
Scar tissue	...	0.7 ± 0.1	0.6 ± 0.1
Interventricular septum	2.9 ± 0.3	2.9 ± 0.2	2.8 ± 0.2
Right ventricle	1.8 ± 0.1	2.3 ± 0.2	2.1 ± 0.2
Atria and large vessels	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
By-pass flow	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.1
Nitroprusside			
Viable LV free wall	11.1 ± 0.7	7.5 ± 0.5	9.0 ± 0.7
Scar tissue	...	1.3 ± 0.3	1.0 ± 0.2
Interventricular septum	5.3 ± 0.4	5.3 ± 0.3	5.8 ± 0.5
Right ventricle	4.4 ± 0.2	5.8 ± 0.6	4.4 ± 0.5
Atria and large vessels	0.8 ± 0.2	1.0 ± 0.2	1.3 ± 0.5
By-pass flow	0.6 ± 0.3	0.7 ± 0.2	0.6 ± 0.1

Distribution of coronary flow at baseline, and during nitroprusside-induced maximal vasodilation over the different regions of hearts from sham-operated rats (SHAM), untreated infarcted rats (MI), and infarcted rats with captopril treatment (MI+CAP).

Table 5.3 Regional tissue perfusion (ml/min.g)

	SHAM	MI	MI+CAP
Baseline			
Viable LV free wall	11.1 ± 0.6	9.8 ± 0.8	12.2 ± 0.7#
Scar tissue	...	4.3 ± 0.5	5.9 ± 0.7
Interventricular septum	12.4 ± 0.7	11.4 ± 0.9	13.4 ± 0.7
Right ventricle	8.2 ± 0.5	9.4 ± 0.8	11.3 ± 1.0
Atria and large vessels	1.1 ± 0.4	1.5 ± 0.4	1.9 ± 0.4
Nitroprusside			
Viable LV free wall	19.3 ± 0.9	16.9 ± 1.1	21.4 ± 1.0#
Scar tissue	...	6.6 ± 1.2	9.7 ± 1.3
Interventricular septum	22.8 ± 1.7	20.5 ± 1.6	27.3 ± 1.7#
Right ventricle	19.9 ± 1.0	22.2 ± 1.3	22.8 ± 2.0
Atria and large vessels	2.5 ± 0.7	2.8 ± 0.4	4.9 ± 1.5

Regional tissue perfusion at baseline, and during nitroprusside-induced maximal vasodilation in hearts from sham-operated rats (SHAM), untreated infarcted rats (MI), and infarcted rats with captopril treatment (MI+CAP). #: $P<0.05$ versus untreated infarcted hearts.

sham hearts, cardiac perfusion was lower in MI hearts throughout the ischemic period, which was improved in captopril-treated MI hearts (at 30 min of ischemia: 0.81 ± 0.05, 0.52 ± 0.06, 0.68 ± 0.05 ml/min.g, in sham, untreated MI and captopril-treated MI hearts, respectively). Reperfusion of the ischemic myocardium was associated with a prompt increase of coronary flow above baseline values, settling back to baseline values within 5 minutes. Ischemia caused a marked bradycardia (57 ± 13, 61 ± 15, 70 ± 12 beats/min in sham, untreated MI, and captopril-treated MI hearts, respectively, after 30 min of ischemia). Reperfusion quickly restored heart rate to baseline values (254 ± 22, 315 ± 17, 301 ± 35 beats/min in sham, untreated MI, and captopril-treated MI hearts, respectively). Pressure-rate product (left ventricular systolic pressure x heart rate), as an index of cardiac work, was lower in MI hearts throughout the ischemic phase (at 30 min of ischemia: 1253 ± 199 versus 2127 ± 331 mmHg/s), but after captopril therapy this was less pronounced (1734 ± 209 mmHg/s). $+(dP/dt)_{max}$ and $-(dP/dt)_{max}$, indicating peak velocity of contraction and relaxation, respectively, were significantly decreased in MI hearts, irrespective of treatment. However, when comparing the ischemic values to the baseline values, LV function was more resistant to effects of ischemia in hearts from captopril-treated rats ($+(dP/dt)_{max}$ 74 ± 3% versus 85 ± 2% decrease from baseline values; $-(dP/dt)_{max}$ 79 ± 3 versus 88 ± 2% of baseline values, in MI hearts from rats with or without

Table 5.4 Concentrations (μM) of purines and lactate in coronary effluent

		SHAM	MI	MI+CAP
Purines	Baseline	2.2 ± 0.4	1.5 ± 0.2	1.4 ± 0.2
	Ischemia	7.9 ± 1.4	9.6 ± 3.1	4.1 ± 0.5
	Reperfusion	3.3 ± 0.6	3.4 ± 1.2	1.5 ± 0.2
Lactate	Baseline	55 ± 6	72 ± 11	37 ± 7#
	Ischemia	494 ± 78	669 ± 151	298 ± 56#
	Reperfusion	178 ± 30	278 ± 97	109 ± 19

Concentrations of purines and lactate in coronary effluent, collected during baseline perfusion, during the last minute of a 30 min period of low-flow ischemia, and during the first minute of reperfusion. MI, untreated infarcted hearts; MI+CAP, captopril-treated infarcted hearts.
#: $P<0.05$ versus untreated infarcted hearts.

treatment, respectively). During reperfusion, these parameters were quickly restored.

Biochemical response to low-flow ischemia

During baseline perfusion, the concentration of purines in coronary effluent was lower in MI hearts than in sham hearts, which reached statistical significance after captopril treatment (Table 5.4). During ischemia, there was a 3 to 7-fold increase in concentration of purines. During reperfusion purine concentrations in coronary effluent were 50% increased from baseline in hearts from sham-operated rats and 2-fold in untreated MI hearts, whereas

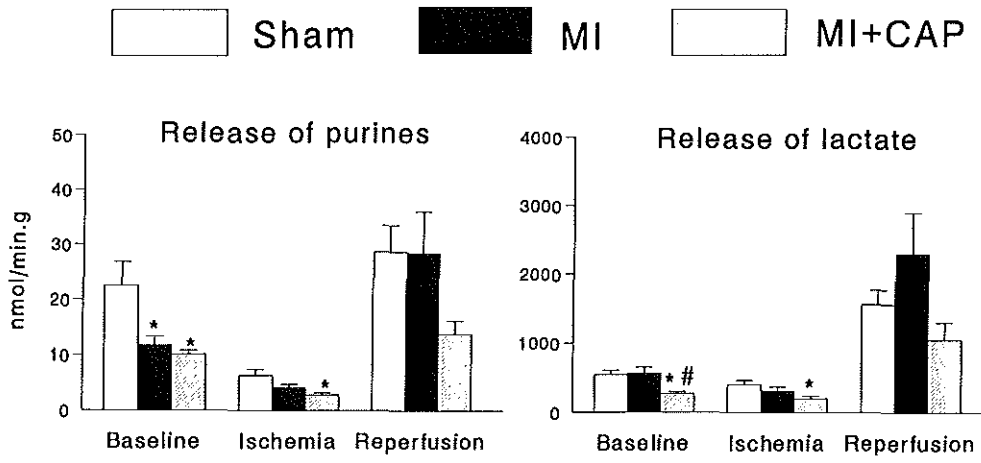

Figure 5.3: Myocardial release (nmol/min.g) of purines (left panel) and lactate (right panel) into the coronary circulation during baseline perfusion, during low-flow ischemia and during reperfusion. *: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI hearts.

reperfusion values in captopril-treated MI hearts were not different from baseline values. Since coronary flow differed between the experimental groups and within the same hearts during the different phases of the experiment, release of purines was calculated as concentration times flow per heart weight (Figure 5.3). Release of purines was lower in MI hearts than in sham hearts during baseline perfusion. During low-flow ischemia, purine release fell below baseline values, whereas it was clearly enhanced during reperfusion. Ischemic release of purines was lower in MI hearts compared to shams. When analyzed for the entire experiment, purine release of MI hearts tended to be lower after captopril treatment compared hearts from untreated rats, although this difference did not reach statistical significance (P=0.088).

Baseline concentrations of lactate in coronary effluent did not differ between MI hearts and normal hearts, but were significantly reduced in captopril-treated MI hearts (Table 5.4). Similar results were obtained during ischemia, increasing lactate concentrations 8- to 10-fold. Cardiac release of lactate into the coronary circulation was not different between normal control hearts and MI hearts. During baseline perfusion, lactate release of captopril-treated MI hearts was reduced compared to both sham-operated hearts and untreated MI hearts (Figure 5.3). Ischemic lactate release of captopril-treated MI hearts was significantly reduced compared to hearts from shams, and tended to be lower than untreated MI hearts.

DISCUSSION

The aim of the present study was to investigate effects of early captopril treatment of MI rats on regional capacity of the coronary vascular bed in relation to regional myocardial mass, as well as to assess the consequences of these structural adaptations for the metabolic response to an additional period of low-flow ischemia. The main findings were: i) MI-induced reactive hypertrophy was most prominent in the LV free wall, which was prevented by captopril treatment. Although weight of right ventricles and interventricular septa of MI hearts were not significantly increased compared to control hearts, captopril treatment still reduced the mass of these regions of MI hearts. ii) Maximal coronary flow during vasodilation, representing vascular capacity, was not affected by early captopril

treatment. iii) Early captopril treatment significantly improved peak perfusion of both the viable part of the LV free wall and the interventricular septum of MI hearts. For interventricular septa this was mainly caused by weight reduction, whereas in LV free walls there was a combined beneficial effect of a slight weight reduction and a tendency towards a higher vascular capacity. iv) Captopril-treated MI hearts subjected to an additional, global low-flow ischemic period released less lactate, suggesting longer preservation of aerobic metabolism.

ACE inhibition and vascular growth

In the present study, vascular capacity of MI hearts was similar to control hearts, despite permanent occlusion of one of the 3 coronary arteries, which acutely reduces coronary flow by approximately 30% (unpublished data). The restored flow capacity at 3 weeks after coronary artery ligation implies angiogenesis in the vascular beds perfused by the two remaining coronary arteries. This is in agreement with previous studies by us and other authors (Chapters 2, 3 and 4; Nelissen-Vrancken *et al.*, 1996). Captopril treatment did not affect vascular capacity, as measured by maximal coronary flow during nitroprusside-induced vasodilation. There was even a tendency towards a higher maximal coronary flow in the spared part of the LV free wall. These findings indicate that early captopril treatment does not impair the vascular growth that occurs in the first phase of post MI cardiac remodeling during scar formation. Absence of interference with vascular growth may be interpreted as a paradoxical finding to the fact that angiotensin II has been recognized as an angiogenic factor (Le Noble *et al.*, 1993; Munzenmaier & Greene, 1996). However, stimulation of vascular growth during ACE inhibition has been reported as well (Cameron *et al.*, 1992; Unger *et al.*, 1992; Olivetti *et al.*, 1993). Unger *et al.* (1992) hypothesized that potentiation of kinins, through ACE inhibitor-related decreased breakdown, would increase vascularization by augmenting myocardial blood flow. Another potential mechanism for ACE inhibitor-induced stimulation of vascular growth is reduction of AT_2 receptor-mediated inhibition of endothelial cell proliferation (Stoll *et al.*, 1995).

Captopril and the metabolic response to an additional period of ischemia

Treatment with ACE inhibitors has been reported to restore biochemical parameters

in hearts of stroke-prone spontaneously hypertensive rats, even at an subantihypertensive dose that did not attenuate LV hypertrophy (Gohlke *et al.*, 1994). Biochemical abnormalities in hearts from SHRs included increased cardiac release of lactate dehydrogenase (LDH), creatine kinase (CK), and lactate into the coronary circulation, and decreased myocardial tissue levels of the energy-rich phosphates ATP and creatine phosphate. Effects of chronic ACE inhibitor treatment on these biochemical parameters, but not the effects on hypertrophy, could be prevented by chronic co-treatment with a bradykinin receptor blocker, suggesting a role for ACE inhibitor-induced potentiation of bradykinin in the biochemical but not in the structural changes. In the present study, ECT decreased myocardial lactate release into the coronary circulation during baseline perfusion, as well as during decreased oxygen supply. A favourable vascularization/myocyte mass ratio, as reflected by the improved tissue perfusion in captopril-treated MI hearts, would decrease oxygen diffusion distance and therefore contribute to a better preservation of aerobic metabolism during ischemia.

Low-flow ischemia resulted in the present experiments in an increased release of ATP catabolites during the reperfusion phase, but not during ischemia, when release of purines was even lower compared to baseline perfusion. If ischemia is regarded as an imbalance between ATP production and ATP consumption, an increased release of ATP catabolites would be anticipated during the state of low coronary flow. Absence of an increase of release of purines during ischemia could have 2 underlying mechanisms: i) Minimalization of myocyte oxygen consumption, as reflected by the decrease in LV work (10-fold decrease in LV systolic pressure-heart rate product), in balance with 90-95% coronary flow reduction, and ii) absence of concentration gradient of purines in intra- and extracellular compartments during the low-flow state (reflected by the high concentration of purines in coronary effluent and the high release during reperfusion). Similarly to previous findings with the same experimental protocol (Chapter 4), hypertrophied MI hearts did not have an increased release of ATP catabolites. In fact, release of purines from MI hearts was significantly reduced compared to shams during baseline perfusion and tended to be lower during ischemia. Since lactate production was not increased, the decreased ATP breakdown cannot be explained by increased ATP production from

anaerobic metabolism, but may be due to lower oxygen expenditure, as reflected by lower velocity of contraction and relaxation in MI hearts. Although not statistically significant, ECT treatment tended to decrease the release of purines during ischemia and reperfusion.

Comparison of early captopril with delayed captopril treatment

Prevention of reactive hypertrophy with early captopril treatment or regression of established hypertrophy with delayed captopril treatment (Chapter 4) led to a similar reduction of ventricular weight. Vascular capacity of MI hearts was affected neither by early nor by delayed captopril treatment. Consequently, impaired peak ventricular perfusion was restored by both early and delayed captopril therapy to values of hearts from sham-operated rats. This suggests that the more favourable vascularization/myocyte mass ratio improves cardiomyocyte oxygenation, which becomes evident as preserved aerobic metabolism during an additional period of low-flow ischemia. This is in agreement with the finding that treatment of MI rats with ACE inhibitors reversed the reduction in mitochondrial oxygen consumption rate (Sanbe *et al.*, 1995).

Implications of in vitro findings for clinical outcome

Clinical trials evaluating early intervention with ACE inhibitors in MI patients have not yielded uniform results. Whereas in some trials a decreased mortality was found (GISSI-3, 1996; ISIS-4, 1995; SMILE [Ambrosioni *et al.*, 1995]), others did not find an improved survival (Sharpe *et al.*, 1991; Swedberg *et al.*, 1992; Ray *et al.*, 1993; CCS-1, 1995). In MI rats, early captopril therapy has been associated with attenuation of LV dilation and with improved survival (J. Pfeffer, 1991). In the rat MI model, an improved heart function was found with delayed captopril treatment, whereas early intervention with captopril failed to do so (Schoemaker *et al.*, 1991). In fact, MI rats that received immediate captopril therapy, had the same cardiac output at rest, resulting from a lower stroke volume and a higher heart rate. Similar results were obtained in anesthetized rats by Gay (1990), and they coincided with an attenuation of LV dilation and decreased LV filling pressures. Whereas the former effects are considered unfavourable, the latter effects are regarded as beneficial. Results from the present study indicate that survival of MI patients could be improved after early intervention with captopril, independent of effects on hemodynamics,

by improved tolerance to additional ischemic episodes. Especially the subgroup of patients with generalized coronary atherosclerosis would benefit, since this group is most at risk of additional ischemic episodes. Delayed captopril treatment was found to reduce the number of further ischemic events (Rutherford *et al.*, 1994). Similarly, MI patients with captopril treatment started after the acute phase of MI (at day 7) were found to have less electrocardiographic signs of ischemia during ambulatory ECG monitoring and ECG monitored exercise testing (Søgaard *et al.*, 1993, 1994). Similar studies have not been performed yet after immediate post MI captopril therapy.

Conclusion

Early captopril treatment of MI rats prevents reactive hypertrophy but not adaptive vascular growth. Hence, it improves peak perfusion of the viable part of the LV free wall by a combination of weight reduction and a tendency towards increased regional vascular capacity, whereas maximal perfusion of the interventricular septum is achieved mainly by weight reduction of this region. The more beneficial vascularization/tissue mass ratio is reflected in a better preservation of aerobic metabolism during an additional low-flow ischemic period. This may provide an explanation why clinical outcome is improved with early captopril treatment in some clinical trials, but cardiac pump capacity is not improved in the rat MI model.

CHAPTER 6

CHRONIC ASPIRIN TREATMENT AFFECTS COLLAGEN DEPOSITION IN NON-INFARCTED MYOCARDIUM DURING REMODELING AFTER CORONARY ARTERY LIGATION IN THE RAT

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ABSTRACT

Low-dose aspirin (acetylsalicylic acid), inhibiting platelet thromboxane production in favour of endothelium formation of prostaglandins, is successfully used as primary or secondary profylaxis against myocardial infarction. Although prognosis may be improved, effects of long-term aspirin treatment on wound healing and cardiac remodeling are not well understood. The aim of the present study was to mimic the clinical situation by inducing myocardial infarction in low-dose aspirin (25 mg/kg/day, i.p.) pretreated rats, and to determine effects on plasma eicosanoid levels, cardiac hypertrophy and collagen deposition, and left ventricular (LV) function during continued aspirin treatment. The effects of this dose were verified to selectively inhibit platelet thromboxane production, and lower plasma levels of thromboxane, but did not affect plasma levels of prostacyclin and prostaglandin E_2 during the acute inflammatory stage following myocardial infarction. As measured by heart dry weight/body weight, cardiac hypertrophy was not affected by aspirin treatment. However, interstitial fibrosis in the spared myocardium as well as perivascular fibrosis, associated with infarction-induced cardiac remodeling, were affected by aspirin treatment. Replacement fibrosis in the infarct itself, considered as representing wound healing, was not significantly influenced by aspirin treatment. Wall thinning following infarction was not aggravated, nor did treatment influence LV cavity diameter in a relaxed state. Results from *in vitro* LV function measurements showed no effects on LV peak velocity of contraction or relaxation after aspirin treatment. In conclusion, although low-dose aspirin may not be expected to have anti-inflammatory action, it did influence post-infarct cardiac remodeling by affecting interstitial and perivascular fibrosis. Aspirin treatment did not have effects on *in vitro* LV dysfunction.

INTRODUCTION

Low-dose aspirin (acetylsalicylic acid) treatment is used in coronary artery disease as primary or secondary profylaxis against myocardial infarction, as it reduces platelet production of pro-aggregatory and vasoconstrictor thromboxane in favour of anti-aggregatory and vasodilator prostaglandins (Patrignani *et al.*, 1982; Coller, 1991). Aspirin impro-

ves prognosis and reduces the chance of reinfarction after myocardial infarction (ISIS-2 Collaborative Group, 1988), and this effect can be attributed to its anti-platelet action (Antiplatelet Trialists' Collaboration, 1988).

At higher doses, aspirin can also exert anti-inflammatory activity by interference with the synthesis of prostaglandins. Anti-inflammatory treatment with corticosteroids and non-steroid anti-inflammatory drugs (NSAIDs) has been shown to retard collagen deposition and to cause infarct thinning (Bulkley & Roberts, 1974; Kloner *et al.*, 1978; Brown *et al.*, 1983; Hammerman *et al.*, 1983a, 1983b; Mannisi *et al.*, 1987; Vivaldi *et al.*, 1987; Jugdutt & Basualdo, 1989). These studies focused on the replacement fibrosis that results in scar formation, whereas effects on interstitial fibrosis that occurs in remote, non-infarcted areas (van Krimpen *et al.*, 1991) are still unknown. Moreover, whether scar formation and cardiac fibrosis in spared myocardium would be affected in patients pretreated followed by continued treatment with low-dose aspirin, which seems to be devoid of anti-inflammatory action, remains to be determined.

The present study was carried out to address this question in a rat model of myocardial infarction (Fishbein *et al.*, 1978a). A dose of aspirin that inhibits thromboxane production from platelets but does not interfere with prostacyclin production from endothelial cells, was carefully selected as a treatment reflecting the aims of low-dose aspirin treatment in man. Plasma eicosanoid levels were evaluated at different timepoints. The effects on interstitial and perivascular fibrosis in spared myocardium, on replacement fibrosis in the infarcted area were investigated, as well as on cardiac remodeling parameters and LV function *in vitro*.

MATERIALS AND METHODS

Male, Wistar rats (270-320 g, Harlan Zeist, The Netherlands) were used in this study. Saline or aspirin 25 mg/kg (lysine-acetylsalicylic acid, Aspégic®, Lorex B.V., Maarssen, The Netherlands) dissolved in saline was administered as daily i.p. injections of 1 ml/kg, starting 2 days before surgery, and the treatment continued until the end of the experiment, at 8, 14 or 21 days after surgery. The injections were administered at the same time each day except on the day of surgery, when the animals were injected immediately after the operation. Rats were housed at a 12 h light/dark cycle with standard rat chow and water available ad lib. The experiments were approved by the University ethics committee for the use of experimental animals.

Validation of the used dose of aspirin To confirm the selectivity of chronic aspirin treatment as inhibiting platelet versus vascular cyclooxygenase activity, a separate group of rats was treated with either aspirin 25 mg/kg body weight (n=6) (based on pilot short-term dose-finding studies) or saline (n=6) as daily i.p. injection. Following 3 weeks of treatment, blood was sampled by heart puncture under pentobarbital (60 mg/kg) anesthesia, 24 hours after the last dose of aspirin. Native blood (1 ml) was allowed to clot in a Vacutainer® glass tube containing SST® Gel and Clot Activator (Becton Dickinson, Meylan Cedex, France) at room temperature for 30 minutes. Serum was separated by centrifugation and, after Sep Pak (Millipore, Milford, USA) extraction, stored at -20°C in methanol until assayed for thromboxane B_2 (TxB_2) produced. A 2 mm-long segment was isolated from the thoracic aorta. The segments were incubated at 37°C for 10 min in 200 µl of Krebs-Henseleit buffer containing 25 µM arachidonic acid (Supelco. Bellefonte, Italy). The rings were removed and weighed, and the supernatant passed through Sep Pak filters and stored at -20°C in methanol until assayed for 6-keto-$PGF_{1\alpha}$ generation, which was expressed as ng/mg wet tissue of aortic segment.

Surgical preparation Under pentobarbital (60 mg/kg, i.p.) anesthesia, a PE-10 catheter filled with heparinized saline (50 IU/ml) was introduced into the thoracic aorta via the left carotid artery for later blood sampling. The catheter was guided subcutaneously to the neck, where it was fixed and exteriorized. Subsequently, left anterior descending coronary artery ligation was performed as described in detail in chapter 2 (page 32).

Blood sampling and measurement of eicosanoids At 4 occasions, 1 ml blood was sampled using syringes filled with 10 µl 0.1 M disodium ethylenedinitrilotetra-acetic acid (EDTA): immediately after implanting the aortic catheter, but before opening the thoracic cavity (day 0), 1 day after surgery, and 8 and 21 days after surgery, when it was obtained by aortic puncture. Day 1 was chosen as a point in time representative of acute inflammation following infarction and day 8 because it represents chronic inflammation, just after peak infiltration of chronic inflammatory cells, and day 21 because inflammation has then waned (Fishbein *et al.*, 1978a). After centrifugation the plasma was passed through Sep Pak C18 cartridges (Waters Ass., USA) and eluted with methanol. Samples were stored at -20°C in methanol. Because of the instability of prostacyclin and thromboxane in biological fluids, their stabile metabolites, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and TxB_2, respectively, as well as prostaglandin E_2 (PGE_2), were measured using radioimmunoassay (antibodies: Advanced Magnetics, USA; Standards: Sigma, USA) as described in detail by Zijlstra *et al.* (1992). To allow comparison of eicosanoid levels between the 3 experimental groups, samples from the same timepoint were assayed together.

Measurement of LV function Under pentobarbital anesthesia, the heart was rapidly excised and mounted for Langendorff perfusion and instrumented for functional measurements as described in detail in chapter 2 (page 32). LV end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume and hearts were allowed to stabilize for at least 20 minutes. LV pressure tracings were recorded at high paper speed (100 mm/s) for graphical determination of positive and negative dP/dt_{max}. At the end of the experiment, hearts were arrested in diastole with a 1 M KCl injection

into the perfusing fluid, and prepared for measurement of infarct size.

Infarct size measurement Large vessels and atria were removed from the heart, and the right and left ventricles were separated and weighed. The left ventricle was quickly frozen (-80°C) and cut into slices of 1 mm from apex to base. The slices were stained with nitro blue tetrazolium (NBT) according to Leprán *et al.* (1983). Briefly, the slices were incubated in 1 mg/ml NBT in 0.1 M Sörensen phosphate buffer (pH=7.4) at 37°C for 15 min, and subsequently put in cold saline (0°C). This procedure stains all tissue that was vital at the time of death, so provides no information about the area at risk. Colour pictures were taken from the slices, and infarct size was determined by planimetry, described in detail elsewhere (Schoemaker *et al.*, 1991). Infarct size was expressed as percentage of LV circumference, calculated as the average of infarct size of endocardial and epicardial surfaces of all slices. Minimal scar thickness as well as thickness of the mid-interventricular septum was measured in all slices containing transmural infarction. Thinning ratio was calculated by dividing the scar thickness by the thickness of the interventricular septum. LV cavity diameter in an undistended state was estimated from mean endocardial circumference. LV cavity diameter to mean wall thickness ratio was calculated as an index of structural LV dilation (Vogt *et al.*, 1987). Dry weights were determined after drying the tissues for 3 days at 37°C.

Measurement of collagen content At 2 weeks after surgery, when a plateau in collagen content is reached in infarcted hearts (van Krimpen, 1991), the amount of interstitial and perivascular collagen was measured in a separate group of rats, using the method as described previously in chapter 2 (page 33). Distribution of collagen fibers in sections examined with normal and with polarized light (Whittaker *et al.*, 1994) was similar, validating the quantification of collagen with normal light (Figure 6.2). In the interventricular septum, as well as in the right ventricle, interstitial collagen was determined as the picrosirius red positive area in 40 high power fields per ventricle per heart. These areas of myocardium did not show signs of replacement fibrosis following focal necrosis. Thus, we indeed measured interstitial fibrosis as the increase of collagen volume in the interstitium between vital myocytes (Figure 6.2). In addition, perivascular collagen of 6-10 septal resistance arteries (diameter approximately 100 µm) was measured (Figure 6.4). In order to evaluate effects on wound healing in infarcted hearts, the picrosirius red positive area was also determined in the central part of the infarct itself.

Data analysis Results comprise data obtained from 6 to 12 animals per group. Data are expressed as group means ± S.E.M., unless indicated otherwise. Rats in the sham group had measured infarct sizes of 0 %. Data from rats with measured infarct sizes less than 20 % were excluded from analysis, because these infarcts are hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Only eicosanoid measurements of animals that survived the complete protocol were used. Because plasma eicosanoid levels did not show a Normal distribution, median values ± 95 % confidence intervals are quoted, and non-parametric data analysis was performed, using the Kruskal-Wallis test. Morphological and functional data were analyzed using one-way analysis of variance (ANOVA), followed by a post-hoc *t*-test (Wallenstein *et al.*, 1980). Differences were considered statistically significant if $P<0.05$.

RESULTS

On average, surgery-related mortality was 32 % (shams 21 %, saline-treated infarctions 37 %, aspirin-treated infarctions 41 %), and occurred mainly during the first 24 h after surgery. After exclusion of data from 3 aspirin-treated and 5 saline-treated infarcted animals because of infarct sizes smaller than 20 %, infarct sizes were comparable in the saline-treated and aspirin-treated infarction groups (Table 6.1). All infarctions were transmural and were located in the lateral (free) wall of the left ventricle.

Validation of the used dose of aspirin

Generation of 6-keto-$PGF_{1\alpha}$ by aortic endothelium stimulated by arachidonic acid (1.35 ± 0.22 ng/mg wet weight in saline-treated rats) was not affected by 3 weeks of aspirin treatment (1.32 ± 0.21 ng/mg wet weight). Production of TxB_2 from platelets during blood clotting was effectively attenuated by aspirin therapy (1.91 ± 0.53 ng/ml serum vs 5.88 ± 0.55 ng/ml serum). Consequently, 6-keto-$PGF_{1\alpha}$ to TxB_2 ratio with aspirin treatment (1.07 ± 0.30) was significantly increased compared to saline-treated animals 0.24 ± 0.03).

Plasma eicosanoid levels

TxB_2 levels were significantly lowered by aspirin treatment at all timepoints except at day 8, when no statistical significance was reached (P=0.07) (Figure 6.1). TxB_2 levels appeared to be higher at 21 days compared to the other timepoints, but these samples were assayed separately. 6-keto-$PGF_{1\alpha}$ levels were significantly decreased by aspirin treatment at day 0 and 21, but not during inflammatory stimulation, at day 1 and 8. This resulted in a comparable presurgical 6-keto-$PGF_{1\alpha}$ to TxB_2 ratio in aspirin- and saline-treated rats (3.5 ± 1.1 and 3.1 ± 1.0, respectively), whereas this ratio was significantly increased in aspirin-treated animals, during acute inflammation, at day 1 (16.4 ± 4.9 vs 5.0 ± 1.7). The ratio returned to presurgical levels at day 8 (3.5 ± 0.7 vs 2.5 ± 0.6). PGE_2 levels were never affected by aspirin treatment, although a tendency towards inhibition might be present after 3 weeks of treatment.

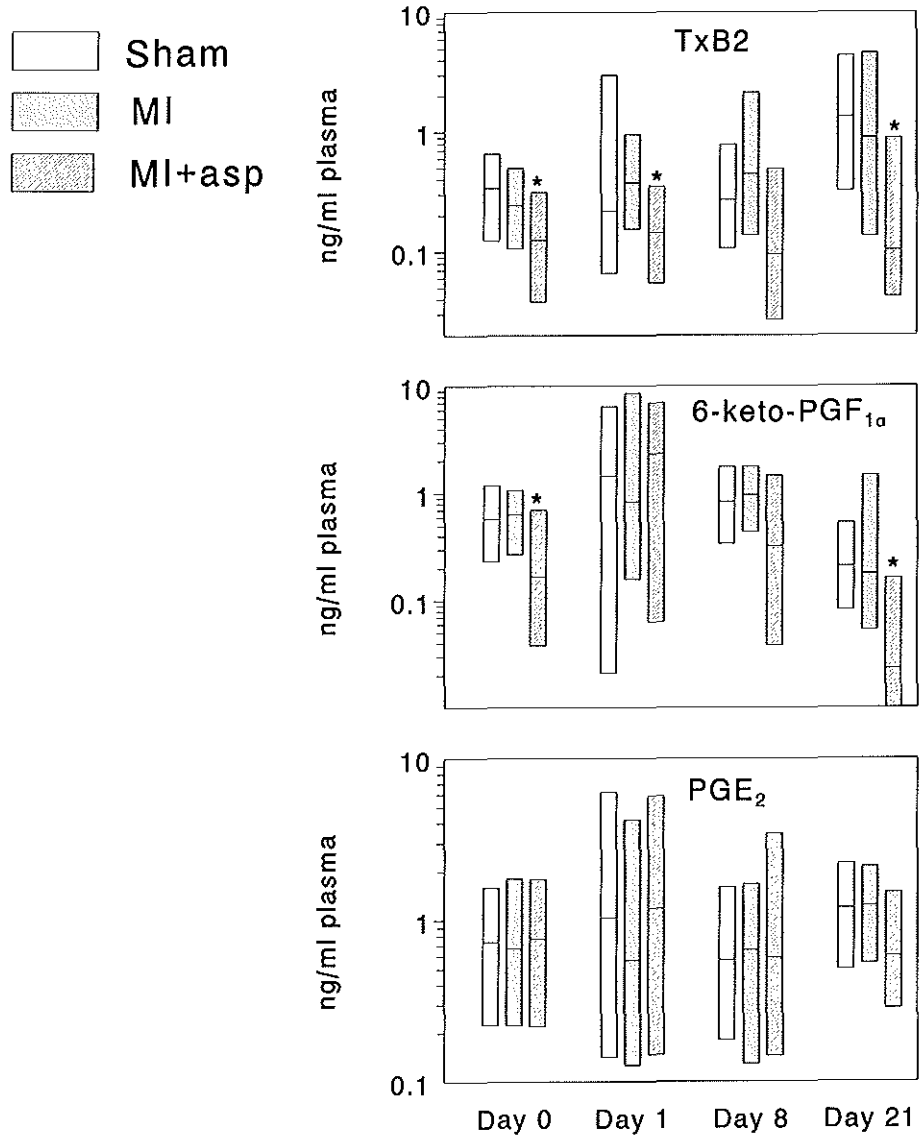

Figure 6.1: Plasma eicosanoid levels (ng/ml) at different timepoints following surgery. Median values and 95% confidence intervals are shown. Open bars: sham (n=9-12); Gray bars: saline-treated infarction (n=10-12); Hatched bars: aspirin-treated infarction (n=8-11).
*: P<0.05 vs saline-treated rats.

Table 6.1 Characteristics of hearts 8 days and 3 weeks after surgery

		Sham	MI	MI+asp
n	Day 8	12	12	11
	Day 21	11	10	8
Infarct size (%)	Day 8	0	41 ± 2	43 ± 3
	Day 21	0	37 ± 1	39 ± 3
Heart wet weight (mg)	Day 8	792 ± 24	954 ± 43*	1046 ± 40*
	Day 21	978 ± 35	928 ± 22	928 ± 23
Wet weight/BW (mg/g)	Day 8	2.81 ± 0.14	3.74 ± 0.30*	4.11 ± 0.24*
	Day 21	2.54 ± 0.10	2.52 ± 0.06	2.60 ± 0.05
Heart dry weight (mg)	Day 8	165 ± 3	154 ± 5	157 ± 4
	Day 21	198 ± 7	179 ± 4	181 ± 5
Dry weight/BW (mg/g)	Day 8	0.58 ± 0.02	0.59 ± 0.03	0.61 ± 0.02
	Day 21	0.51 ± 0.01	0.48 ± 0.01	0.51 ± 0.01
LV thinning ratio	Day 8		0.28 ± 0.02	0.30 ± 0.02
	Day 21		0.31 ± 0.04	0.39 ± 0.10
LV diameter/WT	Day 8	0.46 ± 0.04	1.18 ± 0.09*	1.07 ± 0.07*
	Day 21	0.66 ± 0.04	1.03 ± 0.10*	0.91 ± 0.12*

MI, myocardial infarction; MI+asp, aspirin-treated myocardial infarction; Wet Weight/BW, heart wet weight/body weight; Dry Weight/BW, heart dry weight/body weight; LV, left ventricle; Thinning ratio, minimal scar thickness to midseptal thickness ratio; LV diameter/WT, left ventricle inner diameter/wall thickness. *: Significantly different from sham values.

LV morphology and function

Heart wet weight was significantly increased at 8 days after myocardial infarction, which was mainly attributable to the left ventricle. This observation was even more pronounced in infarcted hearts from aspirin-treated rats. However, for dry weights no differences between the experimental groups were present. Heart dry weight to body weight ratios did not differ between the groups (Table 6.1).

The ratio of LV cavity diameter to wall thickness in a relaxed state was significantly increased. This was the result of an increased LV cavity diameter (3.2 ± 0.2 vs 2.4 ± 0.1 mm) and decreased wall thickness (3.2 ± 0.1 vs 3.7 ± 0.1 mm) at an unchanged outer diameter (9.7 ± 0.2 mm for both groups), at 21 days. These parameters were similar in hearts from saline- and aspirin-treated animals. Scar thickness did not differ between untreated and aspirin-treated rats (1.1 ± 0.1 vs 1.2 ± 0.1 mm at 8 days, and 1.3 ± 0.1 vs 1.5 ± 0.2 mm at 21 days), nor was septal thickness altered by aspirin therapy (4.2 ± 0.2 vs

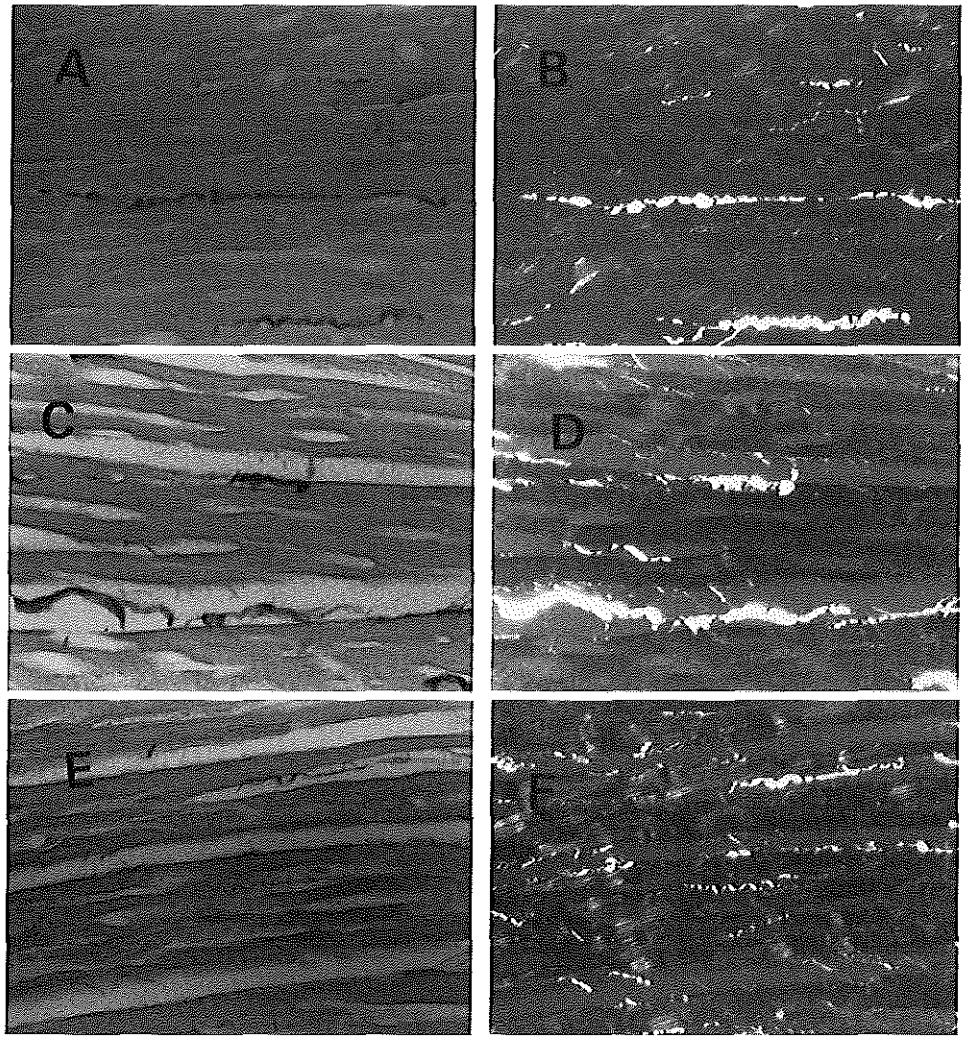

Figure 6.2: Picrosirius red-stained sections of interventricular septum of hearts from A/B: sham operated rats (A with normal light and B with polarized light), C/D: saline-treated rats after infarction of the LV free wall (C with normal light and D with polarized light), E/F: aspirin-treated rats after infarction of the LV free wall (E with normal light and F with polarized light) (original magnification: 62.5x).

Table 6.2 Functional parameters during Langendorff perfusion

		Sham	MI	MI+asp
n	Day 8	9	9	8
	Day 21	11	10	8
Systolic pressure (mmHg)	Day 8	68 ± 4	57 ± 6	64 ± 8
	Day 21	75 ± 5	51 ± 4*	49 ± 4*
$+(dP/dt)_{max}$ (mmHg/s)	Day 8	2807 ± 453	1593 ± 294*	2007 ± 398
	Day 21	3734 ± 432	1695 ± 178*	1696 ± 299*
$-(dP/dt)_{max}$ (mmHg/s)	Day 8	1338 ± 95	991 ± 98*	1136 ± 133
	Day 21	1282 ± 96	885 ± 62*	831 ± 75*
Coronary flow (ml/min)	Day 8	10.0 ± 1.3	8.6 ± 0.6	8.8 ± 0.9
	Day 21	11.3 ± 0.7	10.5 ± 0.6	11.0 ± 1.4
CF/wet weight (ml/min.g)	Day 8	13.0 ± 1.7	9.6 ± 0.9	8.6 ± 0.8*
	Day 21	11.6 ± 0.6	11.1 ± 0.8	11.8 ± 1.4

MI, myocardial infarction; MI+asp, aspirin-treated myocardial infarction; Systolic pressure, LV systolic pressure; $+(dP/dt)_{max}$ and $-(dP/dt)_{max}$: maximum velocity of pressure rise and decline, respectively. CF/wet weight: coronary flow per g of wet cardiac tissue.
*: Significantly different from sham values.

4.3 ± 0.1 mm at 8 days, and 4.3 ± 0.2 vs 4.2 ± 0.3 mm at 21 days, in untreated and aspirin-treated rats, respectively). Thus, thinning ratio was not influenced by aspirin treatment (Table 6.1).

In vitro LV dysfunction of infarcted hearts became evident from a depressed peak velocity of contraction and relaxation, demonstrated by a significantly depressed peak +(dP/dt) and -(dP/dt). Aspirin treatment delayed the development of left chamber dysfunction; contractility and relaxation were not significantly depressed until 3 weeks after infarction. A similar tendency, though less pronounced, could be seen for systolic pressure developed during Langendorff perfusion. Coronary flow in infarcted hearts was not decreased compared to sham values. However, at 8 days after surgery, coronary flow corrected for wet cardiac tissue weight, but not for dry weight, was significantly decreased in infarcted hearts after aspirin treatment (Table 6.2).

Collagen deposition in infarcted and non-infarcted myocardium

In the infarcted area itself, 56.0 ± 3.3 % of total tissue area was found to be picrosirius red positive, which was not significantly influenced by aspirin treatment (49.4 ± 1.0 %). Non-infarcted, interventricular septum showed a considerably higher

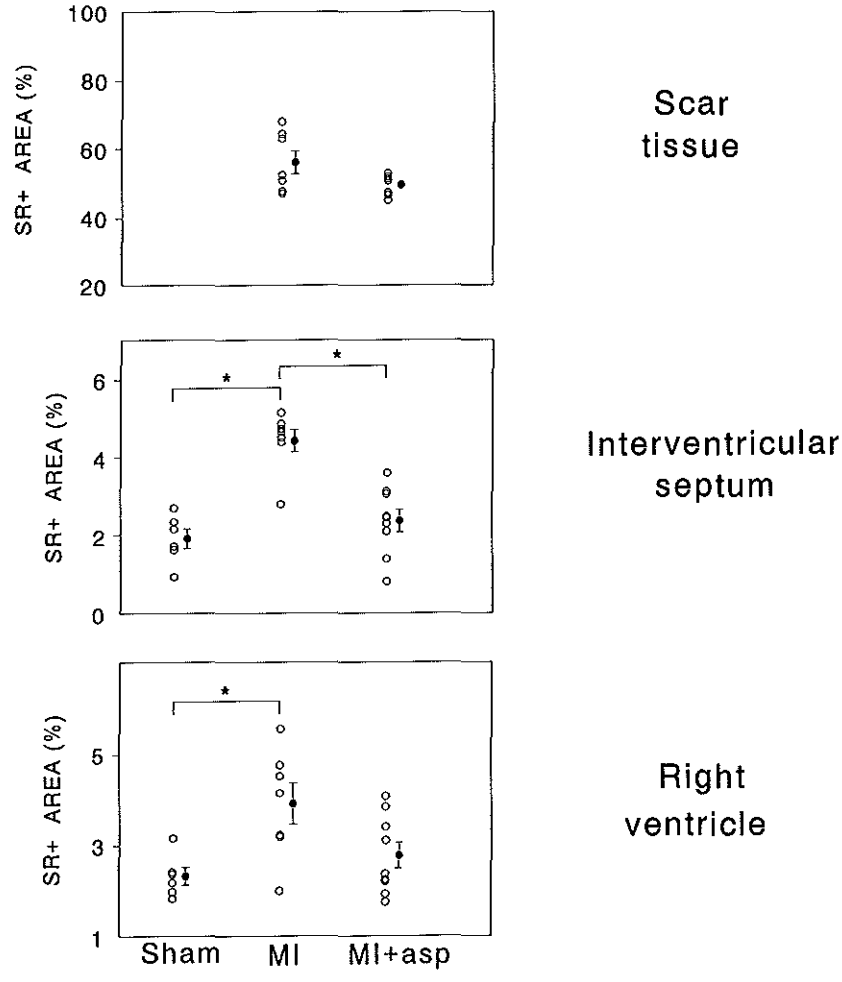

Figure 6.3: Collagen content, as measured by picrosirius red stained (SR+) tissue area and expressed as percentage of total tissue area, in infarct centre (upper panel), interventricular septum (middle panel) and right ventricle (lower panel). MI: myocardial infarction; MI+aspirin: aspirin-treated myocardial infarction. Open symbols: values in individual hearts; Black symbols: group means ± SEM. *:$P<0.05$.

content of picrosirius red positive material after coronary artery ligation than corresponding areas in hearts from sham-operated rats, which was almost normalized after aspirin treatment (Figures 6.2 and 6.3). Also in right ventricular myocardium, infarcted hearts showed a higher interstitial collagen content, which was slightly but insignificantly reduced by aspirin treatment (Figure 6.3).

Perivascular collagen of resistance arteries in the interventricular septum was significantly increased after infarction (0.96 ± 0.09 vs 0.63 ± 0.09 picrosirius red positive area/luminal area). Aspirin treatment significantly affected this collagen deposition (0.71 ± 0.05, P=0.04) (Figure 6.4).

DISCUSSION

Aspirin treatment and eicosanoid profile

In the primary and secondary profylaxis of myocardial infarction low-dose aspirin is successfully used as anti-platelet therapy because it inhibits platelet production of thromboxane in favour of endothelium formation of prostaglandins. In this study, firstly, we evaluated the effects of aspirin treatment in normal healthy animals with regard to platelet and vascular endothelium cyclooxygenase potential to generate thromboxane and prostacyclin, respectively. This is important because inter-species differences in sensitivity and metabolism make dose extrapolation from animal to man on a body weight basis questionable. Corresponding to the rationale behind the clinical use in man, chronic aspirin treatment did not affect vascular endothelium cyclooxygenase, while TxB_2 generation by platelets during blood clotting was effectively blocked. However, measurements in plasma during our experiments using the same dose of aspirin showed some interference with prostaglandin synthesis as well, as indicated by decreased prostacyclin levels at those timepoints that were not associated with the inflammatory response to infarction. This discrepancy may be explained by the fact that the dose of aspirin, that selectively inhibits thromboxane synthesis, was evaluated *in vitro* only in those tissues in which the major production was expected (Gambino *et al.*, 1988). The site of production of plasma prostacyclin may not be limited to vascular endothelium (Mehta *et al.*, 1985; Vergara-Dauden *et al.*, 1985; Weber *et al.*, 1989).

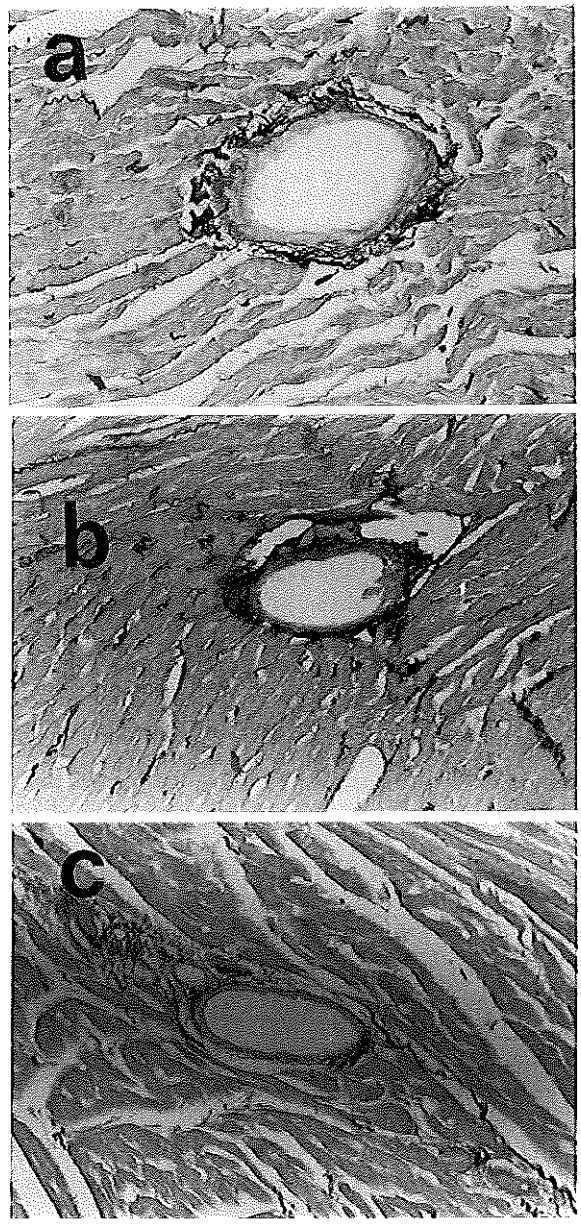

Figure 6.4: Picrosirius red stained sections of interventricular septum containing resistance arteries, from A: sham operated rats, B: saline-treated rats after infarction of the LV free wall, C: aspirin-treated rats after infarction of the LV free wall (original magnification: 62.5x).

Prostacyclin levels during the inflammatory stage (days 1 and 8) were not affected by aspirin treatment. Although inflammation did not result in higher plasma concentrations of prostacyclin, there may be a greater proportion of total release into plasma attributable to the inducible cyclooxygenase-2 (COX-2) relative to the constitutional cyclooxygenase-1 (COX-1) (Vane *et al.*, 1994). The COX-2 isoform is less sensitive to inactivation by aspirin (Mitchell *et al.*, 1993). Plasma eicosanoid levels during this inflammatory stage showed a high interanimal variability. Since variation of plasma eicosanoid levels rather than absolute values were increased at day 1 compared to day 0, and no differences were observed between sham and infarcted rats, we concluded that the surgical procedure rather than the infarction was responsible for the variability in plasma levels and may represent individual inflammatory responses to surgery. The variation may decline with time, indicative for acute and later waning inflammatory response.

Left ventricle morphology and in vitro function

A major finding in the present study is that low-dose aspirin therapy affected collagen build-up in spared myocardium after infarction. High-dose aspirin treatment, providing plasma levels associated with anti-inflammatory action in humans, has been shown to inhibit both the synthesis and degradation of collagen in rat skin (Solheim *et al.*, 1986a), as well as the synthesis of collagen in rat bone (Solheim *et al.*, 1986b). Numerous studies have reported on the effects of steroids and NSAIDs on scar thickness and collagen build-up in the infarcted area (Bulkley & Roberts, 1974; Kloner *et al.*, 1978; Brown *et al.*, 1983; Hammerman *et al.*, 1983a, 1983b ; Mannisi *et al.*, 1987; Vivaldi *et al.*, 1987). Moreover, prostaglandins have been implicated in the regulation of fibroblast proliferation (Otto *et al.*, 1982). Therefore, it seems rational to link the effects of aspirin therapy to its anti-inflammatory action. However, in the present study, aspirin treatment did not affect prostaglandin levels during the inflammatory phase after infarction. Secondly, fibrosis in the spared myocardium, remote from the infarct, rather than in the infarcted area itself was affected; scar thinning and scar collagen content were not significantly altered. On the other hand, inflammation may not be limited to the infarcted area itself (Sulpice *et al.*, 1994). The observation that at 8 days after infarction, when cardiac edema is known to be over its maximum (Fishbein *et al.*, 1978a), increased tissue water content was more

pronounced with aspirin therapy, suggests still some interference with local inflammatory response to infarction. Other possibilities include a role for decreased thromboxane synthesis, direct or indirect through prevention of release of platelet-derived growth factor (Vissinger *et al.*, 1993; Lanas *et al.*, 1994). Finally, effects of medication unrelated to inactivation of cyclooxygenase, for example by metabolites of aspirin (Haynes *et al.*, 1993), cannot be excluded.

Another important aspect of the effect of aspirin is that the prevention of collagen build-up in non-infarcted tissue after infarction did not result in a further increase of LV cavity diameter in a relaxed state. Diastolic pressure-volume curves in infarcted hearts were not altered by aspirin treatment (Chapter 7), indicating that during distension by fysiological diastolic pressures as well, LV dilation was not aggravated by aspirin treatment. Therefore, it is likely that aspirin treatment did not aggravate the initial process of breakdown of pre-existing collagen fibers correlating with post-infarct dilation (Whittaker *et al.*, 1991). Likewise, the slightly lower collagen content of the infarcted tissue did not result in further thinning of this area, which has been described with steroids and NSAIDs (Brown *et al.*, 1983; Hammerman *et al.*, 1983a, 1983b; Mannisi *et al.*, 1987). An effect of aspirin treatment on interstitial fibrosis of spared myocardium, but not on replacement fibrosis of infarcted tissue, suggests that these are indeed two different processes governed by different control mechanisms. Corday *et al.* (1975) pointed out that in dogs, in an ischemia-reperfusion model of myocardial infarction, replacement fibrosis was not limited to the infarcted area, but was also present in areas remote from the infarct, as a result of focal necrosis. Because we have not observed focal necrosis in the sections of spared myocardium analyzed for fibrosis in the rat coronary artery ligation model, this indicates that we indeed quantified interstitial fibrosis in non-infarcted areas, and that replacement fibrosis could be considered limited to the infarcted area itself.

In the present study, only total collagen content was measured. Collagen type, cross-linking, fiber organisation and fiber thickness could also alter the mechanical properties of the myocardium. We cannot exclude that not only the quantity, but also the characteristics of the collagen were affected by aspirin treatment. For example, aspirin-treated hearts could have a higher proportion of thin collagen filaments, as is illustrated by figure 6.2.

Since large myocardial infarction did not decrease heart dry weight to body weight ratios, hypertrophy of spared myocardium is indicated. Although we have not actually measured myocyte dimensions, comparable heart dry weights make it unlikely that aspirin treatment would reduce cardiomyocyte hypertrophy in addition to collagen content, as is the case with ACE-inhibitor and angiotensin II blocker therapy (van Krimpen *et al.*, 1991; Smits *et al.*, 1992). There was no significant effect of aspirin therapy on *in vitro* LV performance. If anything, there may be a tendency to delay the onset of LV dysfunction. After aspirin treatment, the significant depression of peak velocity of contraction and relaxation in infarcted hearts, was limited to the latest timepoint (21 days after infarction). In pressure-overloaded hearts, interstitial fibrosis rather than ventricular hypertrophy has been associated with decreased ventricular compliance (Brilla *et al.*, 1991, 1993). Reduced stiffness of spared myocardium could be one mechanism by which a reduced interstitial collagen might attenuate the incidence and severity of post-infarct heart failure in the long term. Although determination of interstitial collagen content of spared myocardium and the measurements of LV function were not done at the same timepoint, nearly complete prevention of interstitial fibrosis at 14 days makes it unlikely that the treatment effect would be absent at the other timepoints during the healing period. Moreover, retardation of the healing period by aspirin, implying a temporary nature of the observed treatment effects, is unlikely because of the lack of effect on collagen deposition in the infarcted area. However, the effect on interstitial fibrosis of remodeled, spared hypertrophied myocardium by aspirin treatment did not result in an altered diastolic pressure-volume relationship of the whole left ventricle (Chapter 7). Thus, at present, the consequences of pharmacological interference with collagen synthesis during remodeling after infarction, for cardiac function, remain unclear.

In conclusion, 25 mg/kg/day of aspirin inhibited thromboxane synthesis in rats more markedly than prostaglandin synthesis and seems pharmacologically equivalent to chronic low-dose aspirin treatment in patients. This dose affected interstitial and perivascular fibrosis in the spared myocardium, while leaving wound healing (scarring and thinning of the infarcted area) and reactive hypertrophy relatively unaffected. Besides a possible delay in the development, aspirin treatment had no effect on *in vitro* LV dysfunction.

CHAPTER 7

THE COLLAGEN NETWORK IN CARDIAC REMODELING; CONSEQUENCES FOR DIASTOLIC FUNCTION IN DIFFERENT RAT MODELS

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ABSTRACT

Left ventricular (LV) fibrosis in pressure overload-induced remodeling is associated with increased stiffness and impaired LV diastolic function. After myocardial infarction (MI), there is a less distinct accumulation of interstitial and perivascular collagen. To determine if pharmacological modulation of the collagen network during MI-induced remodeling as well as after pressure overload would change LV diastolic properties, MI rats were treated with aspirin (previously reported to reduce collagen in non-infarcted myocardium after MI) or methylprednisolone (MP). In addition, rats subjected to interrenal aortic banding (IRAB) were treated with aspirin. LV function of isolated hearts was assessed at baseline and with isoproterenol (IP). Volume-pressure curves were obtained after diastolic arrest. Collagen content was determined by morphometry. Collagen content of non-infarcted myocardium was not increased at 3 weeks. The infarcted left ventricles were dilated and had a depressed baseline and maximal function. Aspirin treatment did not alter LV dilation nor function. MP treatment of MI rats reduced interstitial and perivascular collagen content, and aggravated LV dilation. Baseline, but not maximal LV function was improved by MP. Altered LV mechanical properties with MP were associated with a dose-dependent decrease of diastolic pressure with IP. In IRAB rats, LV collagen content was increased due to perivascular fibrosis. Volume-pressure curves were steeper: k-values 168 ± 17 ($x10^{-4}$) vs 115 ± 7 ($x10^{-4}$). Aspirin treatment of IRAB rats did not affect LV hypertrophy nor collagen content. However, volume-pressure curves of hearts from IRAB rats were shifted rightward with aspirin and were less steep: k values 129 ± 14 ($x10^{-4}$). Thus, collagen content of remodeled left ventricles could be dissociated from LV stiffness. Therefore, collagen quality (type, degree of cross-linking) may be the major determinant of LV stiffness, especially if the quantity is not markedly increased.

INTRODUCTION

Pressure overload-induced remodeling is associated with accumulation of interstitial and perivascular collagen (Doering *et al.*, 1988; Pick *et al.*, 1989; Contard *et al.*, 1991; Brilla *et al.*, 1993). Fibrosis increases myocardial stiffness and impairs diastolic function

(Jalil *et al.*, 1989, 1991; Brilla *et al.*, 1991; Conrad *et al.*, 1995). In myocardial infarction (MI)-induced remodeling, early damage to the collagen network is associated with infarct expansion and left ventricular (LV) dilation (Whittaker *et al.*, 1991). Later in the course of post MI remodeling, when the infarcted tissue is replaced by scar tissue with a high collagen content, collagen content of non-infarcted tissue is increased as well (Chapter 6; van Krimpen *et al.*, 1991; Smits *et al.*, 1992; Volders *et al.*, 1993; McCormick *et al.*, 1994). Collagen accumulation in non-infarcted tissue is a potential target for treatment, since it is associated with increased stiffness of non-infarcted myocardium (Raya *et al.*, 1988; Litwin *et al.*, 1991b) and could therefore adversely affect LV function. However, a too large reduction of the tensile strength of the LV collagen network could result in aggravation of chamber dilation, as has been reported with NSAIDs and steroids (Bulkley & Roberts, 1974; Brown *et al.*, 1983; Hammerman *et al.*, 1983a, 1983b; Mannisi *et al.*, 1987; Jugdutt & Basualdo, 1989).

In chapter 6, we have shown that aspirin treatment of MI rats prevented collagen accumulation in non-infarcted myocardium in the first two weeks after infarction, without aggravation of LV dilation. However, consequences for cardiac function have yet to be determined. The present study investigated the effects of aspirin treatment on LV contractility and relaxation, as well as on diastolic LV volume-pressure relationships of MI hearts. The effects of aspirin treatment were compared with the effects of treatment with steroids, which can aggravate post MI dilation of the left chamber (Mannisi *et al.*, 1987). Although low-dose aspirin did not reduce plasma levels of prostaglandins (Chapter 6), aspirin may still modulate the local inflammatory response to tissue damage resulting in the observed effects on collagen build up. Therefore, in addition effects of aspirin therapy were also assessed in a model of pressure-overload hypertrophy, in which cardiac remodeling occurs without inflammation of the myocardium due to acute tissue damage.

MATERIALS AND METHODS

Myocardial infarction and interrenal aortic banding models MI was induced by coronary artery ligation as described in detail in chapter 2 (page 32). In rats that were randomised for interrenal aortic banding (IRAB), a midline laparotomy was performed under pentobarbital (60 mg/kg, i.p.) anesthesia. The intestines were kept aside with gauzes, and the abdominal aorta was

exposed. In the segment between the left and right renal artery, a 23 Gauge needle was positioned alongside of the aorta. To make a fixed stenosis, the aorta was tied off together with the needle, except in sham operation. The needle was then removed, and the abdomen was sutured. Before isolation of the heart, at 4-5 weeks after surgery, polyethylene catheters were inserted into the carotid (PE-50) and femoral (PE-10) artery under pentobarbital anesthesia, and connected to a pressure transducer (Viggo-Spectramed, Oxnard, USA), in order to measure the pressure gradient over the banded aortic segment. To evaluate unilateral renal atrophy due to chronic ischemia, the ratio of left to right kidney weight was determined. Only animals with a left to right kidney weight ratio of <0.9, indicative of left kidney atrophy, were included in analysis.

Experimental groups and treatment protocols Rats were subjected to one of the following protocols: (1) 'Thoracotomy-sham' operation + saline from 2 days before to 3 weeks after surgery. (2) MI + saline from 2 days before to 3 weeks after surgery. (3) MI + aspirin 25 mg/kg from 2 days before to 3 weeks after surgery. (4) MI + methylprednisolone 5 mg/kg (Karr *et al.*, 1995) from 1 week (at the end of the acute inflammatory phase, Fishbein *et al.*, 1978a) to 3 weeks after surgery. (5) 'Laparotomy-sham' operation + saline from 2 days before to 4-5 weeks after surgery. (6) IRAB + saline from 2 days before to 4-5 weeks after surgery. (7) IRAB + aspirin 25 mg/kg from 2 days before to 4-5 weeks after surgery. Treatment was given as daily intraperitoneal injection. Aspirin (lysine-acetylsalicylate, Aspegic®) was purchased from Lorex, Maarsen, The Netherlands, and methylprednisolone (methylprednisolone sodiumsuccinate, Methypresol®) from Pharmachemie B.V., Haarlem, The Netherlands.

Left ventricle diastolic function in infarcted hearts At the end of the protocol, the hearts were isolated under pentobarbital anesthesia. Hearts were mounted for Langendorff perfusion and instrumented for functional measurements as described in detail in chapter 2 (page 32). The end-diastolic LV pressure was set to 10 mmHg (instead of 5 mmHg as used in the other experiments in this thesis) in order to fully monitor ß-agonist-mediated decrease of LV end-diastolic pressure. After a stabilization period of 15 min, baseline functional parameters were measured. In order to determine maximal LV performance during ß-agonist stimulation, responses to increasing doses of isoproterenol (ranging from 10^{-9} to 10^{-5}M) of isoproterenol (L-isoproterenol hydrochloride, Sigma Chemicals, St. Louis, USA) were determined. For each dose, 100 µl of isoproterenol solution (dissolved in saline) was injected into the perfusing medium just before it entered the coronary arteries. Whereas hearts were paced at 350 beats/min during stabilization, pacing was set to 450 beats/min during isoproterenol administration to minimize arrhythmias. After a re-stabilization period, hearts were arrested with a 0.5 ml injection of a 1M potassium chloride solution into the perfusing buffer. At 10 to 20 different LV balloon volumes, diastolic pressures in the range of 0-40 mmHg were measured. For each heart, values were fitted into: $Pressure=c.e^{(k.volume)}+a$, ($r>0.99$).

Collagen content After the functional measurements, hearts were fixated by perfusion with 3.6% phosphate-buffered formaldehyde and processed for collagen measurements as described in chapter 2 (page 32). Quantity of collagen in left ventricles was assessed by morphometry as described in detail in chapter 2 (page 32). For interstitial collagen in non-infarcted LV myocardium, the

Table 7.1 Characterization of aortic banding groups

	SHAM	IRAB	IRAB+asp
n	9	7	9
BW (g)	394 ± 11	363 ± 18	393 ± 16
Total ventricular			
weight (mg)	1066 ± 50	1261 ± 71*	1259 ± 65*
weight/BW (mg/g)	2.7 ± 0.1	3.5 ± 0.1*	3.2 ± 0.1
Systolic blood pressure			
Carotid artery (mmHg)	136 ± 4	164 ± 9*	169 ± 13*
dP over IRAB (mmHg)	-7 ± 2	49 ± 16*	48 ± 12*
L/R kidney weight	0.99 ± 0.02	0.48 ± 0.07*	0.41 ± 0.08*

SHAM: hearts from sham-operated rats, IRAB: interrenal aortic banding, asp: aspirin, BW: body weight, dP over IRAB: systolic blood pressure gradient over aortic banded segment (carotid artery systolic blood pressure minus femoral artery systolic blood pressure), L/R kidney weight: ratio of left kidney weight to right kidney weight. *: $P<0.05$ versus sham values. Values in IRAB and IRAB+asp groups did not differ significantly.

interventricular septum opposite (and thus remote) of the infarct scar was used. High power fields including coronary arteries or focal areas of fibrosis, as encountered in hearts from IRAB rats, were ignored for the measurement of interstitial collagen. However, since the focal areas of fibrosis often occurred around resistance arteries, they were included in the perivascular collagen measurements. Perivascular collagen was measured around 12 resistance arteries (lumen diameter <150 μm) per heart, in the non-infarcted part of the left ventricle (interventricular septum and viable left ventricular free wall). Perivascular collagen area was corrected for luminal area of the vessel (Brilla *et al.*, 1991).

Data analysis Data are expressed as group means ± S.E.M., unless indicated otherwise. Only data from MI hearts with an infarcted area comprising the major part of the LV free wall were included in the study, since smaller infarctions are known to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Data were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc *t*-tests. Differences were considered statistically significant if $P<0.05$.

RESULTS

Cardiac hypertrophy

Interrenal aortic banding resulted in hypertension: mean blood pressure (measured in the carotid artery) was raised from 115 ± 4 mmHg in control animals to 140 ± 8 mmHg in IRAB rats. A similar increase was obtained in systolic arterial blood pressure (Table 7.1). Successful banding was indicated by the considerable pressure gradient over the banded

Table 7.2 Body weight and cardiac mass in rats after coronary artery ligation

	SHAM	MI	MI+MP	MI+asp
n	10	12	7	6
BW (g)	346 ± 8	347 ± 8	300 ± 10*#	354 ± 11
Total ventricular				
weight (mg)	1061 ± 52	1211 ± 54*	1059 ± 64	1261 ± 61*
weight/BW (mg/g)	3.1 ± 0.1	3.5 ± 0.1*	3.5 ± 0.1*	3.6 ± 0.2*

SHAM, hearts from sham-operated rats; MI, myocardial infarction; MP, methylprednisolone; asp, aspirin; BW, body weight. *: $P<0.05$ versus sham values; #: $P<0.05$ versus values of untreated infarcted hearts.

segment of the abdominal aorta. Left kidney atrophy was substantiated by a more than 50% weight decrease of the ischemic compared to the well-perfused kidney. Hypertension-induced cardiac hypertrophy was documented by an 18% rise in total ventricular mass. Aspirin treatment of IRAB rats did not affect any of the above mentioned consequences of this procedure.

Compensatory hypertrophy in MI hearts was indicated by a 14% rise of ventricular mass (Table 7.2), despite replacement of the major part of the LV free wall by lighter scar tissue. Similar to aspirin treatment of IRAB rats, aspirin did not affect cardiac hypertrophy in MI rats. However, methylprednisolone treatment of MI rats reduced both heart weight and body weight, resulting in an unaltered heart weight/body weight ratio.

Collagen content in remodeled hearts

LV interstitial collagen as well as perivascular collagen of resistance arteries was quantified by morphometry. MI-induced remodeling was not associated with an increase in interstitial collagen nor perivascular collagen (Figure 7.1). Treatment of MI rats with methylprednisolone reduced both interstitial and perivascular collagen of non-infarcted LV

Table 7.3 Tissue perfusion (ml/min.g) in MI hearts

	SHAM	MI	MI+MP	MI+asp
Baseline	10.2 ± 0.7	8.7 ± 1.0	7.3 ± 0.6	9.7 ± 0.6
Isoproterenol 10^{-5}M	17.6 ± 1.0	16.0 ± 1.1	12.1 ± 0.8*#	16.4 ± 1.2

SHAM, hearts from sham-operated rats; MI, myocardial infarction; MP, methylprednisolone; asp, aspirin; BW, body weight. *: $P<0.05$ versus sham values; #: $P<0.05$ versus values of untreated infarcted hearts.

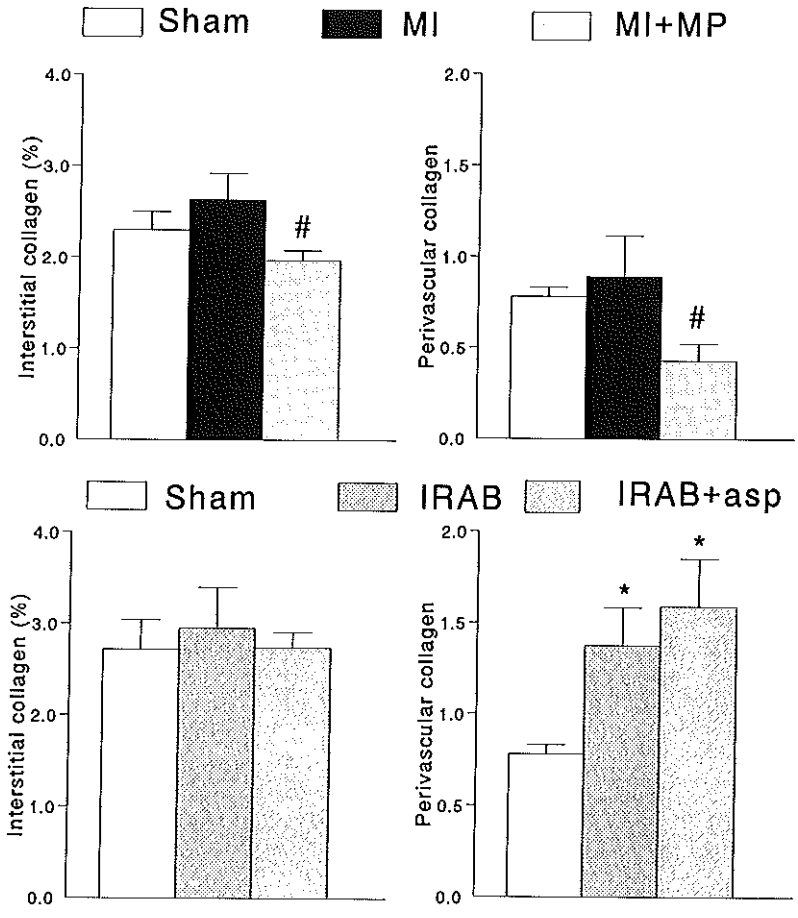

Figure 7.1: Interstitial collagen (left panels) as volume fraction (%) and perivascular collagen (right panels) as collagen to lumen area ratio of resistance arteries. Upper panels: MI hearts; Interstitial and perivascular collagen are measured in non-infarcted LV myocardium. MP: Methylprednisolone. Lower panels: Hearts from rats subjected to IRAB (interrenal aortic banding). Asp: aspirin. *: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI hearts.

myocardium. Similar to aspirin treatment, which did not significantly reduce scar collagen content but only affected collagen quantity of non-infarcted myocardium (Chapter 6), methylprednisolone therapy did not significantly reduce collagen volume fraction of scar tissue: 34.0 ± 3.7% (n=5) versus 42.7 ± 5.2% (n=5) in MI hearts from untreated rats.

Remodeling of myocardium in IRAB rats was associated with focal areas of scar tissue, often around resistance arteries. Interstitial collagen was not increased after IRAB if these scar areas were not included in morphometric analysis. However, there was a distinct increase in perivascular collagen of resistance arteries, which were sometimes located within fibrosed areas (Figure 7.1). Chronic aspirin treatment of IRAB rats did not influence interstitial nor perivascular collagen amount.

Diastolic volume-pressure curves of remodeled left ventricles

MI-induced LV remodeling was associated with a rightward shift of the LV cavity

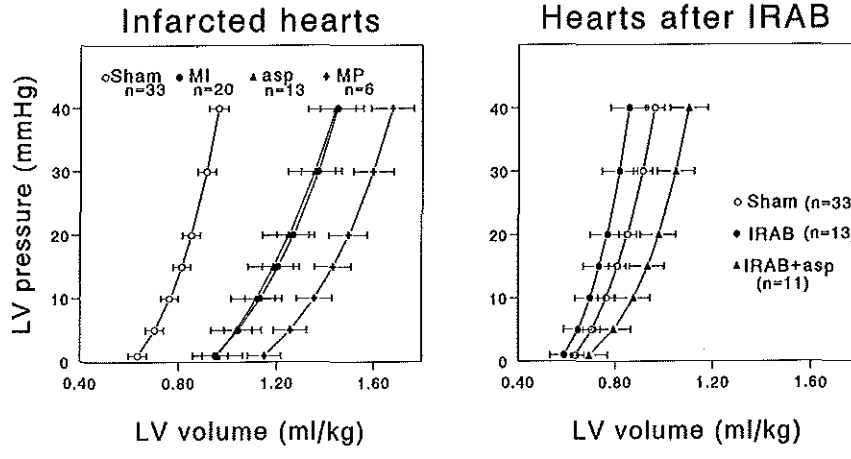

Figure 7.2: Pressure-volume relationships of hearts arrested in diastole. Left panel: MI hearts. Asp: hearts from MI rats treated with aspirin. MP: hearts from MI rats treated with methylprednisolone. Right panel: hearts from rats subjected to IRAB (interrenal aortic banding).

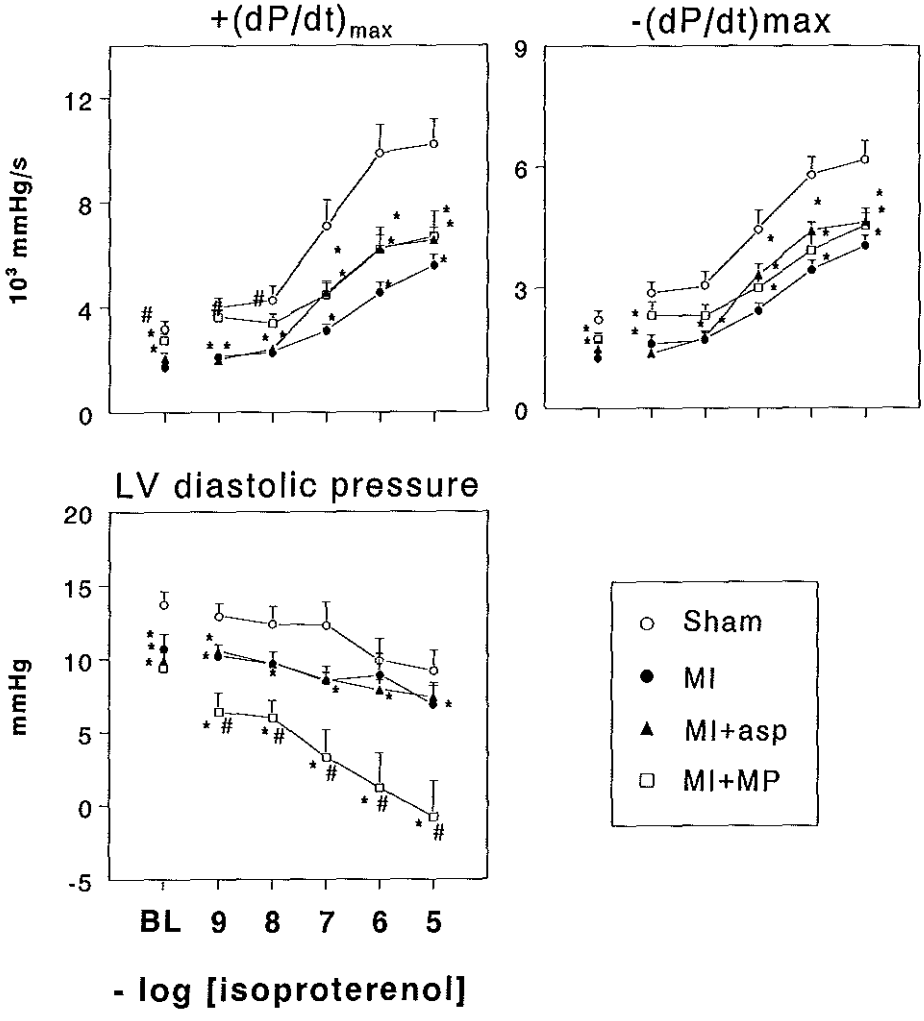

Figure 7.3: LV functional parameters at baseline (BL), and after bolus injection of increasing concentrations of isoproterenol. Upper left panel: peak velocity of contraction (10^3 mmHg/s). Upper right panel: peak velocity of relaxation (10^3 mmHg/s). Lower panel: end-diastolic pressure (mmHg). *: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI hearts.

volume-pressure curve of hearts arrested in diastole (Figure 7.2). In addition, the curves were less steep, as indicated by significantly reduced k values of the exponential volume-pressure relationship: 71 ± 6 ($x10^{-4}$) versus 115 ± 7 ($x10^{-4}$) in hearts from sham-operated rats. Aspirin treatment of MI rats did not alter the volume-pressure relationship, but treatment of MI rats with methylprednisolone resulted in a further rightward shift of the curve.

Cardiac remodeling in rats subjected to IRAB resulted in a slight leftward shift of the volume-pressure relationship, with a significantly steeper curve: k values 168 ± 17 ($x10^{-4}$) versus 115 ± 7 ($x10^{-4}$). After aspirin therapy, curves of hearts after IRAB were shifted to the right side of the control curve. In addition, steepness of the curves, as compared to hearts from untreated IRAB rats, was normalized as indicated by significantly reduced k values: 129 ± 14 ($x10^{-4}$).

Function of infarcted left ventricles with ß-agonist stimulation

Peak velocity of LV contraction, $+(dP/dt)_{max}$, and relaxation, $-(dP/dt)_{max}$, were significantly depressed in MI hearts compared to normal control hearts, at baseline as well as after different doses of isoproterenol (Figure 7.3). This could not be explained by differences in cardiac perfusion, which were not significantly different between MI hearts and hearts from sham-operated rats at any timepoint (Table 7.3). Methylprednisolone treatment of MI rats improved baseline $+(dP/dt)_{max}$, but maximal values were not different compared to hearts from untreated rats, due to a decreased functional reserve. At maximal ß-agonist stimulation, tissue perfusion in methylprednisolone-treated MI hearts was significantly reduced compared to untreated MI hearts (Table 7.3). In aspirin-treated MI hearts, LV function nor myocardial perfusion was significantly different from untreated MI hearts at any timepoint.

Although LV balloon volume was adjusted at the beginning of each experiment to set end-diastolic pressure to 10 mmHg, it appeared that LV diastolic pressure after the stabilization phase had slightly increased in control but not in MI hearts, resulting in a lower diastolic pressure in MI hearts even before the first dose of isoproterenol was given. ß-Adrenoceptor stimulation only had a substantial lowering effect on end-diastolic pressure in MI hearts from methylprednisolone-treated rats, significantly decreasing end-diastolic

pressure compared to the other experimental groups. End-diastolic pressures of MI hearts from aspirin-treated rats were not different from untreated MI hearts at any timepoint.

DISCUSSION

In chapter 6 we showed that chronic treatment of MI rats with aspirin (in a dose that inhibited platelet thromboxane production but did not interfere with endothelial cell prostacyclin production) prevented collagen accumulation in non-infarcted myocardium during the first 2 weeks after MI. The present study was conducted to: i) Determine the effects of aspirin treatment on diastolic function of infarcted left ventricles. ii) Compare the effects of aspirin treatment on diastolic function of infarcted left ventricles with the effects of steroid treatment, during which inhibited collagen synthesis has been reported to aggravate LV dilation (Bulkley & Roberts, 1974; Mannisi *et al.*, 1987). iii) Evaluate the effects of aspirin treatment on collagen content and diastolic volume-pressure relationship in an experimental model of cardiac remodeling without prominent myocardial inflammation due to acute tissue necrosis, such as is observed after MI (Fishbein *et al.*, 1978a; Sulpice *et al.*, 1994).

The main results were: i) Aspirin treatment of MI rats did not aggravate LV dilation at 3 weeks. Impaired baseline and maximal LV function in MI hearts were not altered by

Table 7.4 Collagen content and diastolic volume-pressure relationships

	treatment	int. coll.	P.V. coll.	shift V/P curve	steepness
MI	none	↑/- (vs sham)	↑/- (vs sham)	→ (vs sham)	↓ (vs sham)
	aspirin	↓[1]	↓[1]	-	-
	MP	↓	↓	further →	-
IRAB	none	- (vs sham)	- (vs sham)	~ ← (vs sham)	↑ (vs sham)
	aspirin	-	-	normalization	normalization

Comparison of collagen content and LV volume-pressure curves of hearts from rats subjected to different experimental models of cardiac remodeling (treatment: 'none') and normal hearts, and effects of treatment on these parameters. Int. coll.: interstitial collagen content. P.V. Coll.: Perivascular collagen content. Shift V/P curve: left- or rightward shift of the volume-pressure curve with volume on the horizontal axis. MI: myocardial infarction. IRAB: interrenal aortic banding. MP: methylprednisolone. ↑: increase. ↓: decrease. →: rightward shift. ←: leftward shift. - : no effect. ~: slight effect. Vs sham: versus values of sham-operated hearts. [1] : data from chapter 6.

aspirin. LV diastolic volume-pressure curves of MI hearts were unaltered by aspirin treatment, indicating unchanged LV compliance in the diastolically arrested heart. ii) Steroid treatment of MI rats reduced LV interstitial and perivascular collagen, which was associated with aggravation of LV dilation. LV baseline function was improved by steroid therapy, but maximal LV performance was unchanged. iii) Hypertension due to IRAB caused LV hypertrophy. Interstitial collagen content was not raised, but distinct perivascular fibrosis was observed. Aspirin treatment of IRAB rats did not affect hypertension or LV hypertrophy, nor did it change interstitial collagen content or perivascular fibrosis. However, LV compliance of the hypertrophied hearts, which was reduced after IRAB, was normalized after aspirin treatment.

Relation between collagen and diastolic volume-pressure relationship

In table 7.4, the effects of changes in collagen content on the LV volume-pressure relationships of diastolically arrested hearts are summarized. In infarcted left ventricles, dilation is indicated by a rightward shift of the volume-pressure curves. Increased collagen content of non-infarcted myocardium, similar to that observed in chapter 6, would increase tissue stiffness (Litwin *et al.*, 1991b). However, steepness of volume-pressure curves of the entire left ventricle is decreased, probably due to an effect of LV geometry (shape and size) on the steepness of the volume-pressure relation (Mirsky *et al.*, 1983). In contrast with the increased collagen content in spared tissue of MI hearts as reported by us (Chapter 6) and by other authors (van Krimpen *et al.*, 1991; Smits *et al.*, 1992; McCormick *et al.*, 1994), in the present study collagen volume fraction in non-infarcted myocardium of MI hearts was similar to normal myocardium of control hearts. Total collagen amount of non-infarcted myocardium, however, may still be the same as in chapter 6, since the hypertrophic response in MI hearts, as judged from heart weight, was more pronounced than that observed in chapter 6, and collagen content is expressed as volume fraction.

Despite similar reduction of collagen content in non-infarcted myocardium of MI hearts by aspirin (Chapter 6) and methylprednisolone treatment, only the latter caused a further LV dilation. This deleterious effect of steroid treatment could have been explained by its interference with the replacement fibrosis in the infarcted area (Bulkley & Roberts, 1974; Hammerman *et al.*, 1983a; Mannisi *et al.*, 1987; Vivaldi *et al.*, 1987) in addition to

its effects on collagen in non-infarcted myocardium. However, absolute collagen content in scar tissue, at the end of the healing phase, was not significantly reduced by methylprednisolone, similar to aspirin therapy (Chapter 6). An alternative explanation may be found in a greater effect of steroids than aspirin on physical properties of the collagen network in MI hearts. Despite similar reduction of collagen content, aspirin and methylprednisolone treatment could result in different tensile strength of the collagen weave by different effects on the prevalence of the different types of collagen (Weber *et al.*, 1988) or in the degree of collagen maturation (cross-linking) (Bing *et al.*, 1978; Thiedemann *et al.*, 1983; Iimoto *et al.*, 1988; Kato *et al.*, 1995). The importance of these qualitative factors is supported by the normalization by aspirin treatment of volume-pressure relationships in hearts from rats subjected to IRAB. In this experimental model of cardiac pressure-overload, normalization of volume-pressure curves by aspirin was achieved without effects on interstitial or perivascular collagen content, nor did aspirin affect the hypertrophic response of the myocardium, suggesting effects of aspirin on collagen characteristics such as degree of cross-linking or prevalence of the different collagen types.

Modulation of the collagen network by aspirin treatment

Although minor inflammatory response may occur in pressure overload-induced cardiac hypertrophy following focal myocyte necrosis (Weber *et al.*, 1988), major inflammation after segmental myocardial necrosis such as occurs after MI in both infarcted (Fishbein *et al.*, 1978a, 1978b) and non-infarcted (Sulpice *et al.*, 1994) myocardium, is unlikely. Aspirin treatment normalized volume-pressure curves in aortic banding-induced cardiac hypertrophy without affecting the hypertrophic response of the myocardium, implying effects of treatment on the collagen network. Therefore, the prominent favourable effects of aspirin treatment on volume-pressure curves are probably related to effects on the collagen network dissociated from anti-inflammatory properties of aspirin at a higher dose. This is in agreement with work by Solheim *et al.* (1986b), who reported decreased collagen content and increased collagen solubility in non-inflamed tissue (intact growing femora) of young rats treated with aspirin. Aspirin could interfere with collagen characteristics by 2 mechanisms: i) Aspirin can acetylate free amino groups of the collagen molecule. This inhibits glycation and therefore reduces cross-link formation (Malik &

Meek, 1994). ii) Inhibited production of prostaglandins, which may be involved in the regulation of fibroblast proliferation, even in the absence of a distinct inflammatory reaction (Otto *et al.*, 1982). This would be in agreement with reports on inhibition of collagen accumulation during post MI remodeling by steroids and NSAIDs (Bulkley & Roberts, 1974; Kloner *et al.*, 1978; Brown *et al.*, 1983; Hammerman 1983a and 1983b; Mannisi *et al.*, 1987; Vivaldi *et al.*, 1987; Jugdutt & Basualdo, 1989). Although plasma levels of PGE_2 were not reduced by the used dose of aspirin (chapter 6), myocardial tissue levels of PGE_2 and other prostaglandins might still be affected.

***In vitro* function of infarcted left ventricles after aspirin or steroid treatment**

The reduction of interstitial and perivascular collagen observed in the present study with methylprednisolone treatment of MI rats led to aggravation of LV dilation and an enhancement of diastolic pressure decrease to *in vitro* ß-adrenergic stimulation. Despite similar reduction of collagen content in MI rats at 2 weeks (Chapter 6), aspirin treatment did not aggravate LV dilation nor alter the *in vitro* response to ß-adrenergic stimulation. These differential effects of aspirin and steroid treatment indicate that steroid treatment has a more pronounced effect on the tensile strength of the collagen network, even though steroid injections were not started until 1 week after infarction, when acute inflammatory response is over (Fishbein *et al.*, 1978a).

Conclusions

Both aspirin and methylprednisolone treatment can similarly reduce collagen content of non-infarcted myocardium in MI hearts. However, aspirin did not alter LV volume-pressure relation, whereas methylprednisolone aggravated LV dilation. In hearts from hypertensive rats LV compliance was reduced, and this was associated with perivascular fibrosis of resistance arteries. Aspirin treatment of hypertensive rats did not alter hypertrophy nor collagen content, but normalized LV compliance. Therefore, altered collagen characteristics (type, degree of cross-linking) may determine myocardial stiffness when collagen content is not markedly increased. The beneficial effects of aspirin treatment of hypertensive rats may be related to normalization of collagen characteristics.

CHAPTER 8

LOW-DOSE ASPIRIN IMPROVES IN VIVO HEMODYNAMICS IN CONSCIOUS, CHRONICALLY INFARCTED RATS

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Submitted for publication

ABSTRACT

Low-dose aspirin, which inactivates cyclooxygenase of platelets but not of vascular endothelium, is commonly used in patients with coronary atherosclerotic disease to prevent myocardial infarction or the repeat of this event in patients with established myocardial infarction. In rats with myocardial infarction, aspirin treatment inhibited collagen build-up in non-infarcted myocardium but not reactive hypertrophy, in addition to its anti-platelet effect. We investigated the *in vivo* hemodynamic effects of long-term low-dose aspirin in conscious, chronically instrumented infarcted rats at 3 weeks after myocardial infarction. Aspirin did not improve cardiac output at rest nor after maximal stimulation (rapid intravenous volume loading). However, the increased heart rate in untreated infarcted rats was restored to sham values, with a slight (but statistically not significant) increase in stroke volume, at similar cardiac loading conditions. The lower heart rate after aspirin was a reflection of a reduced intrinsic heart rate (studied in isolated perfused hearts) rather than to a lower sympathetic activation of the heart (unaltered ß-adrenergic responsiveness and plasma catecholamine levels). The lower heart rate in aspirin-treated infarcted rats caused a prolonged diastolic time, with ratio of diastolic to systolic time not significantly altered. The present data show that pharmacological inhibition of the build-up of the collagen network of non-infarcted myocardium in infarcted hearts may result in a favourable reduction of heart rate with a prolonged diastolic time.

INTRODUCTION

Low-dose aspirin (acetylsalicylic acid) reduces platelet production of pro-aggregatory and vasoconstrictor thromboxane in favour of anti-aggregatory and vasodilator prostaglandins (Patrignani *et al.*, 1982; Coller, 1991). The anti-platelet action of low-dose aspirin is used in primary and secondary profylaxis of myocardial infarction (MI) (ISIS-2 Collaborative Group, 1988; Antiplatelet Trialists' Collaboration, 1988).

In a well-established rat model for the functional and structural consequences of myocardial infarction, we have recently studied the effects of chronic aspirin on cardiac remodeling, at a dose that inhibited platelet but not vascular endothelium cyclooxygenase

(Chapter 6). Early aspirin treatment affected collagen deposition in the non-infarcted part of the myocardium, while leaving collagen deposition in the infarcted area relatively unaltered.

In the present study we investigated the functional consequences of chronic low-dose aspirin, during the early phase after MI. Left ventricular function *in vitro* was not improved by aspirin treatment (Chapters 6 and 7). However, since absence of improvement of *in vitro* left ventricular function (Chapter 4) does not necessarily imply absence of *in vivo* cardiac function (J. Pfeffer *et al.*, 1987; Schoemaker *et al.*, 1991), effects of early aspirin treatment were studied in conscious unrestrained rats, chronically instrumented for hemodynamic measurements (Schoemaker *et al.*, 1990, 1991). Because of the interesting observation of a reduced heart rate in these experiments, two additional experiment were performed to investigate whether this could be attributed to reduced sympathetic drive (plasma catecholamine levels) or to altered intrinsic heart rate (*in vitro* heart rate).

MATERIALS AND METHODS

Animals Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 270-320 gram at the time of operation were used; they were housed in groups of 2 or 3, at a 12h light/dark cycle, with standard rat chow and water available ad libitum. Animals used in the hemodynamic studies were housed separately after implantation of measuring equipment. Left descending coronary artery ligation was performed as described in chapter 2 (page 32). Saline or aspirin 25 mg/kg (lysine-acetylsalicylic acid, Aspégic, Lorex b.v., Maarssen, The Netherlands) dissolved in saline was administered as daily i.p. injections of 1 ml/kg starting 2 days before surgery, and was continued until the end of the protocol 21 days after surgery (Chapter 6). At all stages of the experiments, animals were treated according to the local institutional guidelines.

In vivo hemodynamics Two weeks after coronary artery ligation rats were re-anesthetized with pentobarbital (60 mg/kg, i.p.), and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed on the ascending aorta using previously described techniques (Smits *et al.*, 1982; Schoemaker *et al.*, 1991). Briefly, after intubation and starting positive pressure respiration, the thorax was opened at the third right intercostal space, and the ascending aorta was dissected from surrounding tissues. A 2.6 mm diameter probe was placed around the aorta 1-2 mm above the outlet of the heart. The cable was fixed to the ribs, the thorax was closed in layers, and the connector was exteriorized in the neck, where it was sutured to the skin. Five days later, rats were re-anesthetized and implanted with a catheter (PE-10 heat-sealed to PE-50) in the abdominal aorta through the femoral artery to measure arterial blood pressure (MAP). Furthermore, through the

femoral vein, a catheter (PE-10 heat-sealed to PE-50) was implanted into the abdominal vena cava for infusion and a Silastic (602-175, Dow Corning, Midland, MI, U.S.A.) catheter was placed in the thoracic vena cava for measurement of central venous pressure (CVP). All catheters were exteriorized in the neck, filled with heparinized saline, and closed with metal plugs. Animals were allowed to recover 2 days before measurements were done. On the day of measurements, rats were connected to the measuring equipment. Signals were fed into a 68B09-based microprocessor and AT-compatible microcomputer, sampling at 500 Hz. Mean values were obtained for arterial (MAP) and central venous pressure (CVP). From the aortic flow signal, besides cardiac output (CO), heart rate (HR), duration of the ejection time (ET) as a measure of systolic time, and stroke volume (SV) were obtained. Total peripheral resistance was calculated as (MAP-CVP)/CO. Heart period (HP) and diastolic time were calculated as 60,000/HR and HP-ET, respectively. All derivations (except the latter two) were made on-line and stored on disk for later analysis. After 45-60 min stabilization time, baseline values were obtained for 10 min. Then 12 ml (37°C) of a Ringer's solution was rapidly infused in 1 min through the abdominal vena cava catheter. This (Schoemaker *et al.*, 1990; 1991) and a very similar method in anesthetized rats (M. Pfeffer *et al.*, 1979, J. Pfeffer *et al.*, 1987) has been shown to increase CO to a plateau level, which can be used as an indicator for maximal cardiac function, and is referred to as CO_{max}.

In vitro heart rate Hearts were dissected and perfused as described before. LV end-diastolic pressure was set to 5 mmHg by adjusting the volume of the balloon in the left ventricle. When hearts had stabilized (±30 min), baseline values for heart rate were obtained. Then heart rate was maximally increased with isoproterenol by injecting 0.1 ml of 10^{-5} M solution into the buffer just before it entered the coronary arteries. The dose of isoproterenol was validated to induce maximal effect in complete dose-response curves obtained in pilot experiments.

Catecholamines In a separate group of rats, the left common carotid artery was cannulated under pentobarbital anesthesia (60 mg/kg, i.p.). The catheter was passed to the neck subcutaneously, where it was fixated and exteriorized. Rats were allowed to recover for at least one day. Then, blood samples were taken in the last hour of the light period of the light/dark cycle. For that, the carotid catheter was extended with saline-filled tubing to obtain blood samples without disturbing the rat. After at least 30 min of habituation, 2 ml of blood was obtained, and processed according to Boomsma *et al.* (1993). Briefly, blood was collected in syringes prepared with 20 µl EDTA (0.1M), and put on ice. After centrifugation, plasma was collected in pre-chilled tubes filled with 1.2 mg glutathione and stored at -70°C. Plasma concentration of noradrenaline, adrenaline and dopamine were determined by HPLC and electrochemical detection, as described in detail by Boomsma *et al.* (1993). Plasma noradrenaline concentration can be used as an index of activity of the total sympathetic nervous system, despite limitations to this method (only a fraction of released noradrenaline diffuses to plasma, and plasma concentrations also depend on rate at which it is removed from plasma) (Esler *et al.*, 1985).

Data analysis All data are presented as means ± S.E.M. Data of infarcted rats were only included if the infarction comprised the major part of the left ventricular free wall, since small infarctions

Table 8.1 Characterization of experimental groups

	SHAM	MI	MI+asp
n	11	10	8
Body weight (g)	387 ± 10	371 ± 7	366 ± 5
Ventricular weight (mg)	978 ± 35	932 ± 24	928 ± 23
Ventricular/body weight (mg/g)	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1

n: number of animals in each group, Body weight: body mass at the end of the experimental protocol, Ventricular weight: total wet weight of left + right ventricle, MI: myocardial infarction, asp: aspirin.

are found to be hemodynamically fully compensated (M. Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Differences between groups were analyzed using one-way analysis of variance and Bonferroni's *t*-tests for multiple group comparisons (Wallenstein *et al.*, 1980). Differences are regarded statistically significant if $P<0.05$.

RESULTS

In vivo hemodynamics

Experimental groups used for hemodynamic studies are characterized in table 8.1. Since values in the other experiments did not show substantial differences for the presented parameters, this table is regarded as representative for the other experiments as well, and is described only once. No significant changes in heart weight or body weight were found between the experimental groups. The lack of effect of MI on heart weight, despite replacement of a substantial part of the left ventricle (on average ±40% of left ventricular circumference) by much lighter scar tissue implies hypertrophy of the spared myocardium.

Hemodynamics were measured *in vivo* in conscious unrestrained rats. LV dysfunction in these rats was substantiated by decreased CO and SV at rest (Figure 8.1), and after maximal stimulation (volume loading: CO_{max} 120 ± 4 and 98 ± 3 ml/min; SV_{max} 303 ± 15 and 231 ± 9 µl in sham and MI rats, respectively). HR was significantly increased after MI (Figure 8.1). Aspirin treatment did not affect CO at rest nor after volume loading (CO_{max}: 92 ± 4 ml/min). However, although CO at rest was not improved, it was composed of a significantly lower HR and a slightly (but not statistically significantly) higher SV (Figure 8.1). In all experimental groups, hearts operated at comparable loading conditions (Table 8.2): similar preload (CVP) and similar afterload (MAP and TPR). To study the HR

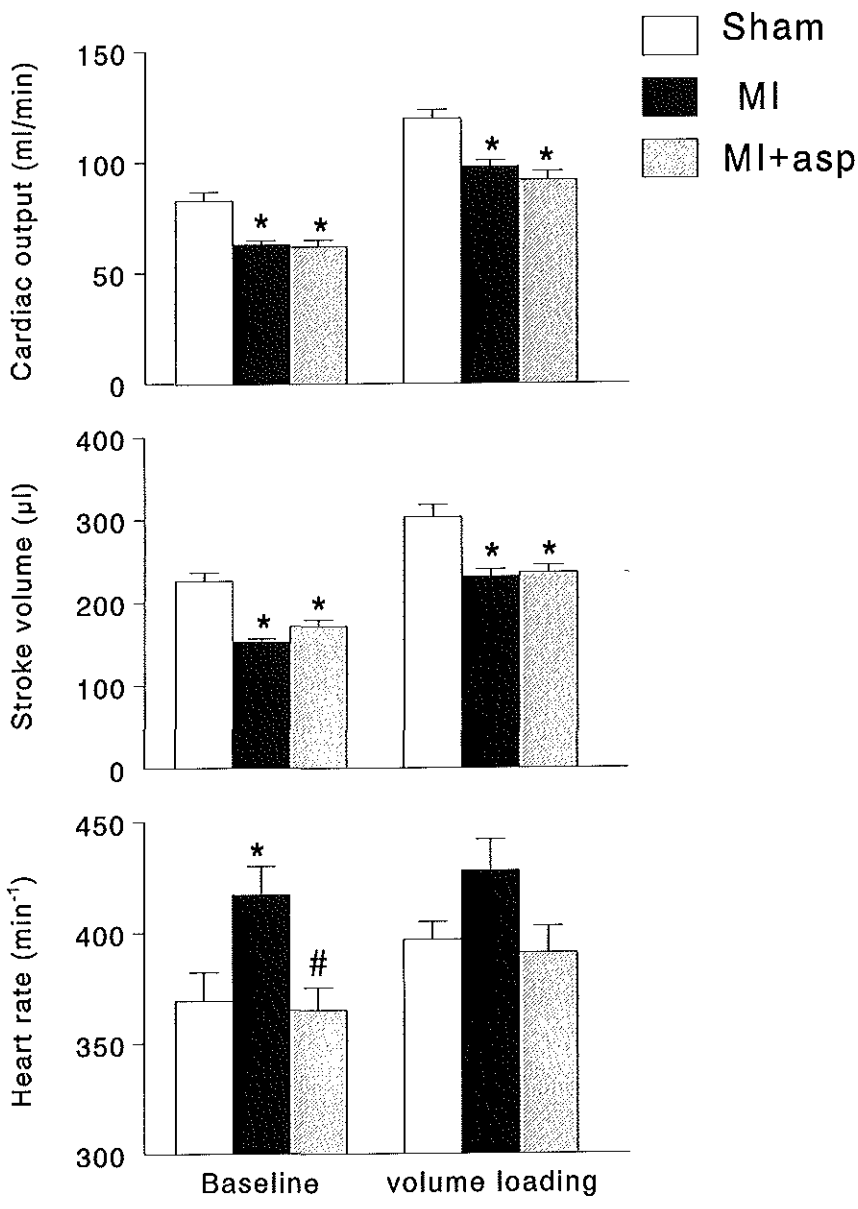

Figure 8.1: Hemodynamic parameters at baseline (left 3 bars) and during rapid intravenous volume loading (right 3 bars). Upper panel: Cardiac output (ml/min); Middle panel: Stroke volume (µl); Lower panel: Heart rate (min^{-1}). MI: myocardial infarction; asp: aspirin.
*: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI rats.

Table 8.2 ***In vivo*** **baseline loading conditions of the heart**

	SHAM	MI	MI+asp
n	7	7	7
CVP (mmHg)	2.4 ± 0.8	1.6 ± 1.1	1.9 ± 0.5
MAP (mmHg)	106 ± 2	99 ± 4	96 ± 2
TPR (mmHg.min/ml)	1.30 ± 0.09	1.56 ± 0.10	1.54 ± 0.09

n: number of animals in each group; CVP: central venous pressure (measured in inferior vena cava); MAP: mean arterial blood pressure; TPR: total peripheral resistance. MI: myocardial infarction; asp: aspirin.

changes in more detail, heart period (HP) was calculated and ejection time (ET) was subtracted to obtain diastolic time. Data are presented in figure 8.2. HP as well as diastolic time were significantly decreased after MI and restored by aspirin treatment. In all groups, time balance between systolic and diastolic time was not significantly different (percentage diastolic time of total heart period: 62.6 ± 0.6, 58.8 ± 2.0 and 62.9 ± 0.7 % in sham, untreated MI rats and aspirin-treated MI rats, respectively).

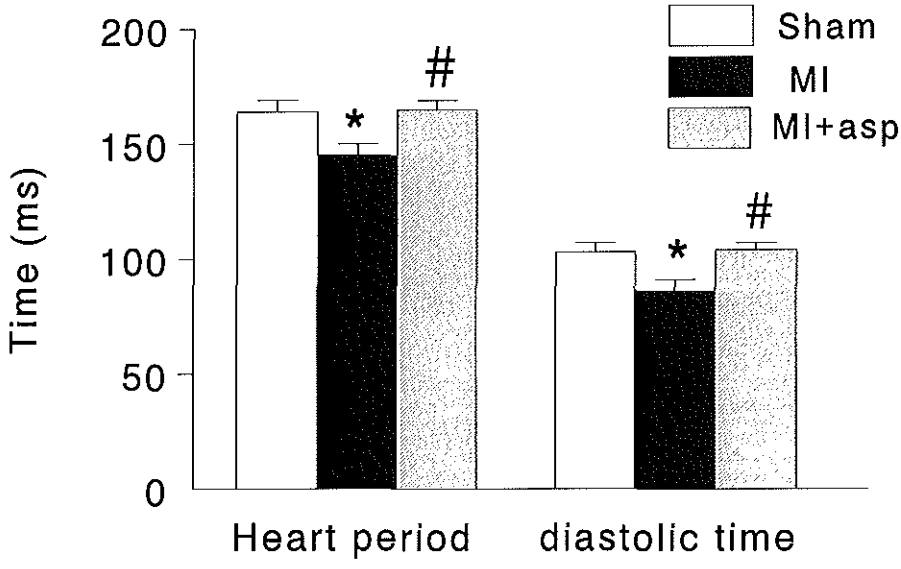

Figure 8.2: Heart period (ms) and diastolic time (ms) as derived from *in vivo* cardiac output signal in the resting animal. MI: myocardial infarction; asp: aspirin. *: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI rats.

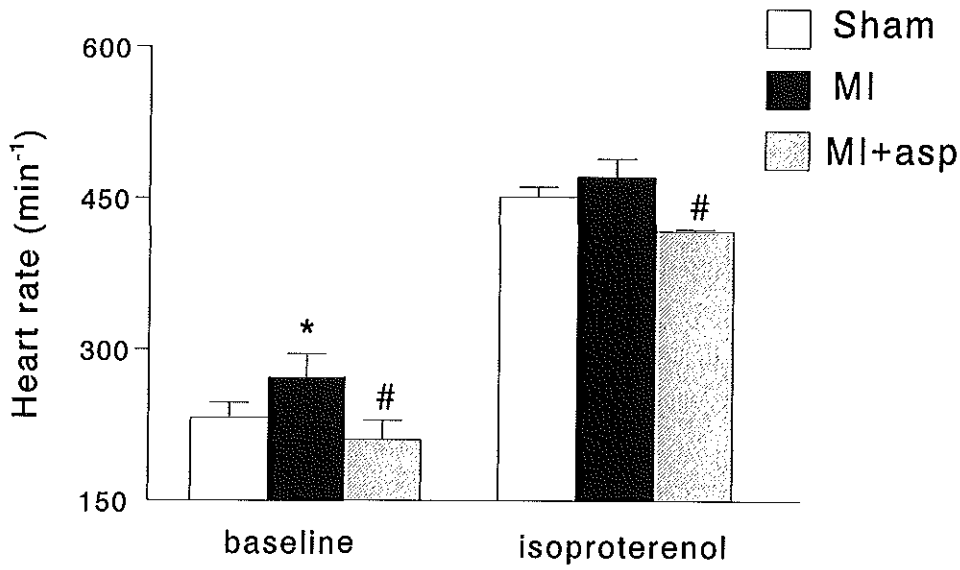

Figure 8.3: Heart rate (assessed in isolated, perfused hearts). Baseline: intrinsic resting heart rate. Isoproterenol: heart rate after maximal ß-adrenergic stimulation (intracoronary isoproterenol administration). MI: myocardial infarction; asp: aspirin. *: $P<0.05$ versus sham values; #: $P<0.05$ versus untreated MI hearts.

The tendency to a relatively lower diastolic period (as percentage of the heart period) in MI rats was restored by aspirin.

In vitro heart rate

Assessment of intrinsic heart rate in isolated, perfused hearts showed similar results as the *in vivo* measurements: Significantly increased HR in MI hearts compared to control hearts, and significant reduction of HR after aspirin treatment (Figure 8.3). After maximal increase with isoproterenol, a similar pattern could be observed, although the significant difference between sham and MI hearts had disappeared (Figure 8.3).

Plasma catecholamines

Plasma concentrations of catecholamines revealed a 43% increase in plasma noradrenaline in MI rats, which was not altered by aspirin treatment (Figure 8.4). Plasma adrenaline and dopamine levels were not different between the experimental groups.

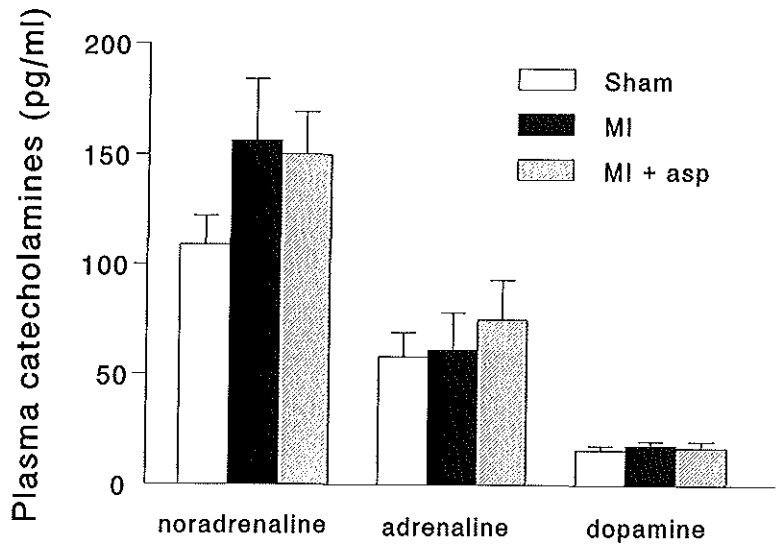

Figure 8.4: Plasma catecholamine concentrations (pg/ml) in arterial blood, sampled from resting animals. MI: myocardial infarction; asp: aspirin.

DISCUSSION

Chronic treatment of MI rats with low-dose aspirin affects collagen deposition in non-infarcted myocardium but not reactive hypertrophy (Chapter 6). The present study was carried out to investigate if the same treatment protocol would improve hemodynamics in MI rats, as a result of its effects on cardiac remodeling. The major findings were: i) Aspirin treatment of MI rats did not improve pump capacity of the heart, but cardiac output at rest was achieved at a significantly lower heart rate compared to untreated MI rats. ii) The reduced heart rate after aspirin treatment was not associated with decreased sympathetic nerve activity (as indicated by unaltered catecholamine levels). Rather, it was a reflection of a lower intrinsic heart rate as assessed in isolated, perfused hearts.

Low-dose aspirin treatment has become an established strategy in the prevention of thrombotic complications of coronary atherosclerotic disease. An equivalent treatment in rats, based on the clinical rationale of low-dose aspirin (inactivation of platelet but not vascular endothelium cyclooxygenase), has been evaluated in chapter 6. Daily

intraperitoneal injections of 25 mg/kg inhibited platelet thromboxane production, while leaving vascular production of prostaglandins intact (Chapter 6, page 96). This treatment, started 2 days before induction of MI, did not alter infarct size and had no significant effect on infarct collagen content, indicating no major anti-inflammatory effects. However, low-dose aspirin affected collagen deposition in the non-infarcted myocardium: Morphometrically assessed interstitial and perivascular collagen content was reduced, and inspection of the myocardium with polarizing light microscopy suggested a greater amount of thin fibers with aspirin treatment.

In chapter 7, we determined diastolic LV volume-pressure relationships in MI hearts from untreated as well as aspirin-treated rats. An improved diastolic compliance could be anticipated, since interstitial collagen is associated with left ventricular stiffness (Raya *et al.*, 1988; Litwin *et al.*, 1991b), whereas on the other hand pharmacological interference with the build-up of the collagen network after MI with steroids could result in aggravation of LV dilation (Chapter 7, Bulkley & Roberts, 1974; Mannisi *et al.*, 1987). However, pressure-volume curves of MI hearts from aspirin-treated rats were identical to those of MI hearts from untreated rats, indicating similar diastolic LV compliance and no aggravation of LV dilation. These results, obtained in diastolically arrested hearts, do not exclude effects of treatment on active stiffness in the beating heart.

In the present study, similar baseline cardiac output at a significantly lower heart rate indicates favourable functional effects of altering the post MI build-up of the collagen network in non-infarcted myocardium, with intact reactive hypertrophy. Captopril treatment during the same period prevented both collagen accumulation as well as reactive hypertrophy of non-infarcted myocardium (van Krimpen *et al.*, 1991), and resulted in the unfavourable hemodynamic effect of a reduced SV at increased HR for the same CO (Schoemaker *et al.*, 1991). Prevention of both hypertrophy and interstitial collagen accumulation by blockade of the AT_1 receptor, however, was not associated with these negative hemodynamic consequences (Smits *et al.*, 1992). AT_2 receptor blockade during the healing phase revealed the same adverse higher HR and lower SV as captopril and prevented reactive hypertrophy rather than collagen accumulation (Smits, unpublished data). Altogether, selective reduction of collagen accumulation in spared myocardium with

intact cardiomyocyte hypertrophic response may provide an optimum in pharmacological modulation of the early post MI remodeling stage.

In vivo HR is strongly determined by both sympathetic (and parasympathetic) nerve activity and circulating catecholamines as well as other circulating hormones. Circulating catecholamines were not altered by aspirin treatment, practically ruling this out as an explanation for the observed effects on HR. Measurement in isolated, buffer-perfused hearts circumvent all hormonal and neural influences. The effect of aspirin treatment on HR appeared even stronger *in vitro* than *in vivo*, while increases in heart rate to ß-stimulation were preserved, indicating that the lower heart rate could be attributed to intrinsic changes in the heart, rather than to a changed sympathetic outflow to the heart. Corresponding findings were reported after physical training (Negrao *et al.*, 1992). *In vivo* bradycardia could be (further) unmasked by autonomic blockade, suggesting even a counterregulating effect of the autonomic nervous system. The authors suggest a direct effect on the sinus node. The sinus node consists for a major part of collagen (Kohl & Noble, 1996), which could be affected by aspirin treatment as well. The same authors suggest a strong interrelation between interstitial collagen and sinus node activity, modulated by stretch. Although this provides an attractive mechanistic explanation, further experimental evidence is needed to elucidate the relation between remodeling and heart rate, and whether the lower heart rate is a primary or secondary event in the aspirin-induced altered hemodynamics of MI rats: Increased stroke volume after aspirin treatment, caused by improved myocardial relaxation, could allow a lower heart rate in MI rats.

Independent of its origin, bradycardia is associated with capillary growth (Hudlicka *et al.*, 1995), which could provide high clinical benefit. Maximal coronary flow in isolated, perfused MI hearts was not increased by aspirin treatment (data not shown), but increased capillarization of non-infarcted myocardium would enhance tissue oxygenation, even without changes in maximal coronary flow (Hudlicka *et al.*, 1995). Moreover, in our assessment of maximal coronary flow in isolated hearts, untreated MI hearts and aspirin-treated MI hearts were paced at the same frequency (350 beats/min), whereas the present study suggests a lower heart rate in aspirin-treated MI rats. The lower heart rate associated with prolonged diastole, could in itself increase myocardial perfusion, especially of the

subendocardial layer, which would be of benefit in coronary atherosclerotic disease (Gordon, 1974). Finally, mechanical efficiency decreases at higher heart rate, already in normal hearts and even more in patients with coronary artery disease (Stewart *et al.*, 1993).

Conclusion

Low-dose aspirin treatment of rats during the early post MI remodeling phase provides a pharmacological tool to inhibit collagen accumulation in non-infarcted myocardium while leaving the compensatory myocyte hypertrophic response intact. Aspirin-treated MI rats had a lower heart rate than untreated MI rats, which was due to a reduced intrinsic heart rate rather than to a decreased sympathetic nervous system activity. The reduced intrinsic heart rate after aspirin could be a reflection of the effects of treatment on cardiac remodeling. Further investigations should elucidate the exact mechanisms of the relationship between remodeling and heart rate, and evaluate if similar favourable effects of low-dose aspirin, additional to its antiplatelet action, could be observed in patients as well.

CHAPTER 9

CONCLUDING REMARKS AND OUTLOOK

Increased hemodynamic load on the myocardium leads to structural changes at organ, tissue and cellular level, which are called cardiac 'remodeling'. Loss of a major part of the LV contractile myocardium due to a myocardial infarction is a frequent cause of cardiac remodeling. Although compensatory hypertrophy can restore the amount of contractile myocardium, pump function of the remodeled infarcted heart may not be normalized. This can lead to persistent neurohumoral activation with sustained cardiac overload, progressive LV dilation and deterioration towards heart failure. Recent publications have established that contractility of non-infarcted hypertrophied myocardium is decreased (Litwin *et al.*, 1991a, 1991b, 1995; Litwin & Morgan, 1992; Cheung *et al.*, 1994; Kramer *et al.*, 1996; Melillo *et al.*, 1996), while relaxation is hampered (Litwin *et al.*, 1991a). Therefore, permanently depressed cardiac output, even despite restored amount of contractile tissue, may indicate intrinsic functional defects at tissue, cellular or subcellular level of remodeled hypertrophied myocardium.

The objective of this thesis was investigation of possible determinants of progressive deterioration of cardiac function in remodeled hypertrophied MI hearts. Ligation of the left anterior descending (LAD) coronary artery in rats, resulting in transmural infarction of the LV free wall, was used to study the structural and functional consequences of a large myocardial infarction.

MYOCARDIAL PERFUSION

Impaired coronary reserve has been described in different forms of cardiac hypertrophy and has been implicated in the decompensation from left ventricular dysfunction to failure (Vatner & Hittinger, 1993). The first part of this thesis (Chapters 2-5) focused on the perfusion of viable hypertrophied myocardium. Peak perfusion of remodeled hypertrophied myocardium was found to be decreased in the most hypertrophied region, that is the remaining viable part of the infarcted LV free wall. Reduced perfusion was related to a greater increase in tissue mass than in vascular capacity, rather than to remodeling of resistance arteries in non-infarcted myocardium. An insufficient adaptation of the coronary vascular bed to the increase in cardiac muscle mass that has to be supplied is consistent with the decreased capillary density found in the rat MI model (Turek *et al.*,

1978; Anversa *et al.*, 1985b, 1986a). Decreased vascular density, in combination with increased interstitial volume and cardiomyocyte cell volume, will lengthen oxygen diffusion pathway (Anversa *et al.*, 1985b). Hampered oxygen supply and decreased myocyte intracellular concentration of mitochondria (Anversa *et al.*, 1986a) will decrease the potential for ATP-production. Imbalance between ATP production and consumption can be counteracted by lower myofibrillar ATP consumption, as is found in hypertrophied rat (Geenen *et al.*, 1989) and human (Anderson *et al.*, 1992) myocardium, and by down-regulation of other ATP-consuming systems such as the sarcoplasmic calcium pump (van Heugten *et al.*, 1996). However, reduction of ATP consumption by down-regulation of myofibrillar ATPase and sarcoplasmic ATP-dependent calcium pumps may be achieved at the expense of velocity of contraction and relaxation. In our experiments, lower ATP consumption was suggested by increased tolerance to low-flow ischemia (Chapter 4) despite the aforementioned decreased vascularization of remodeled hypertrophied myocardium (Chapters 2 and 3), and by decreased peak +(dP/dt) and -(dP/dt) in isovolumetrically beating left ventricles (Chapters 4-7).

In addition to disturbed energy homeostasis within the cardiomyocyte, enhanced cardiac load might interact with reduced oxygen supply (Tanaka *et al.*, 1994) to trigger programmed cell death (apoptosis). Apoptosis has been reported in dogs with heart failure after rapid ventricular pacing (Liu *et al.*, 1995) or multiple coronary microembolization (Sharov *et al.*, 1996) and in non-infarcted myocardium of MI rats (Anversa *et al.*, 1996). After the initial loss of a substantial part of contractile myocardium, ongoing loss of cardiomyocytes could lead to the vicious circle of progressive heart failure.

Effects of captopril

Reduced vascularization of non-infarcted myocardium after MI can be reversed by inhibiting reactive hypertrophy in the surviving part of the heart. Our studies with the angiotensin I converting enzyme (ACE)-inhibitor captopril showed regression (Chapter 4) or prevention (Chapter 5) of compensatory hypertrophy. Vascular growth during remodeling was not affected, as maximal coronary flow was unaltered by treatment. Consequently, peak tissue perfusion was improved by captopril, reflecting improved tissue vascularization. Despite the decreased amount of contractile myocardium, isovolumic LV

function (measured in isolated, perfused hearts) was not affected. Improved vascularization of MI hearts after captopril treatment resulted in a better preservation of aerobic metabolism during an additional period of ischemia, in agreement with the reported lower number of ischemic events in MI patients treated with captopril (Rutherford *et al.*, 1994) and less electrocardiographic signs of ischemia during ambulatory ECG monitoring and exercise testing (Søgaard *et al.*, 1993, 1994). Ameliorated cardiomyocyte oxygenation with captopril is consistent with the restored content of the fast isomyosin of rat MI hearts reported by Michel and associates (1988), improved calcium-handling (Litwin & Morgan, 1992) and restored intracellular energy homeostasis (Sanbe *et al.*, 1995). Thus, impaired tissue perfusion due to decreased vascularization may be the main factor leading to intracellular adaptive changes in the cardiomyocyte, which can eventually lead to cellular dysfunction and additional cell loss. Restored tissue perfusion, such as can be achieved by chronic treatment with ACE inhibitors, appears to reverse the intracellular changes within the cardiomyocyte.

CARDIAC INTERSTITIUM

Besides impaired tissue perfusion, increased interstitial and perivascular collagen in non-infarcted myocardium may be involved in the gradual deterioration of cardiac function, since it has been associated with increased tissue stiffness (Litwin *et al.*, 1991b). In the second part of this thesis, interstitial collagen accumulation in non-infarcted myocardium of MI hearts was studied. Increased collagen content of the spared part of MI hearts has been demonstrated in rats (Smits *et al.*, 1992; McCormick *et al.*, 1994) and humans (Volders *et al.*, 1993), and leads to increased stiffness of spared myocardium (Litwin *et al.*, 1991b). Collagen fraction of non-infarcted tissue was increased in chapter 6, whereas it was comparable to sham-operated rats in chapter 7. However, the amount of collagen may still be increased with an unaltered fraction depending on the degree of hypertrophy. Low-dose aspirin, devoid of distinct anti-inflammatory activity, inhibited collagen build-up in non-infarcted myocardium (Chapter 6) but left compensatory hypertrophy unaffected. This action of aspirin on collagen has been demonstrated before in rat skin (Solheim *et al.*, 1986a) and rat bone (Solheim *et al.*, 1986b). Moreover, low-dose aspirin normalized LV

stiffness in hearts that had been subjected to pressure-overload. This occurred without effects on collagen content, suggesting modulation of stiffness by interference with molecular characteristics of the collagen. Aspirin treatment has been shown to inhibit sugar-induced enhanced cross-linking, probably by acetylation of free amino acids available for cross-link formation on collagen molecules (Malik & Meek, 1994). This would be in agreement with the observation of thinner collagen fibers at microscopic inspection of MI hearts from rats treated with aspirin (Chapter 6).

In contrast to more aggressive inhibition of collagen synthesis with methylprednisolone (Chapter 7) or total blockade of cross-link formation (Kato *et al.*, 1995), low-dose aspirin did not aggravate LV dilation or infarct thinning. LV diastolic compliance nor active diastolic or systolic function, measured in isolated hearts, was altered by aspirin treatment (Chapter 7). Despite the lack of treatment effects *in vitro*, studies in conscious rats revealed a lower baseline heart rate with the same cardiac output as untreated rats (Chapter 8). The lower heart rate is probably related to altered mechanical properties of the heart rather than decreased sympathetic stimulation, since heart rate in isolated MI hearts was also lower after aspirin treatment, and plasma levels of catecholamines were not altered by treatment. A lower heart rate may improve the balance between oxygen demand and supply of hypertrophied myocardium by 3 mechanisms: i) Enhanced tissue perfusion through longer diastolic time (Gordon, 1974). ii) Improved energetic efficiency at a lower heart rate (Stewart *et al.*, 1993). iii) Decreased heart rate has been associated with increased capillarization of the myocardium (Brown *et al.*, 1994; Hudlicka *et al.*, 1995). A reduced heart rate can also be achieved with beta-blockers, but although they are widely prescribed as secondary prevention after MI (Levy, 1990), their use in post MI heart failure is still controversial due to their potential negative inotropic action (Neubauer *et al.*, 1994). Thus, modulation of collagen build up in non-infarcted myocardium, such as described with chronic low-dose aspirin, may beneficially affect clinical outcome after myocardial infarction.

FUTURE OUTLOOK

Following major loss of contractile myocardium due to infarction, normalization of the relation between cardiac muscle growth and vascular growth is associated with improved function of non-infarcted myocardium, and may thus beneficially affect clinical outcome. Vascularization in remodeled MI hearts can be increased by inhibition of reactive hypertrophy or by stimulation of vascular growth. Prevention or regression of compensatory hypertrophy after MI can be achieved by ACE-inhibitors, but immediate and complete prevention of hypertrophy was shown to have deleterious effects on cardiac function (Schoemaker *et al.*, 1991). Therefore, treatment strategies that enhance vascular growth should be further developed and evaluated. Angiogenesis may be stimulated by the application of growth factors (Battler *et al.*, 1993; Banai *et al.*, 1994) or by regular aerobic exercise (Przyklenk & Groom, 1985; Orenstein *et al.*, 1995). From the present thesis still another approach to improve tissue perfusion has come forward: Inhibition of collagen accumulation in non-infarcted myocardium with unaltered hypertrophy by low-dose aspirin treatment resulted in a reduced resting heart rate of MI rats. The exact mechanisms by which aspirin inhibits collagen accumulation and of the coupling between remodeling and intrinsic heart rate still have to be elucidated. In addition, it is valuable to investigate if aspirin has similar effects in MI patients. Heart rate reduction improves tissue perfusion by increasing diastolic time (Gordon, 1974), while energetic efficiency is improved (Stewart *et al.*, 1993). Moreover, long-term reduction of heart rate has been associated with improved capillarization (Brown *et al.*, 1994; Hudlicka *et al.*, 1995). Similar heart rate reduction as with aspirin treatment could be achieved by beta-blockers, but their use remains controversial due to their negative inotropic action (Neubauer *et al.*, 1994). In conclusion, reversal of the disturbed relation between vascularization and muscle mass as well as modulation of collagen deposition in non-infarcted myocardium would improve tissue perfusion and may be aims for pharmacotherapeutical intervention in post MI remodeling.

SUMMARY

The aim of this thesis was to investigate aspects of myocardial infarction (MI)-induced remodeling relevant to the development of heart failure. Ligation of the left anterior descending (LAD) coronary artery in rats, resulting in transmural infarction of the LV free wall, was used to study the structural and functional consequences of a large myocardial infarction. The first part of this thesis (*Chapters 2-5*) evaluated whether vascular growth and remodeling affect tissue perfusion. The effects of treatment with the angiotensin I converting enzyme (ACE) inhibitor captopril during post MI remodeling were also examined. In the second part of this thesis (*Chapters 6-8*) interstitial and perivascular collagen accumulation in non-infarcted tissue were studied, as well as modulation of this collagen build-up by low-dose aspirin treatment and its consequences for *in vitro* and *in vivo* heart function.

Chapters 2-5: Vascular growth and remodeling

In *chapter 2*, vascular remodeling after MI in relation to impaired perfusion of viable myocardium was investigated. Remodeling of resistance arteries in non-infarcted myocardium was confined to a slight and transient increase of perivascular collagen. Reduced maximal tissue perfusion was unrelated to the amount of perivascular collagen. Instead, greater increase in tissue mass than vascular capacity was the main cause of reduced tissue perfusion.

In *chapter 3*, the distribution of coronary flow and regional perfusion of MI hearts was measured. Peak tissue perfusion (after nitroprusside) of non-infarcted myocardium was reduced in the most hypertrophied area, the viable part of the LV free wall. Scar tissue was less perfused than contractile myocardium, but still received a substantial portion of total coronary flow. Resistance arteries had a much greater wall/lumen ratio in scar tissue than in non-infarcted myocardium. This aberrant vascular structure was associated with decreased vasodilation (nitroprusside) and increased vasoconstriction (arginine-vasopressin).

In *chapter 4*, consequences of insufficient vascular growth (reflected in a decreased maximal tissue perfusion) for the sensitivity of MI hearts to an additional ischemic period were determined. Despite reduced peak perfusion, the myocardium of MI hearts was less sensitive (release of ATP catabolites) to low-flow ischemia. Lower ATP consumption may explain the increased tolerance of MI hearts to additional ischemia. Delayed treatment of MI

rats with captopril (3-8 weeks) regressed hypertrophy but did not affect vascular capacity, resulting in improved peak tissue perfusion. Reduced release of ATP catabolites by MI hearts during an additional period of ischemia was not affected by long-term captopril treatment, but lactate release was further reduced, suggesting better preservation of aerobic metabolism.

In *chapter 5*, early captopril treatment of MI rats (1 day-3 weeks) resulted in reduced tissue weight of all parts of MI hearts, both of viable regions (regardless the degree of hypertrophy) and scar tissue. Regional vascular capacity was not affected by treatment. Prevention of hypertrophy but not vascular growth resulted in improved tissue perfusion. The normalized ratio of vascularization and tissue mass resulted in a better preservation of aerobic metabolism during additional ischemia, as reflected by attenuated lactate release.

Chapters 6-8: Remodeling of the collagen network

In *chapter 6*, interstitial as well as perivascular collagen was found to be increased in the viable part of MI hearts. Aspirin treatment was applied in a dose that blocked platelet but not endothelial cell cyclooxygenase. Treatment with low-dose aspirin affected collagen build-up: at histological examination collagen fibers appeared to be thinner and collagen volume fraction was reduced. Collagen content of scar tissue was not significantly affected by aspirin, and infarct thinning nor LV dilation were aggravated by aspirin.

In *chapter 7*, effects of pharmacological modulation of the collagen network during MI- as well as pressure overload-induced remodeling on LV diastolic properties were studied. Treatment of MI rats with methylprednisolone reduced interstitial and perivascular collagen content and aggravated LV dilation. In aspirin-treated MI rats, LV dilation was not aggravated nor was *in vitro* diastolic function influenced. In hearts subjected to experimental pressure overload, increased myocardial stiffness was illustrated by steeper diastolic volume-pressure curves, despite normal LV collagen content. Aspirin treatment of banded rats did not change LV collagen content but normalized the steepness of volume-pressure curves. Therefore, changed collagen quality rather than quantity may determine LV diastolic mechanical properties if LV collagen content is not dramatically increased.

In *chapter 8*, the effects of aspirin treatment of MI rats on *in vivo* cardiac function was investigated in conscious rats. Depressed cardiac function in MI rats (compared to sham

operation) was indicated by a lower baseline and volume loading-stimulated cardiac output. Baseline cardiac output in aspirin-treated MI rats was reached at a lower heart rate, in the absence of reduced activity of the sympathetic nervous system (plasma catecholamine levels), but was rather attributable to a lower intrinsic heart rate (isolated hearts). The exact mechanism by which altered cardiac structure and intrinsic heart rate are related still has to be elucidated.

In *chapter 9*, concluding remarks and suggestions for future research are made. Hypertrophy of non-infarcted tissue after MI is associated with impaired tissue perfusion, which may be explained by decreased vascular density. Reduced tissue perfusion of viable remodeled myocardium might play a role in the intracellular changes within the cardiomyocyte, which appear to be adaptations to hampered oxygenation but can eventually lead to cellular dysfunction and additional cell loss. Besides inhibition of reactive hypertrophy after MI, decrased collagen build up in spared myocardium may beneficially affect clinical outcome after MI. Chronic treatment of MI rats with low-dose aspirin resulted in modulation of the collagen network, which was associated with a reduced intrinsic heart rate and a prolonged diastolic time. The latter may improve tissue perfusion of non-infarcted myocardium.

SAMENVATTING

Het doel van dit proefschrift was om aspecten van remodeling van het hart na een myocardinfarct te onderzoeken die van belang zijn in de ontwikkeling van hartfalen. De anterior descendens tak van de linker coronairarterie werd afgebonden in ratten, met als gevolg een transmuraal infarct van de vrije wand van de linker kamer, als experimenteel model voor de structurele en functionele gevolgen van een groot myocardinfarct. In het eerste deel van dit proefschrift (*hoofdstuk 2-5*) werd onderzocht of onvoldoende vaatgroei of veranderde vaatstructuur de weefselperfusie negatief beïnvloedt. Tevens werden de effecten van de angiotensine I converting enzyme (ACE)-remmer captopril onderzocht. In het tweede deel van dit proefschrift (*hoofdstuk 6-8*) werden de toename van interstitieel en perivasculair collageen beschreven alsmede de invloed van behandeling met een lage dosering aspirine hierop en de gevolgen hiervan voor *in vitro* en *in vivo* hartfunctie.

Hoofdstuk 2-5: Vaatgroei en vaatremodeling

In *hoofdstuk 2* werd onderzocht of remodeling van bloedvaten betrokken is bij verminderde perfusie van overlevend hartspierweefsel na een myocardinfarct. 'Remodeling' van weerstandsarteriën in niet-geïnfarceerd hartspierweefsel bleek beperkt te zijn tot een lichte en voorbijgaande toename van perivasculair collageen. Afgenomen maximale weefselperfusie werd niet veroorzaakt door de toename van het perivasculaire collageen. Het was eerder de grotere toename van weefselmassa in vergelijking met vaatcapaciteit die de afgenomen maximale doorbloeding van geïnfarceerde harten veroorzaakte.

In *hoofdstuk 3* werd de verdeling van de coronairflow en de regionale perfusie in geïnfarceerde harten gemeten. Maximale perfusie (na nitroprusside) van niet-geïnfarceerd hartspierweefsel was afgenomen in het gebied waar de meeste spiergroei had plaatsgevonden, namelijk het niet-geïnfarceerde deel van de linker kamer vrije wand. Perfusie van het littekenweefsel was minder dan van werkend hartspierweefsel, maar het litteken kreeg wel een substantieel deel van de totale coronaire flow. Weerstandsvaten in littekenweefsel bleken een veel grotere wand/lumen verhouding te hebben dan vaten van vergelijkbare grootte in niet-geïnfarceerd hartspierweefsel. Deze afwijkende vaatstructuur ging samen met verminderde vaatverwijding (nitroprusside) en versterkte vaatvernauwing (arginine-vasopressine).

In *hoofdstuk 4* werden de gevolgen van onvoldoende vaatgroei (weerspiegeld in een verlaagde maximale weefselperfusie) voor gevoeligheid van niet-geïnfarceerd hartspierweefsel voor een additionele ischemische periode bepaald. Ondanks de gedaalde maximale weefselperfusie was het niet-geïnfarceerde weefsel minder gevoelig voor 'low-flow' ischemie (minder verlies van ATP afbraakprodukten door het ischemische myocard). Lager ATP verbruik (door lagere ATPase activiteit) zou de verhoogde tolerantie van geïnfarceerde harten kunnen verklaren. Laat-gestarte behandeling van infarctratten met captopril (3-8 weken) liet het gegroeide niet-aangedane deel van de hartspier weer teruggaan in massa maar liet de vaatcapaciteit onaangetast, wat resulteerde in een verbeterde maximale weefselperfusie. Het lagere verlies van ATP afbraakprodukten tijdens ischemie van het infarcthart werd niet beïnvloed, maar de vrijzetting van lactaat werd verder verlaagd door chronische behandeling met captopril, wat een beter behoud van de aerobe stofwisseling suggereert.

In *hoofdstuk 5* wordt beschreven hoe vroege behandeling van infarctratten met captopril (1 dag-3 weken) resulteerde in een verlaging van het gewicht van alle delen van het hart, zowel de niet-geïnfarceerde delen (ongeacht de mate van compensatoire spiergroei) als het infarctgebied zelf. De vaatcapaciteit in de verschillende gebieden werd niet beïnvloed door de behandeling. De preventie van reactieve groei van de hartspier bij onveranderde vaatgroei leidde tot een beter behoud van aeroob metabolisme tijdens een additionele ischemische periode, wat weerspiegeld werd in een beteugelde lactaatvrijzetting.

Hoofdstuk 6-8: Remodeling van het collageennetwerk

In *hoofdstuk 6* wordt beschreven dat de hoeveelheid interstitieel en perivasculair collageen rond weerstandsvaten in niet-geïnfarceerd hartspierweefsel verhoogd zijn in geïnfarceerde harten. Aspirinebehandeling werd gegeven in een dosis die cyclo-oxygenase van bloedplaatjes maar niet van endotheelcellen remt. Behandeling met een dergelijke dosis beïnvloedde de collageenopbouw: bij histologisch onderzoek bleken de collageenvezels dunner te zijn en het collageengehalte lager. Het collageengehalte van het littekenweefsel werd niet significant beïnvloed door aspirine, en het dunner worden van het litteken noch het verwijden van de linker kamer werden verergerd door aspirine.

In *hoofdstuk 7* worden de effecten van modulatie van het collageennetwerk door

medicamenten tijdens de structurele reactie van het hart op een hartinfarct of een drukoverbelasting op de diastole eigenschappen van de linker kamer beschreven. Behandeling van infarctratten met methylprednisolone verlaagde de hoeveelheid interstitieel en perivasculair collageen en verergerde het wijder worden van de linker kamer. In ratten die behandeld waren met aspirine, was het wijder worden van de linker kamer niet verergerd, noch was de diastole functie *in vitro* veranderd. In harten na drukoverbelasting was de stijfheid van het hartspierweefsel verhoogd wat leidde tot stijlere diastole volume-druk curves, ondanks een normaal collageengehalte van de linker kamer. Aspirinebehandeling van ratten met een drukoverbelasting van het hart leidde niet tot een veranderd collageengehalte, maar normaliseerde de stijlheid van volume-druk curves. Het lijkt er daarom op dat de eigenschappen van het collageen, en niet de hoeveelheid, de diastolische mechanische eigenschappen van de linker kamer bepalen in het geval dat de collageenhoeveelheid in de linker kamer niet drastisch is veranderd.

In *hoofdstuk 8* worden de effecten van het behandelen van infarctratten met aspirine op de hartfunctie *in vivo* beschreven in wakkere ratten. Verlaagde hartfunctie bij infarctratten bleek uit een verlaagd basaal en gestimuleerd (intraveneuze volumebelasting) hartminuutvolume. Het basale hartminuutvolume in infarctratten na aspirinebehandeling werd bereikt met een lagere hartfrequentie, hetgeen niet te verklaren was door een verlaagde activiteit van het sympathische zenuwstelsel (concentratie van catecholamines in plasma), maar werd veroorzaakt door een lagere intrinsieke hartfrequentie (gemeten in geïsoleerde harten). De exacte relatie tussen de veranderde structuur van het hart en de intrinsieke hartfrequentie moet nog worden opgehelderd.

In *hoofdstuk 9* worden afsluitende opmerkingen gemaakt en suggesties voor toekomstig onderzoek gedaan. Hypertrofie van het niet-geïnfarceerde hartspierweefsel na een infarct gaat gepaard met een gedaalde weefselperfusie, wat waarschijnlijk te verklaren is door een gedaalde vaatdichtheid. De gedaalde perfusie van niet-geïnfarceerd weefsel speelt mogelijk een rol in de intracellulaire aanpassingen aan verminderde oxygenatie. Deze veranderingen in de hartspiercellen kunnen uiteindelijk leiden tot dysfunctie van de cel of celdood. Naast normalisatie van de verhouding tussen vascularisatie en hartspiermassa kan remming van collageenafzetting in het niet-geïnfarceerde hartspierweefsel na het infarct de prognose gunstig

beïnvloeden. Langdurige behandeling met een lage dosis aspirine moduleerde het collageennetwerk, wat gepaard ging met een lagere intrinsieke hartfrequentie en een verlengde diastole tijd. Het laatste zou de perfusie van het niet-geïnfarceerde hartspierweefsel kunnen verbeteren.

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List of Publications

Bergmeijer JH, Nijman R, Kalkman EAJ. Stenting of the ureterovesical anastomosis in pediatric renal transplantation. Transplant international (3), 146-148, 1990

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Kalkman EAJ, van Haren P, Saxena PR, Schoemaker RG. Early captopril prevents myocardial infarction induced hypertrophy but not angiogenesis; consequences for tissue perfusion and metabolism. submitted for publication

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