ADRENALINE AND HYPERTENSION

(ADRENALINE EN HOGE BLOEDDRUK)

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3 AH van den Meiracker, AJ Man in't Veld, F Boomsma, PJ Blankestijn, MADH Schalekamp. Adrenaline-induced amplification of sympathetic activity during rest and stress: inhibition by non-selective and beta$_1$-selective beta-adrenoceptor blockers.
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Cardiovascular, endocrine and psychological function during six hours of continuous infusion of epinephrine or norepinephrine in healthy volunteers. Submitted.

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CONTENTS

Chapter 1

GENERAL INTRODUCTION

The temporal relationships between certain forms of stress and the release of adrenaline from the adrenal medulla on the one hand and between stress and elevation of blood pressure on the other hand, are well known. Consequently adrenaline has been considered for many years as one of the factors contributing to the development of hypertension. The classical role of adrenaline is to prepare the body for the demands during fear, fight or flight reaction by activating ß-adrenoceptors in the heart and blood vessels and by inducing some "metabolic" effects such as a rise in blood glucose. However, adrenaline may also modulate noradrenaline release from sympathetic nerves and may be in that way closely related to the regulation of blood pressure. It is approximately 10 years ago that an hypothesis was formulated for a mechanism by which increases in circulating adrenaline could result in more prolonged increases in blood pressure, which in the end could progress to primary hypertension (1-3). In this chapter, after giving a general description of some aspects of the sympatho-adrenal system, catecholamines, their receptors, receptor subtypes and function, the evidence is reviewed for a mechanism by which adrenaline could modulate noradrenaline release. Special attention will be given to the possible involvement of adrenaline in the pathogenesis of some forms of experimental and human hypertension. Furthermore, some aspects of the metabolism of catecholamines and their urinary metabolites and the nature of the "metabolic" effects are discussed. Since an "inability" to excrete sufficient amounts of sodium seems to be an essential feature of all forms of hypertension, the possible role of catecholamines in this renal abnormality is discussed. This chapter ends with a formulation of the aims of our studies.

THE SYMPATHO-ADRENAL SYSTEM

The sympathetic nervous system and the adrenal medulla are of major importance in controlling the function of smooth, skeletal and cardiac muscle and concur in the regulation of exocrine and endocrine glands. Preganglionic sympathetic nerves connect the central nervous system with the ganglia of the sympathetic trunk, where the neurotransmitter is acetylcholine. Postganglionic sympathetic nerves connect the ganglia with the target organs, where the neurotransmitter is noradrenaline. Some of this noradrenaline spills over into the plasma. The adrenal medulla is the major source of the circulating N-methyl-noradrenaline (adrenaline), which is synthesized from noradrenaline by phenylethanolamine-N-methyltransferase (PNMT). The adrenal glands also release some noradrenaline. Noradrenaline and adrenaline together with dopamine, the precursor of noradrenaline, are the major catecholamines. Both noradrenaline and adrenaline elicit a variety of effects both in the central nervous system and peripherally, which will be discussed in detail in this thesis, whereas dopamine is mainly an important neurotransmitter in the brain.

Ahlquist was the first to suggest that there are two types of catecholamine receptors: designated by him as α- and ß-adrenoceptors (4). He distinguished these receptors by physiological means. The discovery of selective agonists and antagonists for α- and ß-adrenoceptors made it possible to confirm the distinction made by Ahlquist. A further subclassification of these receptors in α_1, α_2, $ß_1$ and $ß_2$ has been made by pharmacological means as more specific and selective agents became available (5-8).

α-Adrenoceptors

The α-adrenoceptors are responsive to noradrenaline and adrenaline, but little responsive to the nonselective ß-adrenoceptor agonist isoprenaline. The α-adrenoceptor mediated effects are antagonized by antagonists, such as phentolamine and phenoxybenzamine. Activation of α-adrenoceptors in smooth muscle is usually excitatory in nature and results, for instance, in constriction of blood vessels and contraction of the uterus.

The α-adrenoceptors have been subdivided in 2 subtypes (5). The α_1-adrenoceptors are predominantly located postsynaptically and mediate smooth muscle contraction or stimulation of glands. They are thought to be located close to the nerve terminals and to be the predominant adrenoceptors mediating vasoconstrictor responses to endogenously released noradrenaline. α_2-Adrenoceptors can be located prejunctionally, where they regulate noradrenaline release in an inhibitory fashion. Postjunctional α_2-adrenoceptors also exist and are present in several organs, such as the kidney, blood vessels, uterus, platelets and certain areas of the brain. The proportion of postjunctional α_1- and α_2-adrenoceptors varies within the vascular bed; the α_1-adrenoceptor predominates in renal vessels, whereas α_2-adrenoceptor predominate in cerebral blood vessels. Postjunctional α_2-adrenoceptors are thought to be located extrasynaptically and to be activated preferentially by circulating catecholamines (6).

α_1-Adrenoceptor agonists increase blood pressure and peripheral resistance with bradycardia through an increase in vagal tone. α_1-Adrenoceptor selective agonists include phenylephrine and methoxamine and their antagonists prazosin and doxazosin (Table 1). Selective α_2-adrenoceptor agonists include clonidine and their antagonist yohimbine. Noradrenaline and adrenaline are nonselective agonists, they both stimulate both α_1- as well as α_2-adrenoceptors.

ß-Adrenoceptors

Stimulation of ß-adrenoceptors results in inhibition of contraction of smooth muscle, such as in the vascular (leading to vasodilatation) and uterine musculature, whereas stimulation of ß-adrenoceptors mediate the excitatory effects on the heart (leading to

TABLE 1 α-ADRENOCEPTOR AGONISTS AND ANTAGONISTS

	Agent	Receptor influenced
Agonists	Noradrenaline	$\alpha_1 + \alpha_2 + \beta_1$
	Adrenaline	$\alpha_1 + \alpha_2 + \beta_1 + \beta_2$
	Phenylephrine	$\alpha_1 > \alpha_2$
	Clonidine	$\alpha_2 > \alpha_1$
	Guanfacine	$\alpha_2 > \alpha_1$
Antagonists	Phentholamine	$\alpha_1 + \alpha_2$
	Prazosin	α_1
	Doxazosin	α_1
	Labetolol	$\alpha_1 + \beta_1 + \beta_2$
	Yohimbine	α_2
	Idazoxan	α_2
	Rauwolscine	α_2

Adapted from reference 7

tachycardia and increase in force of contraction), and lipolysis and gluconeogenesis in hepatic and skeletal muscle tissue.

Lands et al (8) proposed the subdivision of β-adrenoceptors into β_1- and β_2-subtypes. This concept has been confirmed later in studies with specific agonists and antagonists. β_1-Adrenoceptors are present in cardiac, intestinal and adipose tissue, whereas β_2-adrenoceptors predominate in bronchi, vascular beds, the uterus, skeletal muscle and the liver. Noradrenaline and adrenaline both stimulate β_1-adrenoceptors, whereas the affinity of adrenaline for β_2-adrenoceptors is much higher than the affinity of noradrenaline for these receptors. Isoprenaline is generally considered to be a pure nonselective β-adrenoceptor agonist. Selective β_2-adrenoceptor agonists, such as salbutamol, relax bronchial smooth muscle. A great number of β-adrenoceptor blocking agents are now available, both non-selective ($\beta_1 + \beta_2$, such as propranolol), β, selective (such as atenolol and metoprolol) and β_2 selective (such as ICI 118551) (Table 2).

Postjunctional versus prejunctional adrenoceptors

Postjunctional adrenoceptors are located on the target organ. Their stimulation leads to an effect in the target organ. For instance stimulation of vascular postjunctional α_1-adrenoceptors induces vasoconstriction, it increases vascular resistance and therefore

TABLE 2 ß-ADRENOCEPTOR AGONISTS AND ANTAGONISTS

	Agent	Receptor influenced
Agonists	Noradrenaline	$ß_1 + \alpha_1 + \alpha_2$
	Adrenaline	$ß_1 + ß_2 + \alpha_1 + \alpha_2$
	Dobutamine	$ß_1 + \alpha_1$
	Isoprenaline	$ß_1 + ß_2$
	Fenoterol	$ß_2 » ß_1$
	Salbutamol	$ß_2 » ß_1$
	Terbutaline	$ß_2 » ß_1$
Antagonists	Propranolol	$ß_1 + ß_2$
	Pindolol	$ß_1 + ß_2$
	Timolol	$ß_1 + ß_2$
	Sotalol	$ß_1 + ß_2$
	Atenolol	$ß_1 » ß_2$
	Metoprolol	$ß_1 » ß_2$
	Acebutolol	$ß_1 » ß_2$
	ICI 118,551	$ß_2 » ß_1$
	Labetolol	$ß_1 + ß_2 + \alpha_1$

Adapted from reference 7

blood pressure rises.

Prejunctional adrenoceptors modulate the release of neurotransmitters in response to nerve stimulation. Several types of these prejunctional adrenoceptors may be present on the nerve ending where they reinforce or inhibit noradrenaline release. Prejunctional α_2-adrenoceptors exert an inhibitory action on noradrenaline release, when a critical concentration within the synaptic cleft is reached. The α-agonist clonidine, besides its effects on the central nervous system, inhibits noradrenaline release through such a mechanism. Antagonists like phenoxybenzamine and phentholamine potentiate noradrenaline release by inhibition of the prejunctional α_2-adrenoceptors. However, for blockade of this inhibition much higher concentrations are required than for the effects on postjunctional α-adrenoceptors (9).

Prejunctional adrenoceptors capable of facilitating noradrenaline release have been clearly demonstrated. These adrenoceptors are suspected to play a role in the pathogenesis of some forms of hypertension and they are discussed in more detail below.

ADRENALINE MEDIATED MODULATION OF NORADRENALINE RELEASE

Prejunctional ß-adrenoceptors

The first suggestion for the presence of prejunctional ß-adrenoceptors came from the observation of Adler-Graschinsky and Langer (10). They showed that isoprenaline enhanced the nerve stimulation induced release of noradrenaline from guinea-pig isolated atria and that this effect was blocked by propranolol. Almost at the same time Stjärne and Brundin (11,12) also demonstrated the existence of an enhancing effect of ß-adrenoceptor agonists on stimulation induced noradrenaline release. Since then many investigators reported similar observations with several ß-adrenoceptor agonists in a range of isolated cardiac and vascular tissues and other sympathetically innervated tissues from a number of different species. On the basis of a range of studies with selective and non-selective agonists and antagonist the prejunctional ß-adrenoceptors appeared to be of the $ß_2$-subtype (review 13-16).

Noradrenaline is a weak $ß_2$-adrenoceptor agonist whereas adrenaline is a potent agonist for this receptor. Indeed adrenaline activates $ß_2$-adrenoceptor mediated noradrenaline release in *in vitro* studies in concentrations of 100-1800 pg/ml (15). Higher concentrations of adrenaline can inhibit noradrenaline release, through activating prejunctional α_2-adrenoceptors (15). Under physiological conditions noradrenaline has no action on $ß_2$-adrenoceptors.

Adrenaline as co-transmitter.

Adrenaline can be actively taken up into sympathetic nerve terminals, retained in the transmitter stores and subsequently released by nerve stimulation just like noradrenaline. The small amount of adrenaline normally present in the sympathetically innervated tissues is probably derived largely from that released from the adrenals, since adrenal medullectomy results in a decrease in adrenaline levels in the tissues. The amount of adrenaline taken up by the neurons is increased when plasma adrenaline is elevated (17). Once accumulated in the nerves, adrenaline can be co-released along with noradrenaline upon nerve stimulation. The adrenaline released as co-transmitter can mediate a positive feedback autofacilitating loop resulting in increased noradrenaline release. This effect is observed in *in vitro* experiments in rat atria (18). Also *in vivo* in the rabbit, adrenaline given intravenously (17) or endogenously released by splanchnic nerve stimulation (19) resulted in an increase of noradrenaline release into the plasma, even well after the adrenaline has been cleared from the plasma but when levels in sympathetically innervated tissues were still raised (17,19). Pretreatment with the neuronal uptake blocker desipramine, abolished the effect of adrenaline injection and adrenaline released by splanchnic nerve stimulation in producing a subsequent increase in the rate of noradrenaline release. These observations are entirely consistent with the view that adrenaline

produced its effects as a cotransmitter and that its incorporation into the transmitterstores was prevented by neuronal uptake blockade by desipramine. Intravenously administered ^{3}H-adrenaline in rats has a short half life (approximately 1 minute), whereas once adrenaline is accumulated in the tissues, the half live of its disappearance is several hours (18). So in this way occasional bursts of adrenaline secretion by the adrenal medulla can have a more prolonged effect on sympathetic neurotransmission.

Also in humans there is evidence for the facilitating effect of adrenaline on noradrenaline release from sympathetic nerves. In isolated tissues including omental blood vessels (11,12) and digital arteries (20) adrenaline and other ß-adrenoceptor agonists have been found to enhance noradrenaline release. Our group observed dose-dependent increments in plasma noradrenaline during graded infusions of isoprenaline in normotensives and borderline hypertensive subjects. The rise was completely abolished by concomitant infusion of propranolol. During atenolol the dose response curve for plasma noradrenaline was shifted to slightly higher infusion rates of isoprenaline (21). These results are completely compatible with the above described mechanism of prejunctional $ß_2$-adrenoceptor mediated enhancement of noradrenaline release. Similar results were later reported by several other investigators (22,23).

ADRENALINE AND HYPERTENSION

Adrenaline and experimental hypertension.

The effect of chronic administration of adrenaline in producing increases in blood pressure that outlasted the period of adrenaline administration has been demonstrated in a series of experiments. The way of administration of adrenaline was a subcutaneous implant (24), intermittent intravenous infusion (25) or intraperitonally from osmotic minipumps (26). The rise in blood pressure was prevented by concomitant administration of metoprolol (24,26), whereas in rats implanted with pumps delivering noradrenaline, blood pressure did not differ from control (26). Borkovski and Quinn (27) also used subcutaneous implants and produced a sustained increase in blood pressure in SHR rats, which had their adrenal medullas removed. This effect was prevented by the selective $ß_2$-adrenoceptor blocker ICI-118,551. Several other groups have also provided data indicating that prolonged infusion of adrenaline can induce hypertension in the rat (28,29). There is a number of animal studies on the association between stress and hypertension and stress and adrenaline. Exposure to continuous stimulation with flashing lights, oscillation of the cage and noise for several weeks, but also sound deprivation, have been reported to produce hypertension in rats (30,31). Treatment with propranolol prevented the development of isolation-induced hypertension (32,33). Stress-induced increases in

adrenaline secretion have been reported. Forced immobilization of rats by taping the legs to a board, results in a marked increase in urinary excretion of catecholamines and a depletion of the adrenal contents (34). Plasma adrenaline is also markedly increased by this procedure (35). Rats subjected to the chronic stress of individual housing and the binding of their rear legs has produced a rise in blood pressure when compared to control rats. In rats, which have been adrenal medullectomized prior to the stressing procedure, given desipramine or propranolol, the stressing procedure did not elevate blood pressure (36). The amount of adrenaline accumulated in the heart was also measured and was found to be increased by the stress. Desipramine treated stressed rats did not have elevated heart adrenaline levels. In adrenal medullectomized rats, the heart adrenaline levels were reduced in both stress and control rats by more than 90% (36). Taken together, these findings suggest that adrenaline has a role in initiating stress-induced increases in blood pressure in the rat. The results of blockade experiments with desipramine and propranolol are consistent with the hypothesis that the neuronal accumulation of adrenaline and its subsequent activation of prejunctional ß-adrenoceptors on sympathetic nerve endings are also involved.

Adrenaline in human hypertension

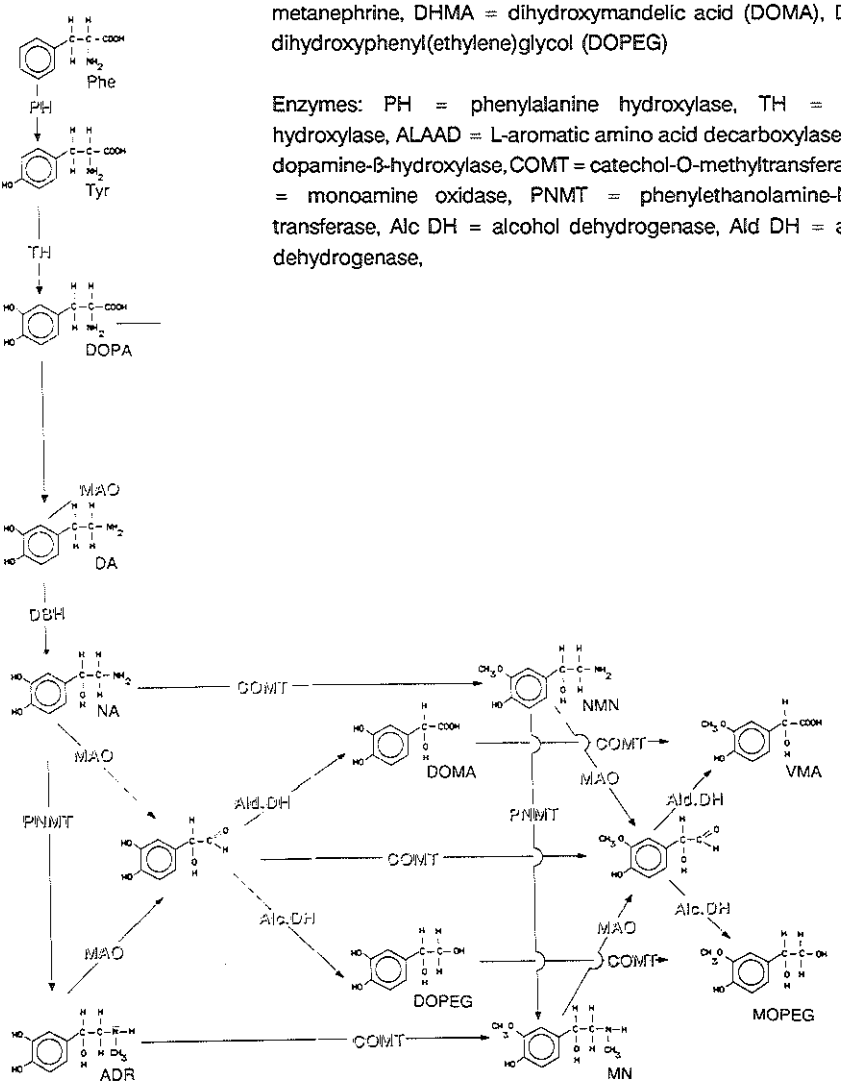
Adrenaline has also been implicated in the pathogenesis of human hypertension. Several investigators have reported elevated plasma levels of adrenaline in patients with borderline hypertension (37-40), both at rest and during activities that raise blood pressure. Many patients with borderline hypertension have the characteristics of increased ß-adrenoceptor activity, such as an increase in heart rate, cardiac output and forearm blood flow. These changes are antagonized by ß-adrenoceptor blockade (41,42).

The concept that adrenaline, by stimulating prejunctional ß-adrenoceptors, facilitates the release of endogenous noradrenaline from sympathetic nerves leading to an increase in blood pressure, is also investigated in humans. As already mentioned, our group and others have provided evidence that ß-adrenoceptor agonists can increase plasma noradrenaline (20,21). In a later study our group demonstrated that infusion of adrenaline, which raised plasma adrenaline into the high physiological range, augmented the pressor response to reflex sympathetic nervous system stimulation by standardized cold pressor and isometric exercise test (3,43,44). This amplification was abolished by concomitant administration of propranolol. Plasma noradrenaline was higher during adrenaline infusion both before and after the tests, and this effect of adrenaline was antagonized by propranolol. So these results seem to support the view that adrenaline amplifies sympathetic pressor responses in humans by facilitation of the release of noradrenaline.

Figure 1. Pathways of synthesis and metabolic breakdown of catecholamines.

Phe = phenylalanine, Tyr = tyrosine, DOPA = dihydroxy phenylalanine, DA = dopamine, NA = noradrenaline, NMN = normetanephrine, VMA = vanillylmandelic acid, MHPG = 3-methoxy-4-hydroxyphenyl(ethylene)glycol (MOPEG), ADR = adrenaline, MN = metanephrine, DHMA = dihydroxymandelic acid (DOMA), DHPG = dihydroxyphenyl(ethylene)glycol (DOPEG)

Enzymes: PH = phenylalanine hydroxylase, TH = tyrosine hydroxylase, ALAAD = L-aromatic amino acid decarboxylase, DBH = dopamine-ß-hydroxylase, COMT = catechol-O-methyltransferase, MAO = monoamine oxidase, PNMT = phenylethanolamine-N-methyl transferase, Alc DH = alcohol dehydrogenase, Ald DH = aldehyde dehydrogenase,



Ten years ago an hypothesis has been put forward, which suggested a role for adrenaline in the pathogenesis of human hypertension (1-3). The mechanism involved was though to be as follows: increased release of adrenaline by the adrenals, for instance induced by "stress", leads to enhanced noradrenaline release either directly through stimulation of prejunctional β_2-adrenoceptors, or indirectly after adrenaline had been taken up in the sympathetic neurons and after it was subsequently co-released with noradrenaline during periods of sympathetic stimulation which further increases noradrenaline release.

At the time we started our experiments described in this thesis (1986), two studies were available on the effect of adrenaline during, but particularly also after its administration. Brown and Dollery studied 6 normotensive volunteers and infused 100 ng/kg/min of adrenaline over 2 hours (45). Indirectly measured blood pressure was higher up to 2½ hours after cessation of the infusion. However, the authors reported an absence of statistical significance for this pressor effect. Nezu et al (23) showed that 30 minutes of adrenaline infusion (1.25 - 1.5 µg/min) resulted in a significant rise in blood pressure after cessation of the infusions, which lasted at least for 90 minutes. Pretreatment with propranolol completely abolished this effect.

At that time no data were available on more long term (several hours) pressor effects of adrenaline and no studies existed examining the question whether the effect observed in the two studies just mentioned (23,45), was unique for adrenaline. This have led us to perform the studies described in this thesis.

CATECHOLAMINES AND THEIR URINARY METABOLITES

Understanding the metabolism of catecholamines has proved to be important in gaining insight in the mechanism of their function. Since it was shown that only a small part of infused catecholamines was excreted unchanged in the urine, much work has been done to clarify the pathways of synthesis and metabolic breakdown (Figure 1). It is now clear that vanillyl mandelic acid (VMA) and 4-hydroxy-3-methoxy-phenylglycol (MHPG) are quantitatively the most important metabolites of both noradrenaline and adrenaline and that only a small fraction of released adrenaline and noradrenaline *in vivo* are excreted unchanged or as metanephrine or normetanephrine (46). Measurements of plasma and urinary concentrations of adrenaline, noradrenaline and various metabolites have proved to be useful in the diagnosis and management of certain diseases such as pheochromocytoma, Parkinson's disease, neuroblastoma and several psychiatric disorders (47-51). In this study we measured the amounts of adrenaline, noradrenaline, metanephrine, normetanephrine, VMA and MHPG excreted in the urine in an attempt to find an answer to the question whether or not any of the catecholamines or their metabolites in urine could reflect the plasma levels of noradrenaline and adrenaline during the infusions.

OTHER EFFECTS OF ADRENALINE

Sodium excretion

Since Claude Bernard observed an increased urine flow following section of the splanchnic nerve in the anaesthetized animal, the influence of the sympathetic nervous system on renal sodium and water handling has been the subject of extensive research (52-55). It is now clear that the renal sympathetic nervous system and circulating catecholamines can exert profound effects on renal haemodynamics and in that way on sodium and water excretion. However, it is also apparent that adrenaline and noradrenaline also have a direct and distinct effect on renal sodium handling independent of their renal haemodynamic effects. In this matter the primary site in the nephron influenced by catecholamines appears to be the proximal tubule. In this study we measured urinary sodium excretion in order to gain insight in the effects of alterations of plasma concentrations of adrenaline and noradrenaline within the physiological range.

Metabolic effects

It was again Claude Bernard, who was the first to describe a "metabolic" effect which appeared to be mediated by the sympathetic nervous system, although he did not recognize it as such. He observed a transitory outpouring of sugar in the urine after he punctured rabbits into the forth ventricle (56). It is now appreciated that besides haemodynamic effects, adrenaline and noradrenaline exert a variety of "metabolic" effects. They play a role in the regulation of carbohydrate, lipid, ketone and protein metabolism. They contribute to the regulation of the plasma concentrations of electrolytes and hormones as well as thermogenesis. In this study we evaluated whether circulating catecholamines exert any effect on some of the above mentioned metabolic pathways, when they are infused intravenously in such doses that increments within the physiological range are reached.

AIM OF THE STUDIES

The questions we hoped to answer by the studies described in this thesis, were:

1 Does adrenaline, when infused intravenously in normotensive subjects leading to plasma levels in the high physiological range, cause a sustained and protracted rise in blood pressure, which outlasts the duration of the increments in circulating adrenaline? And if so, does this effect on blood pressure occurs at rest or during periods of activation of the sympathetic nervous system? In view of the data of Vincent et al (43), we hypothesized that the latter would be the case.

2 If the questions under number 1 are positively answered, does intravenous administered noradrenaline have the same effect? When the effect of

adrenaline is indeed mediated through prejunctional β_2-adrenoceptors, we hypothesized that this would not be the case.

3 Do the pressor responses to standardized sympathetic nervous system stimulation by cold pressor and isometric exercise testing before, during and 18 hours after cessation of infusions of adrenaline, noradrenaline or dextrose 5% differ? Again we hypothesized that infusion of adrenaline but not noradrenaline would lead to an amplification of the blood pressure responses.

4 Are the changes in plasma concentrations of adrenaline and noradrenaline during the infusions of adrenaline and noradrenaline also detectable in alterations in the amounts of catecholamines and their metabolites excreted in the urine?

5 Do the infusions of catecholamines, which lead to alterations in plasma concentrations within the physiological range, have any effect on urinary sodium excretion or on plasma levels of several hormones, potassium or glucose?

6 What is the effect of non-selective and β_1-selective β-blockade on the adrenaline mediated facilitation of noradrenaline release and the adrenaline induced enhancement of reflex sympathetic nervous system activity?

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Chapter 2

STUDY PROTOCOL, SUBJECTS AND METHODS

In this chapter a description is given on the study protocol, the subjects and methods concerning the data presented in chapters 3 to 7. A separate description on the study protocol, subjects and methods is given for chapter 8.

SUBJECTS

The studies were performed in 10 male non-smoking healthy volunteers, aged 25 years (range 21-28). All subjects were normotensive, they were taken no medication and showed no evidence of cardiovascular disease, diabetes mellitus or any other serious disease based on their medical history, physical examination and electrocardiogram at the time of the study. Blood urea nitrogen and electrolytes were within the normal range of our laboratory. Urinalysis revealed no abnormalities and mean serum creatinine was 93 μmol/L (range 83-103). They used an unrestricted diet on the days prior to the study and during the study. The purpose and procedures of the study were explained to the subjects. All gave their written informed consent and the study was approved by the Hospital Ethical Review Committee.

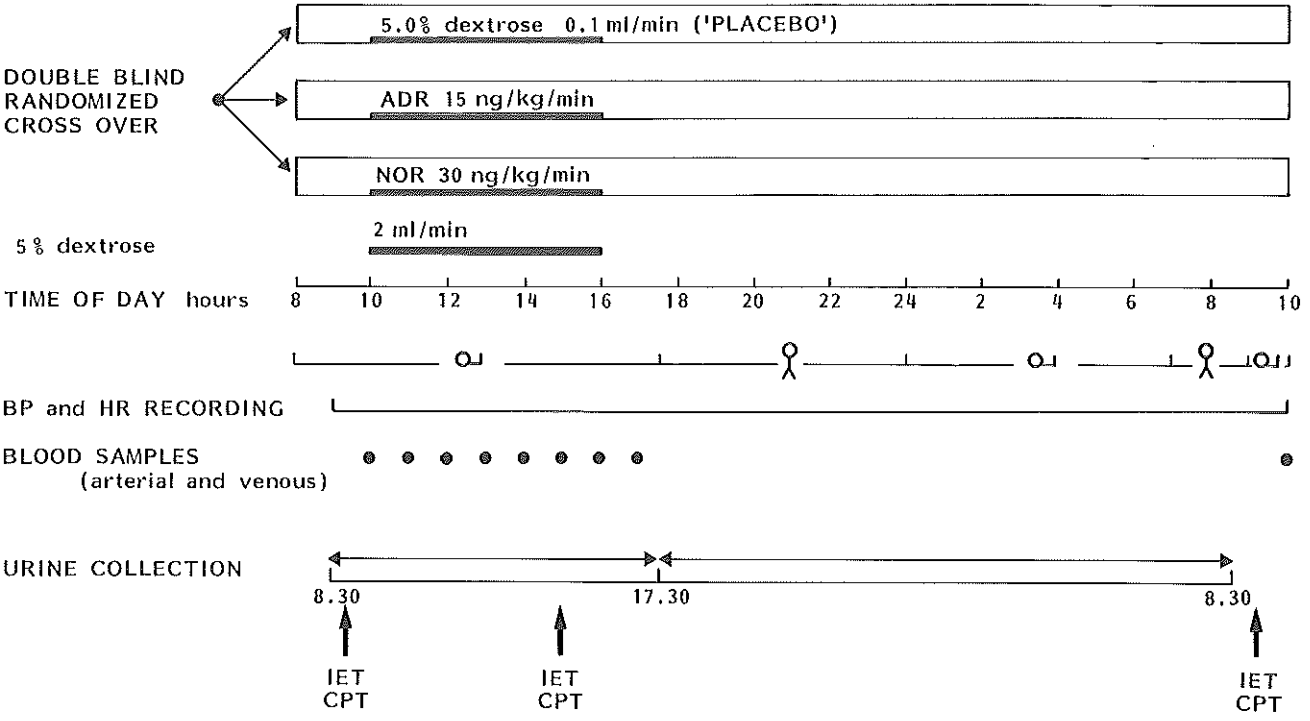
STUDY PROTOCOL

The study was carried out according to a double-blind crossover design. Adrenaline (15 ng/kg/min), noradrenaline (30 ng/kg/min) or 5% dextrose (5.4 ml/hr) were infused for 6 hours in random order 2 weeks apart (Figure).

Subjects arrived in the laboratory at 8.00 h after an overnight fast. After they had used a standardized breakfast containing approximately 25 mmol of sodium, they emptied their bladder. The brachial artery of the nondominant arm was cannulated with a catheter (Seldicath Teflon, 1.0 mm thick, 11 cm long, Laboratoire Pharmaceutique Saint-Leu-La-Foret, France) under local anaesthesia with 2% lidocaine. Venous catheters (Venflon 1.2 mm thick, 45 mm long, Viggo Products, Helsingborg, Sweden) were introduced in deep forearm veins of both forearms for infusion and blood sampling. After obtaining baseline values of blood pressure and heart rate from 9.00 to 10.00 h, the infusions were given between 10.00 h and 16.00 h. During the infusions the subjects rested supine and were not allowed to eat or drink. A 5% dextrose solution was infused at a rate of 2 ml/min. Arterial and venous blood samples were taken simultaneously at regular time intervals. All passed urine was collected. The subjects left the hospital at 17.30 h after they had emptied their bladder. They were requested to perform similar activities during the evenings of the three study days and to go to bed at approximately the same time. They returned the next morning at 8.30 h. Before ending the blood pressure registration, they rested supine for 60 minutes. Again all passed urine was

STUDY PROTOCOL

3 studies of 25 hours 2 weeks apart



ADR = adrenaline
NOR = noradrenaline

IET = isometric exercise
CPT = cold pressure test

= supine
= ambulatory

BP = blood pressure
HR = heart rate

collected.

The effect of the infusions on the blood pressure and heart rate response to isometric exercise and cold exposure (cold pressor test) was studied three times during each study day: approximately 30 minutes before the start of the infusion, after 4½ hours of infusion, while the infusion was still running, and the next morning, approximately 18 hours after cessation of the infusion.

Blood was sampled at hourly intervals for the following assays: adrenaline and noradrenaline (arterial and venous), renin, aldosterone, growth hormone, prolactin, insulin and cortisol (venous), before, after 1 hour and after 6 hours of infusion for glucose and potassium (venous). Venous and arterial catecholamines were also measured at 5 minutes interval during the first 20 minutes after cessation of the infusions. Arterial catecholamines were measured the next morning after 60 minutes of bedrest before the subjects performed the cold pressor and isometric exercise test.

All passed urine was collected from 8.30 to 17.30 h (infusion period, urine 1) and from 17.30 to 8.30 h the next morning (postinfusion period, urine 2). This second urine always included the morning voiding.

HAEMODYNAMIC MEASUREMENTS

24 hr blood pressure and heart rate registration

Arterial pressure was continuously monitored in the brachial artery of the non-dominant arm by means of the Oxford technique (1). The intra-arterial cannula was connected to a perfusion-transducer device (Northwick Park Hospital, London, UK) suspended in front of the chest. The transducer signal was recorded on magnetic tape by means of an analogue taperecorder (Medilog Recorder II, Oxford Medical Instruments, Oxford, UK). The analogue signal was digitized during replay of the tape at 60 times real time with a sampling frequency of 33⅓ samples/s. Traces were analyzed beat by beat and were scrutinized for beat loss, clipping of the amplifier, damping and of moving artefacts by means of the computer system. These events amounted to <2% of all data and were excluded from analysis (2,3,4). Hourly means of systolic and diastolic arterial pressure and heart rate were calculated.

Cold pressor and isometric exercise test

The isometric exercise test was carried out by handgrip with one hand for 60 seconds at 50% of maximal voluntary contraction, which was estimated by the subjects just after arrival in the laboratory. The cold pressor test was done by immersion of one hand up to the wrist into ice-water for 60 seconds. During all tests the subjects remained in the supine position. The tests were always done in

the same sequence, i.e. first the isometric exercise test and 10 minutes later the cold pressor test. The start and the end of the tests were marked on the tape with an event marker. The means of blood pressure and heart rate of the five minutes immediately preceding the tests were taken as baseline values. The changes in blood pressure and heart rate from these baseline values were computed as 10 seconds averages. The changes in blood pressure and heart rate are presented as 10-seconds means during the infusions and 18 hours after the cessation of the infusions as compared to the data for the tests done before the start of the infusions. The results of the dextrose study were subtracted from the results of the adrenaline and noradrenaline study. So the zero mmHg respectively zero beats per minute are in fact the results of the dextrose study. Also the mean differences with standard error of the means (SEM) in response of systolic and diastolic arterial pressure and heart rate to isometric exercise and cold pressor test during and after adrenaline and noradrenaline as compared to dextrose are presented.

ANALYTIC PROCEDURES OF PLASMA

For measurements of plasma catecholamines 10 ml venous and arterial blood was collected simultaneously in chilled tubes containing 19 mg EGTA and 12 mg gluthathione. After centrifugation at 0°C, samples were stored at -70°C until assay. Plasma noradrenaline and adrenaline were measured by high-performance liquid chromatography with electrochemical detection. Results are expressed in pg/ml (5).

For determination of plasma renin concentration and plasma aldosterone venous blood was collected in tubes containing EDTA in a final concentration of 2 mg/ml of blood. Samples were centrifuged immediately at 0°C and stored at -20°C until assay. Renin was measured by radioimmunoassay of angiotensin I formed at neutral pH in the presence of saturating concentrations of sheep renin substrate. Results are expressed in milli-units of a standard of purified renin from human kidney (Medical Research Council standard 68/356, National Institute for Biological Standards and Control, London, UK) per litre (6). Plasma aldosterone was measured by commercially available radioimmunoassay (AldoKit, Labservice Benelux, Apeldoorn, the Netherlands).

Cortisol and insulin were measured by commercially available radioimmunoassay kits (respectively "Coat-a-count", Diagnostic Products Corporation, Los Angeles, USA and Incstar, Stillwater, Minnesota, USA). Growth hormone and prolactin were measured by an immunoradiometric assay (Euro-Diagnostics BV, Apeldoorn, the Netherlands). Glucose was measured in whole blood using the hexosekinase reaction.

ANALYTIC PROCEDURES OF URINE

Urine was collected in polyethylene containers over 0.5 g of Na_2EDTA and 0.5 g of $Na_2S_2O_5$ and kept at 4°C in the dark. After completion of collection, aliquots were stored in a freezer at -70°C until assay. The catecholamines and metabolites have been shown to be stable for at least one year under these conditions (7).

Adrenaline and noradrenaline were assayed by high-performance liquid chromatography with fluorescence detection and precolumn derivatization (8). 3-Methoxy-4-hydroxyphenylglycol (MHPG), vanillylmandelic acid (VMA) and metanephrines were determined by high-performance liquid chromatography with electrochemical detection (9,10).

Sodium was measured by flame photography and creatinine was measured with an EPOS 5060 analyzer (Eppendorf method: alkaline picrate, Merck creatinine kit).

STATISTICAL ANALYSIS

The data are presented as means with SEM unless indicated otherwise. Because values of renin were not distributed normally, mean values were calculated after logarithmic transformation.

Analysis of haemodynamic values

Haemodynamic baseline values, and the post-infusion values were compared using a Student paired t-test. Haemodynamic baseline values were also compared with mean values during infusions using Student paired t-test. In other comparisons of haemodynamic values we used a three-way analysis of variance (ANOVA, SPSS/PC program, Chicago Ill, USA), unless indicated otherwise, for testing the treatment factor (noradrenaline, adrenaline, dextrose) on each of the parameters. No (subject x time) interaction was assumed. A (treatment x time) interaction was taken into account by testing the treatment effect for separate periods. The following periods were defined: baseline (9-10 h), infusion (10-16 h), daytime ambulation (16-24 h), bedtime (0-7 h), daytime ambulation (7-9 h), daytime bedrest (9-10 h).

Analysis of biochemical values

Baseline plasma biochemical values (adrenaline, noradrenaline, growth hormone, prolactin, cortisol, insulin, potassium, glucose) and the post-infusion values were compared using a Student paired t-test. In other comparisons we used a three-way analysis of variance (ANOVA, SPSS/PC program, Chicago Ill, USA), unless indicated otherwise, for testing the treatment factor (noradrenaline, adrenaline, dextrose) on each of the parameters. No (subject x time) interaction was assumed. A (treatment x time) interaction was taken into account by testing the treatment

effect for separate periods. In case baseline values of the three study occasions were different, results of measurements during the infusions were also compared with corresponding baseline values.

Analysis of haemodynamic responses to stress tests

For analysis of the haemodynamic responses during cold pressor test and isometric exercise test the six 10 seconds means of the change in blood pressure and heart rate as compared to the preceding baseline values of the adrenaline, noradrenaline and dextrose study were compared using a repeated measurements analysis of variance (BMTP Statistical software Inc, Los Angeles, CA 90025 USA). When the null-hypothesis (that no difference existed) was rejected, the means of the six 10-seconds values of the change in blood pressure and heart rate were compared using a Student t-test for paired observations.

Analysis of results of urinary excretion of electrolytes and creatinine

The amounts of sodium, potassium, creatinine are presented. The results were also corrected for creatinine excretion, because of the possible failure of complete collection. Differences in urinary excretion of these compounds were analyzed with repeated measurements analysis of variance (SPSS/PC program, Chicago Ill, USA). When the null-hypothesis (that no difference existed) was rejected, the means of the results of the adrenaline and the noradrenaline study were compared with the results of the dextrose study using a Student-t-test.

Analysis of results of urinary excretion of catecholamines and their metabolites

The amounts of adrenaline, noradrenaline, metanephrine, normetanephrine, VMA and MHPG are presented. Differences in urinary excretion of these compounds were analyzed with repeated measurements analysis of variance (BMTP Statistical software Inc, Los Angeles, CA 90025 USA).

Interrelationships between the plasma, the urine and the haemodynamic data.

Interrelationships between results of plasma catecholamines, urinary catecholamines and metabolites and haemodynamic measurements were determined by computing Pearsons correlation coefficients (SPSS/PC program, Chicago Ill, USA).

In most analyses a p value of less than 5% and in some analyses, where considered appropriate, of less than 1%, were considered to indicate statistical significance.

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Chapter 3

SUPPORT FOR THE ADRENALINE-HYPERTENSION HYPOTHESIS: 18 HOUR PRESSOR EFFECT AFTER 6 HOURS OF ADRENALINE INFUSION.

Based on: PJ Blankestijn, AJ Man in't Veld, JHM Tulen, AH van den Meiracker, F Boomsma, P Moleman, HJ Ritsema van Eck, FHM Derkx, P Mulder, SWJ Lamberts, MADH Schalekamp. Support for the adrenaline-hypertension hypothesis: 18 hour pressor effect after 6 hours adrenaline infusion. Lancet 1988; ii: 1386-89.

INTRODUCTION

Adrenaline has been implicated in the pathogenesis of 'stress-linked' hypertension (1-5). After its release into the circulation from the adrenal medulla adrenaline can activate prejunctional ß-adrenoceptors at sympathetic nerve terminals to enhance noradrenaline release and thereby increase α-adrenoceptor mediated vasoconstriction (6-8). In addition to this humoral effect adrenaline may also act as a co-transmitter of noradrenaline. Adrenaline is taken up from plasma by the sympathetic nerves and the amount of adrenaline that is stored together with noradrenaline by the neurons is increased when plasma adrenaline is elevated (9). At a time when plasma adrenaline concentrations are no longer elevated co-released adrenaline can still cause activation of prejunctional ß-adrenoceptors.

In the rat slow release implants containing adrenaline elevate blood pressure for several weeks, an effect which is not seen after pretreatment with ß-adrenoceptor antagonists (10-14). Infusion of adrenaline amplifies the blood pressure response to sympathetic stimulation in humans (15-17), and this amplification can also be prevented by prior ß-adrenoceptor blockade. However, direct evidence in man that adrenaline can cause a sustained and protracted elevation of blood pressure is lacking. Therefore we have decided to study the effects of a 6 h catecholamine infusion (adrenaline: 15 ng/kg/min or noradrenaline: 30 ng/kg/min) as compared to a 5% dextrose solution (5.4 ml/min) in healthy volunteers. After the infusions had been stopped, the subjects were followed for 18 h by means of ambulatory intra-arterial blood pressure monitoring.

SUBJECTS AND METHODS

See chapter 2.

RESULTS

Haemodynamic effects

Mean baseline values of arterial pressure and heart rate were not different on the three study days (Figure 1, t-test). During the infusion of adrenaline arterial pressure fell from 103/62 ± 3/2 mm Hg to 99/57 ± 3/2 mm Hg ($p<0.01$, t-test) after 3 h, but in the second part of the infusion period arterial pressure returned to baseline (Figure 2). Blood pressure during adrenaline infusion was significantly lower than during dextrose infusion ($p<0.001$, ANOVA). Heart rate rose during infusion of adrenaline from 61±3 to 63±3 beats/min ($p<0.001$). During infusion of noradrenaline arterial pressure rose from 104/64 ± 3/2 mm Hg to 113/70 ± 3/2 mm Hg ($p<0.001$) after 5 h of infusion and heart rate declined from 61±2 to 55±2 beats/min ($p<0.001$). Blood pressure during noradrenaline infusion was significantly higher than during dextrose infusion ($p<0.001$, ANOVA). The post-infusion period (16.00-10.00 h) was analyzed as a whole, and also over separate periods, i.e. 16.00 to 24.00 h, 0.00 to 7.00 h, 7.00 to 9.00 h and 9.00 to 10.00 h. For all periods, except for the period between 9.00 and 10.00 h, systolic arterial pressure (16.00-10.00 h: 6%, 16.00-24.00 h: 6%, 0.00-7.00 h: 5%, 7.00-9.00 h: 9%) and mean arterial pressure (16.00-10.00 h: 7%, 16.00-24.00 h: 5%, 0.00-7.00 h: 7%, 7.00-9.00 h: 10%) were higher after adrenaline infusion than after dextrose ($p<0.001$, ANOVA). Diastolic pressure was higher in the period from 16.00 to 10.00 h (7%) from 16.00 to 24.00 h (5%), from 0.00-7.00 h (7%) and from 7.00-9.00 h (13%). Heart rate after adrenaline infusion was not different from values after dextrose in any period. Haemodynamic values after noradrenaline infusion were never different from concomitant values after dextrose.

Effects on venous and arterial catecholamine levels

Mean baseline concentrations of adrenaline and noradrenaline were similar on the three study days (Table 1, t-test). During the infusion of adrenaline both arterial and venous concentrations of adrenaline rose approximately 10-fold (Table 1, Figure 3). Mean baseline venous concentrations of adrenaline increased from 21 ± 4 pg/ml to a mean of 230 ± 28 pg/ml during infusion ($p<0.001$, t-test), and were significantly higher than 30 ± 3 pg/ml, the corresponding value during dextrose (ANOVA, $p<0.001$). During infusion of noradrenaline arterial and venous plasma concentrations of noradrenaline rose 3- to 5-fold. Venous noradrenaline rose from 207 ±17 pg/ml to a mean of 705 ± 58 pg/ml during infusion (t-test, $p<0.001$) and was significantly higher than 219 ± 24 pg/ml, the corresponding value during dextrose, (ANOVA, $p<0.001$). Within 5 minutes after cessation of the infusions catecholamine concentrations had returned to baseline values. At 17.00 h and the

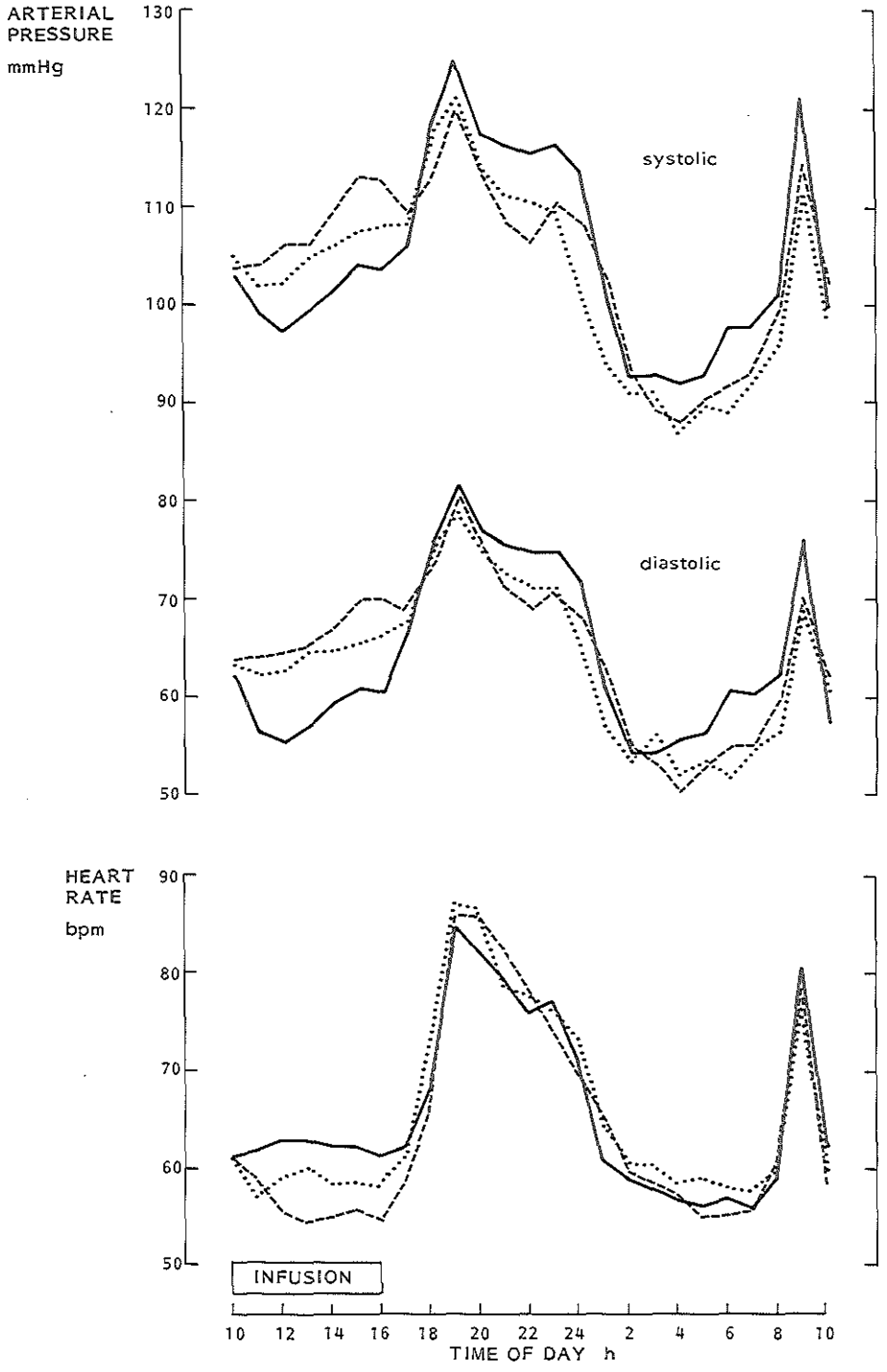

Figure 1. Mean hourly values of arterial pressure and heart rate before, during and after infusion of adrenaline (——), noradrenaline (——) and dextrose (••••).

next morning after 1 h of supine rest, plasma catecholamine concentrations were not different in the three situations and not different from the respective baseline values. Neither venous nor arterial plasma concentrations of noradrenaline were higher during adrenaline infusion than during 5% dextrose infusion. A small, although significant, decrease in both arterial and venous plasma adrenaline occurred during noradrenaline infusion as compared to corresponding values during dextrose infusion; mean arterial and venous adrenaline were 40 ± 7 and 22 ± 3 pg/ml respectively during noradrenaline infusion and 58 ± 5 and 30 ± 3 pg/ml during dextrose infusion ($p<0.001$ for both comparisons, ANOVA).

TABLE 1: ARTERIAL PLASMA CONCENTRATIONS (MEAN ± SEM) OF NORADRENALINE AND ADRENALINE BEFORE, DURING AND AFTER A 6 H INFUSION OF 5% DEXTROSE, NORADRENALINE AND ADRENALINE.

TIME (h)	NORADRENALINE (pg/ml)			ADRENALINE (pg/ml)		
	DEX	NOR	ADR	DEX	NOR	ADR
0	186±24	183±15	198±21	42±7	36±7	34±6
1	161±16	910±51	168±14	60±10	43±13	348±13
2	175±18	999±35	161±11	57±8	42±8	360±18
3	169±18	1038±33	153±13	51±6	40±7	398±26
4	178±21	1049±45	169±17	55±6	44±8	378±19
5	176±21	1061±69	179±19	59±8	42±7	402±23
6	162±15	1094±50	175±22	63±7	45±7	440±31
6.05	156±15	247±23	180±24	76±11	55±9	64±5
6.10	164±14	215±18	190±30	68±8	56±10	58±6
6.15	166±15	219±17	182±28	69±7	59±9	75±10
6.20	162±15	214±16	182±27	70±6	55±7	76±11
7	160±20	202±16	190±22	77±19	57±6	69±5
24	175±22	194±17	234±40	61±5	68±12	77±9

DEX = 5% dextrose, NOR = noradrenaline, ADR = adrenaline

TABLE 2 PLASMA CONCENTRATIONS OF RENIN, GROWTH HORMONE, PROLACTIN AND CORTISOL BEFORE, DURING AND AFTER INFUSION OF ADRENALINE AND NORADRENALINE.

	RENIN (mU/L)			GROWTH HORMONE (μg/L)			PROLACTIN (μg/L)			CORTISOL (nmol/L)		
time(h)	dex	nor	adr	dex	nor	adr	dex	nor	adr	dex	nor	adr
0	13.4(12.0-14.9)	15.5(14.6-21.0)	14.4(11.4-18.1)	1.4(0.6)	0.7(0.1)	0.6(0.1)	4.6(0.2)	4.4(0.2)	4.2(0.3)	425(31)	416(31)	437(53)
1	12.8(10.5-15.6)	15.6(12.0-20.3)	21.5(18.9-24.4)	1.1(0.2)	2.0(0.9)	1.8(0.9)	4.3(0.2)	3.9(0.2)	4.1(0.1)	334(28)	341(38)	311(41)
2	10.7(8.7-13.2)	18.2(13.9-23.9)	19.9(16.6-23.9)	1.5(0.5)	1.3(0.3)	1.1(0.3)	4.6(0.5)	3.9(0.1)	4.0(0.2)	330(46)	308(39)	317(30)
3	11.7(10.0-13.6)	15.7(12.4-19.8)	20.1(16.3-24.8)	1.0(0.2)	0.7(0.1)	0.9(0.2)	4.4(0.4)	3.8(0.1)	4.1(0.2)	318(41)	281(28)	290(31)
4	9.9 (8.6-11.4)	18.4(13.9-24.3)	17.1(14.7-20.0)	1.9(0.7)	0.7(0.1)	0.7(0.1)	4.2(0.2)	4.0(0.2)	4.2(0.2)	326(25)	305(30)	313(33)
5	10.5(8.4-13.0)	18.7(14.1-24.8)	17.2(14.8-19.9)	3.1(1.6)	0.8(0.2)	1.2(0.3)	4.6(0.1)	4.1(0.2)	4.8(0.3)	347(31)	343(33)	391(50)
6	11.1(8.8-14.1)	17.1(13.2-22.2)	16.4(14.1-19.2)	3.0(0.9)	1.9(0.8)	1.9(0.7)	4.8(0.3)	4.5(0.2)	4.8(0.3)	356(34)	287(20)	282(36)
7	10.0(8.2-12.3)	19.7(14.9-26.1)	15.3(12.9-18.0)	3.0(0.9)	1.5(0.4)	1.0(0.2)	5.0(0.4)	5.0(0.4)	5.4(0.7)	260(64)	268(32)	350(30)

Data are mean (SEM), except for renin which are geometric means (range).
Dex = dextrose infusion, nor = noradrenaline infusion, adr = adrenaline infusion

Hormonal changes

Baseline concentrations of active plasma renin were similar on the three study days (Table 2, t-test). Renin rose both during infusion of adrenaline and noradrenaline ($p<0.001$, ANOVA). At 17.00 h, one hour after the infusions had been stopped, the differences from dextrose were no longer statistically significant. Plasma concentrations of cortisol, prolactin and growth hormone before, during and after the infusions were not different during the three studies. The normal fall of cortisol between 9.00 and 17.00 h ($p<0,01$, t-test) was seen on each of the infusion days.

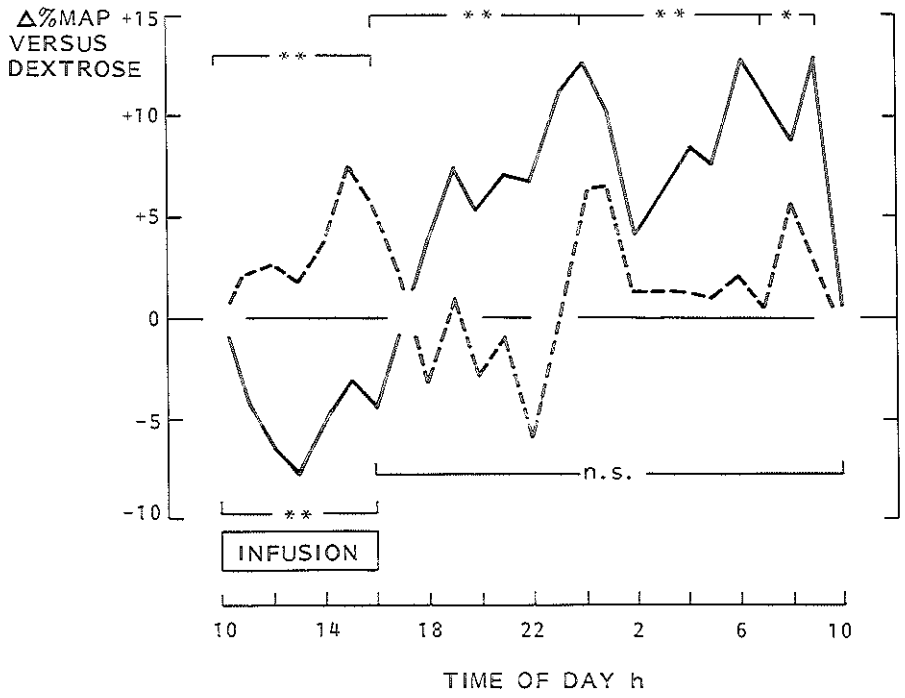

Figure 2. Mean hourly values of percentage change in mean arterial pressure as compared with baseline during and after adrenaline (——) and noradrenaline (– – –) corrected for percentage change from baseline during and after 5% dextrose. MAP = mean arterial pressure.
* $p < 0.01$, ** $p < 0.001$ adrenaline or noradrenaline versus dextrose.

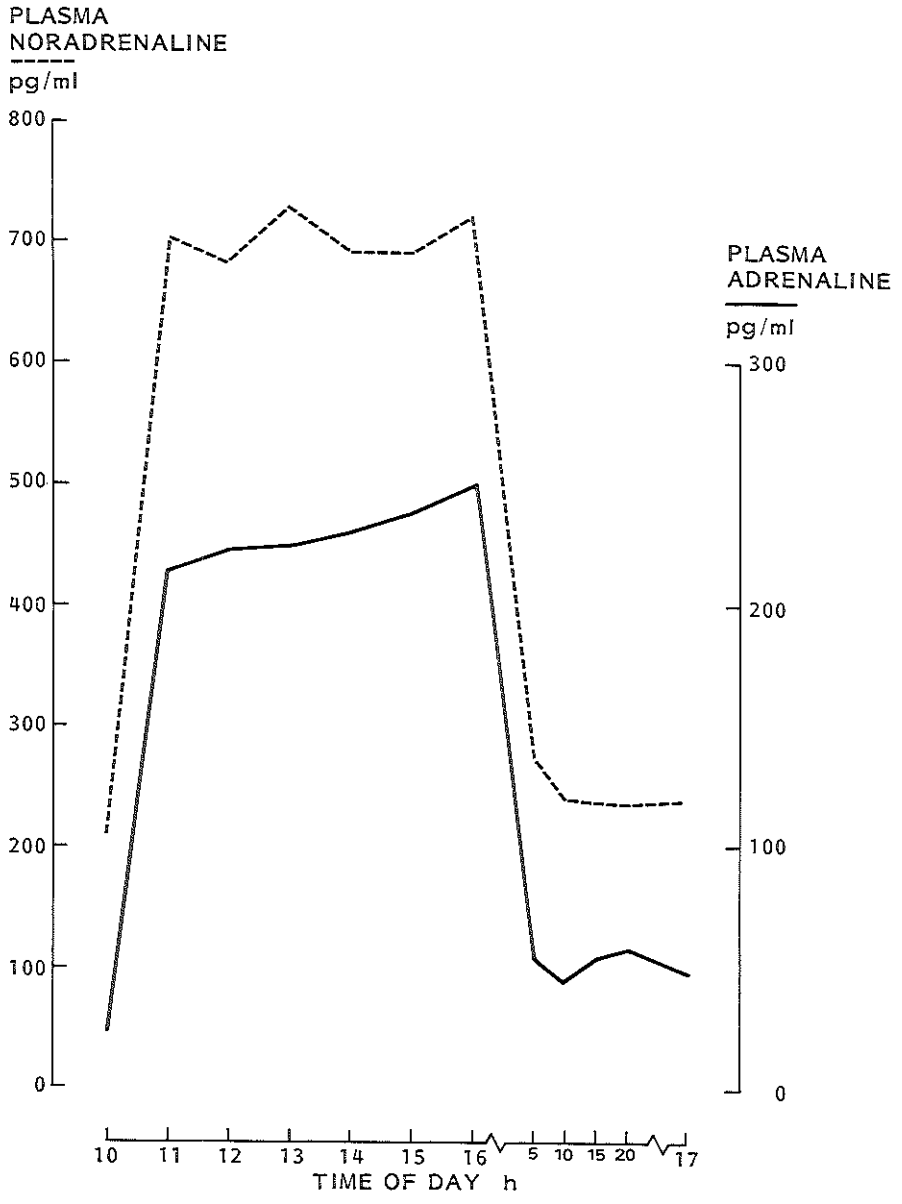

Figure 3. Venous concentrations of adrenaline and noradrenaline before, during and after infusions of adrenaline (——) and noradrenaline (----).

DISCUSSION

The plasma concentrations of catecholamines achieved by the infusions in this study are well within the physiological range. Venous concentrations of noradrenaline of approximately 700 pg/ml can be seen during quiet standing and mild physical exercise (18,19). Plasma concentrations of adrenaline of approximately 200 - 300 pg/ml have been reported during surgery, during moderate to severe physical exercise and during public speaking (18,19).

This study clearly shows, that infusion of adrenaline for 6 hours, at a rate which increases plasma levels of adrenaline into the high physiological range, causes a sustained increase in blood pressure which lasts for at least 18 hours after the infusion has been stopped. Our findings are compatible with the hypothesis that adrenaline taken up by sympathetic nerve terminals can be co-released with noradrenaline during sympathetic stimulation. Infusion of noradrenaline at a rate, which also increases plasma concentrations of noradrenaline into the high physiological range, lacks such an effect. Thus, the pressor effect of adrenaline is mediated by a β_2-adrenoceptor. Obviously, the prejunctional β_2-adrenoceptor, which increases noradrenaline release upon stimulation, is the ideal candidate to explain this delayed pressor effect of adrenaline. Indeed, after the infusion of adrenaline had been stopped, its pressor effect was only evident during periods of increased sympathetic activity, i.e.: 1. during ambulation after the subjects went back home, 2. in the second part of the night when sympathetic activity is known to be increased during REM sleep (20-22) and 3. the next morning when the volunteers came back to the hospital. The pressor effect of adrenaline could not be demonstrated under basal conditions, i.e. 1. in the hour following cessation of the infusion, 2. shortly after the subjects had gone to bed and 3. the following day during one hour recumbency in the laboratory (Figure 4).

Are there alternative explanations for our findings? The renin-angiotensin system might be a candidate for adrenaline's pressor effect. Renin release is mediated by β_1-adrenoceptors and both adrenaline and noradrenaline are β_1-adrenoceptor agonists (23). However, it is adrenaline and not noradrenaline that has a prolonged pressor effect after its infusion had been stopped. Thus, stimulation of the renin-angiotensin system is not a very likely explanation for this effect. Another possibility is that adrenaline, either directly or indirectly through its peripheral effects, stimulates central sympathetic outflow, thereby increasing blood pressure. Release of adrenocorticotropic hormone may be directly stimulated by adrenaline (24). Release of growth hormone and prolactin can be triggered by various forms of

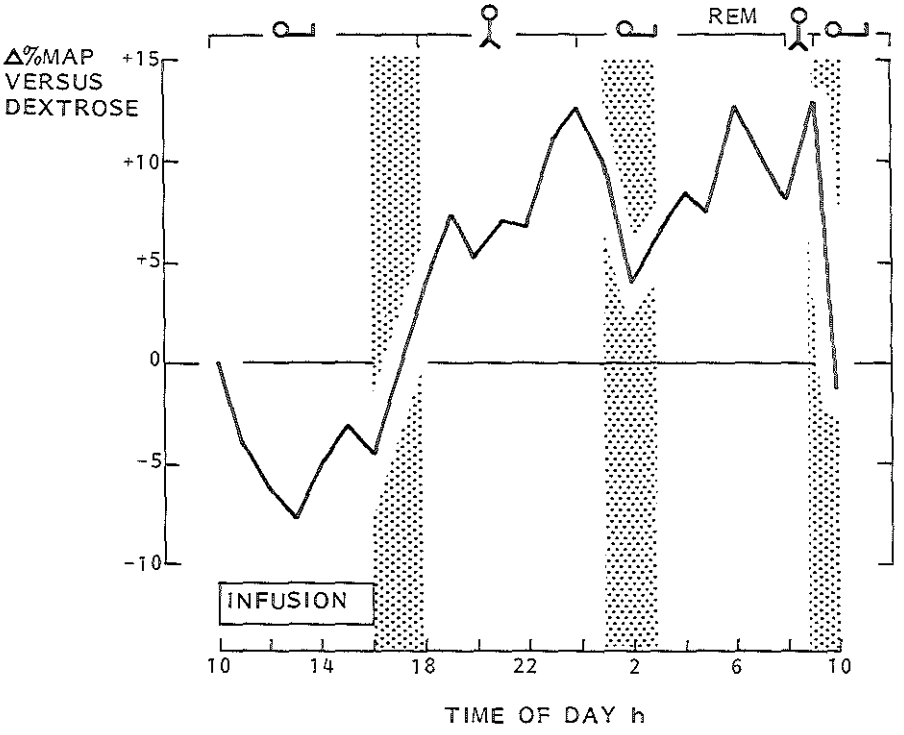

Figure 4. Effect of adrenaline infusion on blood pressure in relation to sympathetic activation. Postinfusion period: ::::: = "basal" conditions, white areas = increased sympathetic activity MAP = mean arterial pressure.

stress (25). However, cortisol values were not different in the three study situations and they showed their normal decline during the day. In addition growth hormone and prolactin concentrations were similar during the different study conditions and they did not rise during the infusions.

In contrast to previous reports (4, 26), we were not able to demonstrate an effect of adrenaline on heart rate in the post-infusion period. In these studies of Brown and co-workers adrenaline had no significant effect on blood pressure, but it caused a long-lasting rise in heart rate after the infusion was stopped. This phenomenon was taken as evidence by the authors for prejunctional ß-adrenoceptor stimulation of cardiac sympathetic nerves. An increase in metabolic rate by adrenaline, offsetting its pressor effect and increasing heart rate, would be an alternative explanation for their finding (27). In Brown's study much higher infusion rates were used than in our study (100 vs 15 ng/kg/min) and indeed at infusion rates above 50 ng/kg/min adrenaline increases the metabolic rate with secondary haemodynamic consequences (27). Also in a more recent study, in which adrenaline was infused at the same rate as in our study (28), an increase in blood pressure was seen for one hour after the infusion had been stopped, whereas heart rate was not increased in this period. The most likely explanation for the absence of an effect of adrenaline on heart rate, at the time it exerted its delayed pressor effect, is the interference of the arterial baroreflex which may offset the effect of adrenaline on neurotransmitter release in cardio-accelerator nerves.

Some studies have shown increments in plasma noradrenaline after infusion of ß-adrenoceptor stimulants, including adrenaline (16, 29-31), whereas others did not (32). We also were unable to detect, either in arterial or venous blood, an increase in plasma noradrenaline during the adrenaline infusion. Again, it should be stressed here, that adrenaline only exerted its delayed pressor effect during periods of sympathetic stimulation. Unfortunately, we only took blood samples from our subjects in the supine resting situation and not during sympathetic activation.

Three recent studies by Floras et al are of particular interest, since they provide further evidence for the functional significance of the mentioned concept. In the first study (17) they compared the effects of adrenaline and isoprenaline on forearm vasoconstrictor responses to a reflex stimulus for noradrenaline release (lower body negative pressure [LBNP]). To localise their action adrenaline (50 ng/min) and isoprenaline (10 - 25 ng/min) were infused for 40 minutes into the brachial artery and caused no systemic pressor effect. The vasoconstrictor response to LBNP was considerably augmented during but also 30 minutes after cessation of adrenaline

infusion. In contrast, prior infusion of isoprenaline, which is not taken up by sympathetic nerves, had no such aftereffect on the vasoconstrictor response to LBNP. Since the plasma half live of adrenaline is not more than a few minutes, these data provided evidence that adrenaline is taken up by sympathetic nervous system and released during subsequent reflex stimulation and augments neurogenic vasoconstriction. In a second study a comparable protocol was studied in borderline hypertensives (33). Again, 30 minutes after adrenaline an enhanced vasoconstrictor response was found, similar to the first study. In a third experiment normotensive subjects were studied (34). Again the forearm vasoconstrictor response to LBNP was measured before and after adrenaline, this time infused systemically. This protocol was carried out at two occasions: after placebo and after pretreatment with the neuronal uptake blocker desipramine. The study showed that the vasoconstrictor response was augmented 30 minutes after adrenaline on the placebo occasion, but not on the desipramine treatment day. Blood pressure was higher after adrenaline during placebo than during desipramine. So these studies clearly showed that the aftereffects of adrenaline are mediated by adrenaline which has been taken up in sympathetic nerves and which is subsequently coreleased with noradrenaline during sympathetic nervous stimulation.

In our study we used intravenously infused adrenaline. To our knowledge no data are available on the long term pressor effect of endogenously released adrenaline in humans. These results would be of great interest since they would substantiate the above suggested role of adrenaline in the pathogenesis of hypertension. Various kinds of stress can induce both a rise in adrenaline and in blood pressure (see extensive review 35), however, whether stress induced adrenaline release is able to mediate a delayed and protracted pressor effect as described in this chapter, is not known. Researchers who would plan to organize a study to evaluate this question, will encounter major methodological problems and problems with standardization. Another way to induce a rise in endogenously released adrenaline, is by inducing hypoglycaemia. A study on the long term pressor effect of hypoglycaemia, does not exist to our knowledge. However, important problems would exist when performing such a study in normal volunteers, for instance problems of ethical nature, but also the fact that hypoglycaemia induces a marked rise in several other hormones except adrenaline, such as growth hormone, cortisol, noradrenaline and glucagon (36). When inducing hypoglycaemia in diabetics a possible problem would be the frequently existing impaired hormonal response to low blood glucose, which is particularly true for adrenaline and glucagon (37). So, we think that, although such studies could potentially further

contribute to our understanding of the role of adrenaline in the pathogenesis of hypertension, these studies can hardly be performed.

In conclusion, man's endogenous β_2-adrenoceptor stimulant adrenaline when elevated into the high physiological range, as seen during several forms of stress, mediates a delayed and protracted pressor effect. Stress levels of noradrenaline are devoid of such activity. Central nervous system activation or stimulation of the renin-angiotensin system can not readily explain the different effects of the two catecholamines. This suggests that activation of prejunctional β_2-adrenoceptors by stored adrenaline is responsible for its delayed pressor effect. When co-released with noradrenaline upon sympathetic stimulation, adrenaline augments noradrenaline release, thereby amplifying vasoconstrictor nerve activity and raising blood pressure.

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Chapter 4

"STRESS" LEVELS OF ADRENALINE MEDIATE A PROTRACTED AMPLIFICATION OF THE BLOOD PRESSURE RESPONSE TO SYMPATHETIC STIMULATION IN MAN.

Based on: PJ Blankestijn, J Tulen, AJ Man in't Veld, AH van den Meiracker, F Boomsma, P Moleman, MADH Schalekamp. "Stress" levels of adrenaline for 6 hours mediate a protracted (18 hours) amplification of the blood pressure responses to sympathetic stimulation in man. J Hypertens 1991, in press

INTRODUCTION

In most healthy human subjects, exposure of the skin to ice-cold water, the cold pressor test, and performance of isometric exercise increase arterial pressure, heart rate and vascular resistance (1-4). This response is generally believed to be the result of sympathetic nervous system stimulation, which gives a rise in plasma noradrenaline, vasoconstriction and an increase in blood pressure. This pressor response is abolished by α-adrenoceptor blockade, but not by ß-adrenoceptor blockade (5,6). The cold pressor and isometric exercise test are used to evaluate sympathetic neural control of peripheral circulation. The cold pressor test has been reported to produce exaggerated responses in hypertension-prone persons (4) and it is impaired in patients with orthostatic hypotension caused by efferent sympathetic failure (7).

In previous studies our group has demonstrated that infusion of adrenaline resulting in plasma levels in the high physiological range, is capable of augmenting the rise in blood pressure induced by the cold pressor and isometric exercise test (8-10). Beta-blockade prevented the facilitation of the pressor responses. Also plasma noradrenaline was higher during adrenaline infusion. It was hypothesized that adrenaline may amplify sympathetic pressor responses by facilitation of the release of noradrenaline through activation of the prejunctional $ß_2$-adrenoceptors.

The aim of the present study was twofold:

1. Adrenaline is likely to be the physiologic agonist of the prejunctional $ß_2$-adrenoceptors, as opposed to noradrenaline which is virtually devoid of $ß_2$-adrenoceptor agonistic properties. Therefore, we measured the responses in blood pressure and heart rate during cold pressor test and isometric exercise test during adrenaline, but also during noradrenaline and dextrose infusion in order to collect further evidence for the hypothesis that the amplification of the pressor response by adrenaline indeed occurs through stimulation of $ß_2$-adrenoceptors.
2. Since it was hypothesized that circulating adrenaline can be taken up in the sympathetic neuron and can be "co-released" along with noradrenaline during sympathetic stimulation and subsequently facilitate noradrenaline release, we also measured the responses of blood pressure and heart rate during cold pressor test and isometric exercise test 18 hours after cessation of the infusions, in order to provide evidence that this mechanism might also be operational in man.

SUBJECTS AND METHODS

See chapter 2.

RESULTS

Plasma catecholamines and haemodynamics

During infusion of adrenaline and noradrenaline arterial plasma concentrations of noradrenaline and adrenaline rose from 34±6 to 387±15 pg/ml and from 183±15 to 1025±38 pg/ml respectively and returned to baseline values within 10 minutes after the cessation of the infusions. Plasma noradrenaline during adrenaline infusion did not change as compared to baseline levels and to plasma levels during dextrose infusion. Results of measurements of venous plasma concentrations of adrenaline and noradrenaline are presented in chapter 3.

TABLE 1 ARTERIAL PLASMA ADRENALINE (pg/ml) CONCENTRATIONS

	baseline	during infusion	18h after infusion
Adrenaline infusion	34 ± 6	387 ± 15 ***	77 ± 9
Noradrenaline infusion	36 ± 7	41 ± 7	68 ± 12
Dextrose infusion	42 ± 7	58 ± 5	61 ± 5

ARTERIAL PLASMA NORADRENALINE (pg/ml) CONCENTRATIONS

	baseline	during infusion	18h after infusion
Adrenaline infusion	199 ± 21	167 ± 15	230 ± 40
Noradrenaline infusion	183 ± 15	1025 ± 38 ***	194 ± 17
Dextrose infusion	186 ± 24	170 ± 17	175 ± 20

data are means ± SEM

*** $p<0.001$, adrenaline infusion: plasma adrenaline during infusion of adrenaline versus dextrose infusion, but also versus baseline of adrenaline study

Noradrenaline infusion: plasma noradrenaline during infusion of noradrenaline versus dextrose infusion, but also versus baseline of noradrenaline study

Mean arterial plasma concentrations of adrenaline and noradrenaline before the isometric exercise and cold pressor test performed 18 hours after the cessation of the infusions were not different during the three study days (Table 1). Venous plasma concentrations of adrenaline and noradrenaline were not measured at that time.

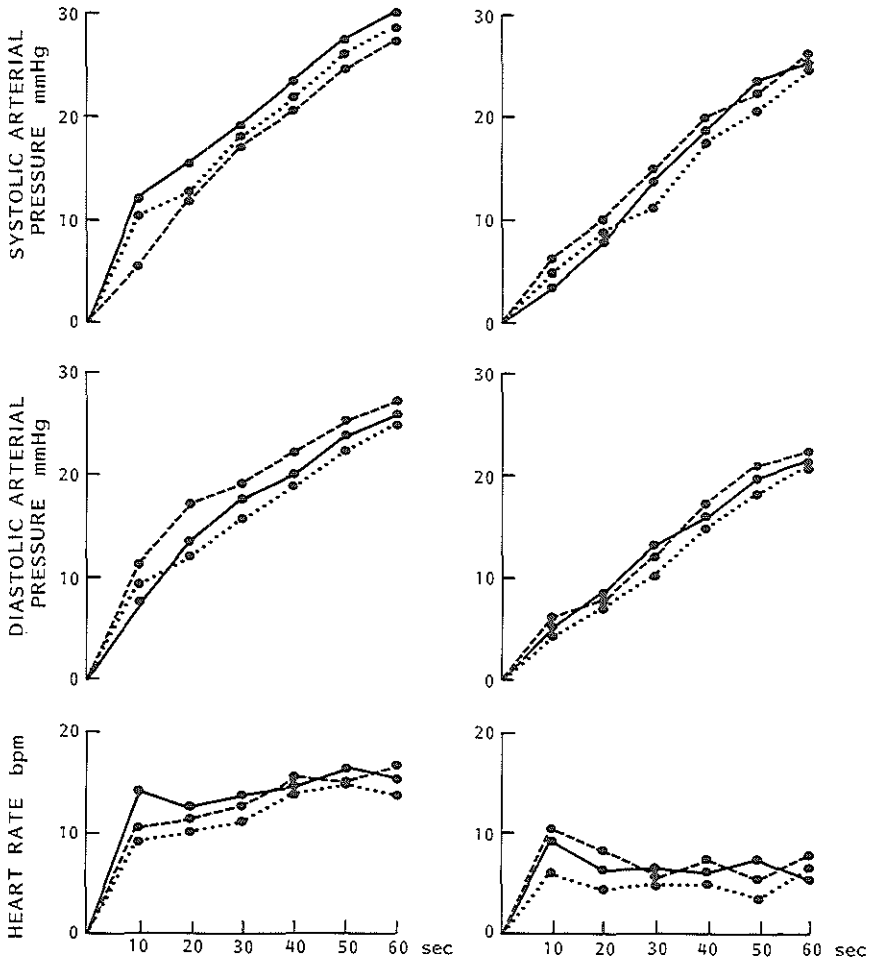

Figure 1. Blood pressure and heart rate response before the start of the infusions of adrenaline (——), noradrenaline (– – –) and dextrose (••••).
Left panel: isometric exercise test, right panel: cold pressor test

Baseline arterial blood pressure and heart rate were not different between the 3 studies. Mean blood pressure decreased 5±1 % during adrenaline as compared to dextrose and increased with 4±1 % during noradrenaline as compared to dextrose. During the post-infusion period systolic and diastolic arterial pressure were 6±2 % and 7±2 % higher after adrenaline than after dextrose, whereas blood pressure after noradrenaline was not different from the dextrose study. No differences in heart rate occurred after adrenaline or noradrenaline as compared to dextrose (see for more detailed information: chapter 3).

Stress tests

The isometric exercise and cold pressor test performed before, during as well as after the infusions induced a gradual increase in systolic and diastolic arterial pressure.

Changes in blood pressure and heart rate during both the isometric exercise and the cold pressor test performed before the start of the infusions were not different on the 3 study days (Figure 1).

TABLE 2 DIFFERENCES IN RESPONSE OF BLOOD PRESSURE AND HEART RATE TO COLD EXPOSURE AND ISOMETRIC EXERCISE DURING AND 18h AFTER ADRENALINE AND NORADRENALINE INFUSION AS COMPARED TO DEXTROSE INFUSION.

ISOMETRIC EXERCISE	during ADR		after ADR		during NOR		after NOR	
SAP (mmhg)	7±2	p<0.01	7±2	p<0.01	-5±3	ns	-2±2	ns
DAP (mmHg)	3±2	ns	3±2	ns	-4±3	ns	-1±1	ns
HR (bpm)	-1±4	ns	2±2	ns	0±3	ns	4±2	ns

COLD EXPOSURE	during ADR		after ADR		during NOR		after NOR	
SAP (mmHg)	4±1	p<0.05	9±3	p<0.05	-3±2	ns	0±2	ns
DAP (mmHg)	4±2	ns	6±2	p<0.01	-3±2	ns	-3±2	ns
HR (bpm)	-2±2	ns	-2±3	ns	-1±1	ns	-3±3	ns

Data are means ± SEM of the 6 ten seconds means during the tests.
SAP = systolic arterial pressure, DAP = diastolic arterial pressure, HR = heart rate
ADR = adrenaline, NOR = noradrenaline

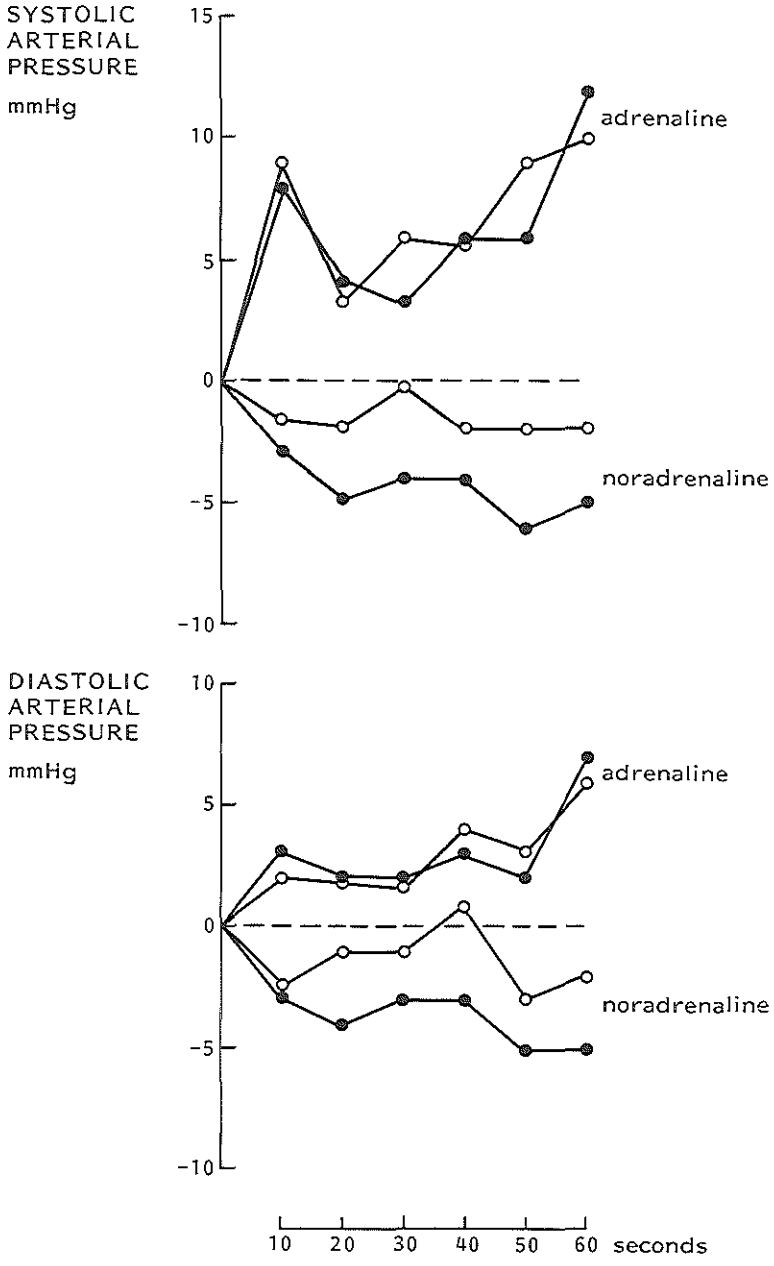

Figure 2. Difference in blood pressure response during isometric exercise as compared to dextrose. ●–● during infusion, O–O after infusion

The increments in blood pressure induced by isometric exercise test were greater during adrenaline infusion than during dextrose infusion (Figure 2 and Table 2). This difference was only statistically significant for systolic arterial pressure. The pressor effect of isometric exercise test 18 hours after cessation of the infusion of adrenaline was still increased as compared to the test done 18 hours after dextrose (Figure 2, Table 2). Changes in heart rate during and after adrenaline and in blood pressure and heart rate during and after noradrenaline infusion during the isometric exercise test were not different from those of the dextrose study.

The effect of adrenaline infusion on the pressor responses to cold pressor test were similar to the effects observed during the isometric exercise test. During, but also after the adrenaline infusion the rise in systolic and diastolic arterial pressure was more pronounced than in the dextrose study (Figure 3, Table 2). Responses in heart rate during and after the adrenaline and in blood pressure and heart rate responses during and after the noradrenaline infusion were not significantly different from the effects of the tests during dextrose.

DISCUSSION

As has been shown in numerous earlier studies, isometric exercise and the cold pressor test induce a rise in both systolic and diastolic arterial pressure and heart rate (1-4). Our results are in concordance with these reports. This increase in blood pressure is a result of an activation of the sympathetic nervous system (1-6,11). That these tests indeed actually increase sympathetic outflow was elegantly shown by the group of Allyn Mark and coworkers (12,13). They measured muscle sympathetic nerve activity with micro-electrodes inserted percutaneously into a peroneal muscle nerve fascicle in the leg before and during cold pressor test. Arterial pressure, plasma noradrenaline and muscle sympathetic nerve activity rose during immersion of the hand in ice-water. The increments in muscle sympathetic activity during the test were well correlated with the increases in blood pressure and peripheral venous noradrenaline (12). In a second series of experiments they demonstrated that also sustained handgrip leads to an increase in muscle sympathetic nerve activity (13).

In a previous studies we have demonstrated that the pressor effect induced by cold pressor test and isometric exercise test was amplified during adrenaline infusion in comparison to placebo (8-10). In those studies adrenaline was infused at a rate of 30 ng/min/kg body weight, which produced arterial plasma levels of approximately 700 pg/ml. The present study in which we infused adrenaline at a rate of 15 ng/min/kg body weight, leading to arterial plasma levels of approximately 380 pg/ml, confirm these earlier findings. This means that also

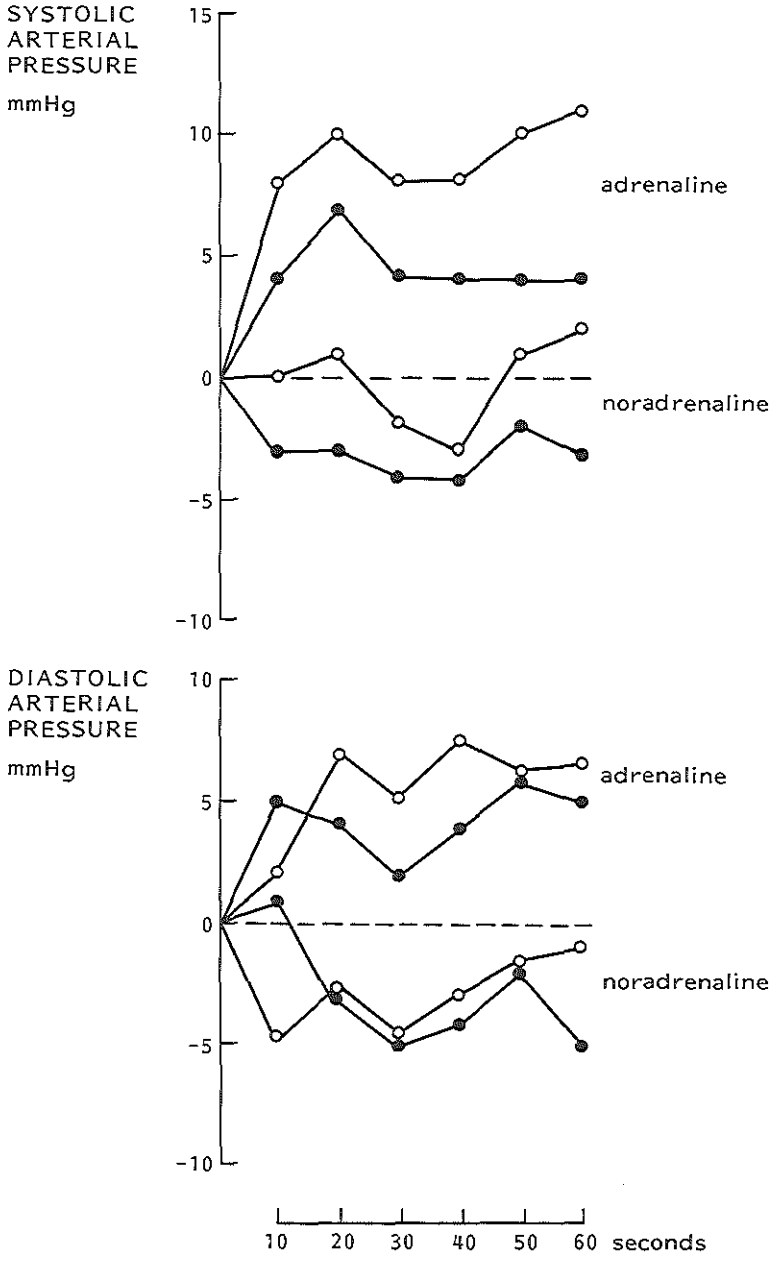

Figure 3. Difference in blood pressure response during cold exposure as compared to dextrose. ●—● during infusion, O—O after infusion

moderately increased plasma concentrations of adrenaline, such as during mild stress, elicit this effect on blood pressure. As was found by Vincent et al (8), we did not detect any difference in responses in heart rate during adrenaline as compared to dextrose.

The present study also demonstrates, that infusion of noradrenaline does not produce an amplification of the pressor response to isometric exercise test and cold pressor test as observed during adrenaline infusion. This finding can be interpreted as an indication through which mechanism the amplification of this pressor response occurs. *In vitro* studies have shown that the release of noradrenaline from sympathetic nerves is enhanced by ß-adrenoceptor agonists (14,15). The receptor involved in this facilitating response, is most likely of the prejunctional $ß_2$ subtype (16-18). These $ß_2$-adrenoceptors are demonstrated to be sensitive to the naturally occurring ß-adrenoceptor agonist adrenaline (15,18-19). These findings have led to the hypothesis that endogenous adrenaline can modulate noradrenaline release through stimulation of these receptors. In that way adrenaline may indirectly increase sympathetic vasoconstrictor tone. Our findings *in vivo* that adrenaline but not noradrenaline can amplify the blood pressure response to isometric exercise and cold pressor test give support to the hypothesis that this effect is $ß_2$-adrenoceptor mediated and that it might also be operational *in man*, since adrenaline is mans endogenous $ß_2$-adrenoceptor stimulant.

Another important finding of our study is the fact that the pressor effect of the isometric exercise and cold pressor test performed the next morning, approximately 18 hours after cessation of the adrenaline infusion, was enhanced as compared to the same tests after the noradrenaline and dextrose infusions. Arterial plasma levels of adrenaline and noradrenaline taken shortly before the isometric exercise and cold pressor test were not different on the three study days (Table 2).

These data support the results of extensive animal and *in vitro* research on the possible function of adrenaline as cotransmitter of noradrenaline. It is almost 30 years ago that is was shown that adrenaline can be taken up into sympathetic nerves (20). Once incorporated into the nerve terminals, adrenaline may be released as cotransmittor of noradrenaline during sympathetic nerve stimulation. When released from sympathetic nerves, adrenaline activates prejunctional $ß_2$-adrenoceptors to further enhance noradrenaline release. Majewski et al have demonstrated in the anaesthetized rabbit, that adrenaline given intravenously (21) or released from the adrenal medulla by splanchnic nerve stimulation (22), resulted in an increase in the rate of noradrenaline release into the plasma well after the adrenaline had been cleared from the plasma. At that time the adrenaline concentrations in the sympathetically innervated tissues were still raised.

Pretreatment of the rabbits with desipramine in order to block neuronal uptake of adrenaline or pretreatment with ß-adrenoceptor antagonist, prevented this facilitating effect of adrenaline on noradrenaline release completely. In other studies by Majewski et al (23) it was shown that rats subjected to the chronic stress of individual housing and binding of their rear legs had elevated blood pressures when compared to control rats. In rats that had undergone adrenal medullectomy prior to the stressing procedure, or giving desipramine or propranolol, the stressing procedure did not elevate blood pressure. After 12 days of "stress" the animals were sacrificed and adrenaline content of the heart was measured. The adrenaline content was higher in stressed rats than in control rats. In desipramine treated rats the cardiac adrenaline content was not different from desipramine treated control rats. All these results taken together suggest that in these animals adrenaline had a primary role in the stress-induced hypertension. The experiments with the neuronal uptake-blocker desipramine and the ß-adrenoceptor blocker propranolol are consistent with the hypothesis that neuronal accumulation of adrenaline and activation of (prejunctional) ß-adrenoceptors are also involved.

Floras et al provided evidence in three recent studies that also in man adrenaline can be taken up and can be "co-released" along with noradrenaline during sympathetic nervous stimulation. In the first study (24) they compared the effects of adrenaline and isoprenaline on forearm vasoconstrictor responses to a reflex stimulus for noradrenaline release (lower body negative pressure [LBNP]). To localise their action adrenaline (50 ng/min) and isoprenaline (10 - 25 ng/min) were infused for 40 minutes into the brachial artery and caused no systemic pressor effect. The vasoconstrictor response to LBNP was considerably augmented during, but also 30 minutes after cessation of adrenaline infusion. In contrast, prior infusion of isoprenaline, which is not taken up by sympathetic nerves, had no such aftereffect on the vasoconstrictor response to LBNP. Since the plasma half live of adrenaline is not more than a few minutes, these data provided evidence that adrenaline is taken up by the sympathetic nervous system and released during subsequent reflex stimulation and that it augments neurogenic vasoconstriction. In a second study a comparable protocol was carried out in borderline hypertensives (25). Again, 30 minutes after adrenaline an enhanced vasoconstrictor response was found, similar to the first study. In a third experiment normotensive subjects were studied (26). Again the forearm vasoconstrictor response to LBNP was measured before and after adrenaline, this time infused systemically. This protocol was done on two occasions: after placebo and after pretreatment with the neuronal uptake blocker desipramine. The study showed that the vasoconstrictor response was augmented 30 minutes after adrenaline on the placebo occasion, but not on the

desipramine treatment day. Blood pressure was higher after adrenaline during placebo than during desipramine. So these studies clearly showed that the aftereffects of adrenaline are mediated by adrenaline which has been taken up in sympathetic nerves and was subsequently coreleased with noradrenaline during sympathetic nervous stimulation.

In our study *in man* we found that the pressor effect induced by isometric exercise and the cold pressor test performed 18 hours after the cessation of the adrenaline infusion, was still enhanced in comparison with the results of the noradrenaline and dextrose study. These data are compatible with the hypothesis which is at the base of the animal research and the studies in humans just mentioned. This is to say that adrenaline can be taken up in sympathetic nerve endings and that it can be can be co-released with noradrenaline during sympathetic stimulation. Subsequently it activates prejunctional β_2-adrenoceptors, it enhances noradrenaline release and increases vasoconstrictor tone and therefore it raises blood pressure. Our results support the view that this mechanism is also operational in man. Furthermore, our finding, that adrenaline particularly increased blood pressure in the periods after its infusion, in which sympathetic activity was supposedly increased (Chapter 3), also accords with the present findings.

In conclusion our results provide further evidence for the hypothesis that adrenaline amplifies the blood pressure response to sympathetic stimulation, both when circulating adrenaline levels are in the high physiological range (during stressful conditions) as well as for a period of at least 18 hours after the raised plasma adrenaline concentrations. This effect is most likely due to stimulation of the prejunctional β_2-adrenoceptors, since noradrenaline is devoid of such an effect.

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Chapter 5

URINARY EXCRETION OF CATECHOLAMINES AND THEIR METABOLITES DURING AND AFTER A 6 HOUR INFUSION OF ADRENALINE AND NORADRENALINE

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Abbreviations used in this chapter

COMT	catechol-O-methyltransferase
DHMA	3,4-dihydroxymandelic acid
DHPG	3,4-dihydroxyphenylglycol
MAO	monoamine oxidase
MHPG	4-hydroxy-3-methoxyphenylglycol
MN	metanephrine
NMN	normetanephrine
PNMT	phenylethanolamine-N-methyltransferase
VMA	vanillyl mandelic acid

INTRODUCTION

The major catecholamines are noradrenaline, adrenaline and dopamine. Noradrenaline is the principal neurotransmitter of the sympathetic nervous system, but it is also an important transmitter in the central nervous system. Adrenaline is almost exclusively secreted by the adrenals and exerts its major actions as a circulating hormone. Dopamine is the precursor of noradrenaline and adrenaline and is an important central nervous system transmitter, but has also some peripheral effects.

When it was shown that only a small fraction of administrated catecholamines is excreted in urine (1,2), interest in their metabolism increased. Studies with radiolabelled catecholamines provided insight in the metabolic transformation and it became clear that deamination by MAO and O-methylation by COMT are the two major routes of metabolism (3). The biosynthetic interrelationships between the catecholamines and their metabolites are shown in Figure 1 of Chapter 1.

In this chapter we describe the results of measurements of the amounts of catecholamines and their metabolites excreted into the urine, collected during the infusions and during the postinfusion periods. The questions which we hoped to answer by this study were, firstly: are increments in plasma catecholamines within the physiological range also recognizable in alterations in the urinary excretion of catecholamines and their metabolites? And if the answer is yes, measurement of which metabolite in which urine is most suitable for answering this question? If the answer on the first question is affirmative, the following question is, whether the enhanced noradrenaline release, which is hypothesized to be responsible for the pressor effect observed in the postinfusion period (see Chapter 3) is also detectable in increased excretion of noradrenaline and/or its metabolites? Since no plasma noradrenaline concentrations were measured in the postinfusion period, we hoped to collect additional support for this concept by measuring the urinary catecholamines and metabolites.

RESULTS

Effects on plasma adrenaline and noradrenaline

Baseline values of arterial and venous adrenaline and noradrenaline did not differ between the three study days. During the infusion of adrenaline venous adrenaline rose from 21 ± 4 to a mean of 230 ± 28 pg/ml and arterial adrenaline rose from 34 ± 6 to 387 ± 15 pg/ml. Both venous and arterial plasma levels during infusion of adrenaline were higher than during dextrose infusion (ANOVA, both $p<0.001$). Plasma noradrenaline did not change during adrenaline infusion.

During noradrenaline infusion venous noradrenaline rose from 207 ± 17 to a mean of 705 ± 58 pg/ml and arterial noradrenaline from 183 ± 15 pg/ml to 1025 ± 38 pg/ml. Both venous and arterial plasma concentrations were higher than during dextrose infusion (ANOVA, both $p<0.001$).

A small although significant decrease in both arterial and venous plasma adrenaline occurred during noradrenaline infusion as compared to corresponding values during dextrose infusion; mean arterial and venous adrenaline was 40 ± 7 and 22 ± 3 pg/ml respectively during noradrenaline infusion and 58 ± 5 and 30 ± 3 pg/ml during dextrose infusion ($p<0.001$ for both comparisons, ANOVA).

Five minutes after cessation of the infusions plasma concentrations of adrenaline and noradrenaline after respectively adrenaline and noradrenaline infusion had returned to baseline values. (see for more detailed description chapter 3). The total amounts of adrenaline and noradrenaline infused on the respective occasions was 5.00 ± 0.45 μmoles of noradrenaline and 2.31 ± 0.21 μmoles of adrenaline.

Effects on urinary excretion of adrenaline and noradrenaline and metabolites

Infusion of adrenaline resulted in a significant increase in excretion of adrenaline and M during the infusion period as compared to dextrose infusion, i.e. during adrenaline and dextrose respectively adrenaline excretion of 149 ± 31 vs 22 ± 7 nmoles and M excretion 559 ± 186 and 291 ± 92 nmoles (Table 1). In the postinfusion period adrenaline excretion after adrenaline infusion was not different from values after dextrose infusion, whereas M excretion was still significantly increased, 556 ± 219 vs 325 ± 192 nmoles respectively (Table 1). The excretion rate of adrenaline and M during the total collection period was significantly higher during the adrenaline study than during the dextrose and noradrenaline study; adrenaline excretion 0.17 ± 0.04 vs 0.04 ± 0.01 μmoles during adrenaline and dextrose study respectively and M excretion 1.13 ± 0.39 vs 0.62 ± 0.27 μmoles respectively (Table 2).

TABLE 1. URINARY CATECHOLAMINES AND THEIR METABOLITES DURING AND AFTER 6 HOURS INFUSION OF ADRENALINE AND NORADRENALINE.

	infusion condition DEXTROSE	NOR	ADR	Condition effect p
ADR				
urine 1, (nmol)	22.0± 7.7 (10)	21.0±9.6 (10)	149 ±31 (9)	<0.001
urine 2, (nmol)	20.1±12.8 (10)	16.9±10.6 (10)	23.4±10.5 (10)	n.s.
M				
urine 1, (nmol)	291 ± 92 (10)	298 ± 67 (9)	559±186 (9)	<0.001
urine 2, (nmol)	325 ±192 (10)	374 ±209 (10)	556±219 (10)	<0.001
NOR				
urine 1, (nmol)	82.5 ±32.1 (10)	290 ±60.3 (10)	91.7 ±33.6 (9)	<0.001
urine 2, (nmol)	159 ±48.3 (10)	156 ±39.7 (10)	154 ±48.9 (10)	n.s.
NM				
urine 1, (nmol)	415 ±106 (10)	521 ± 92 (9)	518 ±143 (8)	0.054
urine 2, (nmol)	559 ±165 (10)	737 ±229 (10)	743 ±221 (10)	0.050
MHPG				
urine 1, (μmol)	6.05 ±2.98 (10)	5.35±1.59 (10)	4.88±1.15 (9)	n.s.
urine 2, (μmol)	8.67 ±3.46 (10)	7.73±4.05 (10)	7.12±3.95 (10)	n.s.
VMA				
urine 1, (μmol)	7.58 ±2.11 (9)	8.40 ±1.35 (10)	8.81 ±1.72 (9)	n.s.
urine 2, (μmol)	10.48±4.96 (9)	11.20±4.04 (10)	10.90±4.70 (9)	n.s.
creatinine				
urine 1, (mmol)	6.9 ±1.15 (10)	6.8 ±1.05 (10)	7.1 ±1.55 (9)	n.s.
urine 2, (mmol)	8.2 ±2.49 (10)	8.0 ±1.79 (10)	8.5 ±2.53 (10)	n.s.
volume				
urine 1, (L)	0.97±0.36 (10)	0.95±0.44 (10)	0.77±0.24 (9)	n.s.
urine 2, (L)	0.76±0.55 (10)	0.58±0.34 (10)	0.60±0.51 (10)	n.s.

ADR indicates adrenaline; NOR, noradrenaline; M, metanephrine; NM, normetanephrine; MHPG,3-methoxy-4-hydroxyphenylglycol; VMA, vanillylmandelic acid.
Urine 1 indicates urine collected during the infusion period (8.30-17.30 h), and urine 2 indicates urine collected during the postinfusion period (17.30 - 8.30 h next morning).
All values expressed as mean ± SD (n).

Infusion of adrenaline resulted in an increase in NM in the infusion and postinfusion period, which was marginally significantly different from values obtained during dextrose study (Table 1). Excretion of NM during the total collection period of the adrenaline study was significantly increased as compared to the dextrose study, 1.29 ± 0.32 vs 0.97 ± 0.22 μmoles (Table 2). Excretion of noradrenaline, VMA and MHPG in the urine of the infusion and the postinfusion period were not different from results of the dextrose study (Tables 1 and 2).

Infusion of noradrenaline resulted in a significant increase in excretion of noradrenaline and NM in the urine collected during the infusion period. Noradrenaline excretion during noradrenaline infusion was 290 ± 60.3 and 82.5 ± 32.1 nmoles as compared to values obtained during dextrose infusion and NM was 521 ± 92 and 415 ± 106 nmoles respectively. Excretion of adrenaline, M, VMA and MHPG in the urine of the infusion period, but also in that of the postinfusion period were not different from the values obtained during the dextrose study. Noradrenaline and NM excretion in the postinfusion period were not different from corresponding results obtained during the dextrose study. However noradrenaline and NM excretion during the total collection period of the noradrenaline study was higher than during the dextrose study, noradrenaline excretion 0.45 ± 0.05 vs 0.24 ± 0.07 μmoles respectively and NM excretion 1.23 ± 0.25 vs 0.97 ± 0.22 μmoles respectively (Table 2).

TABLE 2. URINARY EXCRETION OF CATECHOLAMINES AND METABOLITES DURING THE TOTAL COLLECTION PERIOD.

	infusion condition			condition effect
	DEXTROSE	NOR	ADR	p
ADR	0.04±0.01	0.04±0.01	0.17±0.04	<0.001
M	0.62±0.27	0.67±0.14	1.13±0.39	<0.001
NOR	0.24±0.07	0.45±0.05	0.25±0.08	<0.001
NM	0.97±0.22	1.23±0.25	1.29±0.32	0.024
MHPG	14.7±6.2	13.1±4.3	12.4±4.5	ns
VMA	18.3±6.5	19.6±5.0	19.9±5.7	ns

Abbreviations as in table 1
Values are expressed as μmoles, mean±sd.

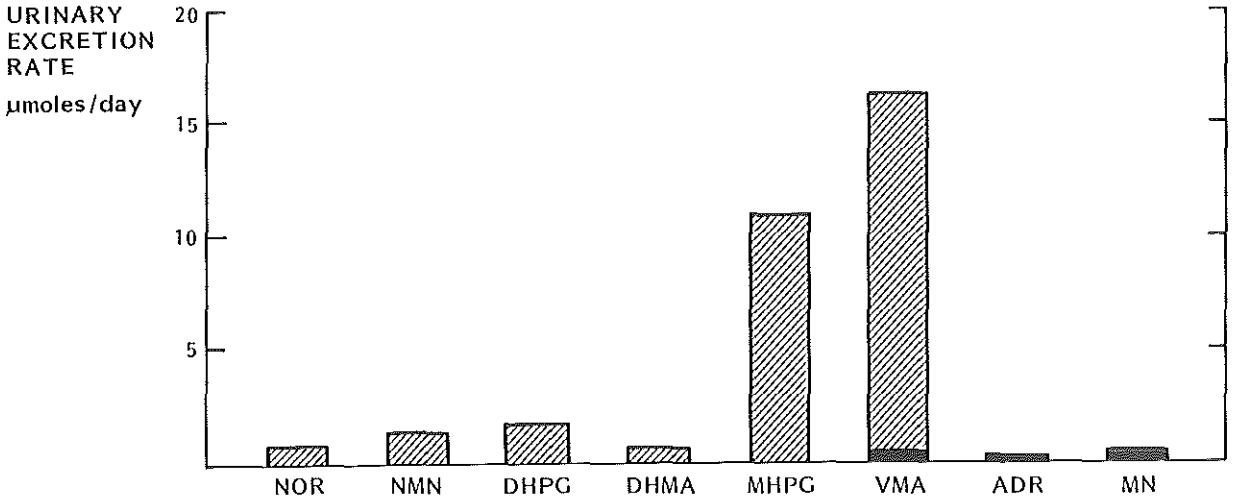

Figure 1. Urinary excretion of catecholamine metabolites derived from adrenaline (■) and noradrenaline (///). (Adapted from reference 3)

DISCUSSION

The kidney participates in the whole body catecholamines metabolism in two ways. Firstly, in keeping with the fact that many renal functions are influenced by the renal sympathetic nervous system, the kidneys are richly innervated and have a high tissue catecholamine content. With the exception of PNMT, the enzyme needed to convert noradrenaline into adrenaline, the enzymes required for synthesis of catecholamines from tyrosine are present in the kidney. The principal enzymes for degradation, COMT and MAO, are also present. Besides this intrarenal synthesis, release and degradation of catecholamines the kidneys participate in other ways in the "whole body" metabolism. Catecholamines are extracted from the plasma and released into the circulation and catecholamines and metabolites are excreted into the urine.

For each catecholamine only a small fraction of the total amount produced is excreted unchanged in the urine. Urinary noradrenaline excretion represents 1-2% of the whole body synthesis rate (4). Urinary noradrenaline is particularly derived from plasma noradrenaline filtered at the glomerulus. The component of urinary noradrenaline derived from renal sympathetic nervous system is at most 30% and probably less, so urinary noradrenaline provides rather an index of "overall" sympathetic nervous system activity, than a measure of renal sympathetic nervous function (5,6). Experimental studies have, however, also provided evidence of a contribution of active secretion of, especially, the metabolites by the renal tubules (5,6).

Adrenaline is excreted in the urine approximately in proportion to its glomerular filtration rate, although possibly tubular secretion contributes to some extend (4).
The major urinary metabolites of noradrenaline and adrenaline are VMA and MHPG, which were firstly described approximately 30 years ago (7,8). In the tissue and body fluids also DHPG and DHMA, and their respective O-methylated derivatives MHPG and VMA exist. The excretion rate of the final products of noradrenaline and adrenaline are shown in the Figure 1. The sum of these metabolites reflects total body production of noradrenaline, since only a very small portion of MHPG and VMA is derived of adrenaline. Clearly VMA and MHPG are the major metabolites. Our findings of the amounts excreted during the 24 hour collection period, are in complete agreement with these data of the literature (Table 2).

When analyzing our data, the first point to be discussed is the question how infused catecholamines are metabolized and what the possible differences are with endogenously released catecholamines. Much of the insight of the metabolic breakdown of catecholamines, is learned from studies, mainly done in the fifties and sixties, with radiolabelled catecholamines and the influence of certain drugs on these processes. When radiolabelled catecholamines are administrated

intravenously their distribution is determined largely by the distribution of blood (9). Adrenaline, which is almost exclusively synthesized by the adrenals, is released into the circulation unmetabolized. The metabolic fate of intravenously administrated adrenaline is therefore suspected to parallel that of endogenously formed and released adrenaline (9). Besides being excreted in the urine unmetabolized, a part of administrated adrenaline will be O-methylated to M, which is either excreted after conjugation or further metabolized (deamination through MAO) to MHPG and VMA. The increase in adrenaline and M excretion in the adrenaline study as compared to the dextrose study was 0.13 μmoles (0.17 - 0.04) of adrenaline and 0.51 μmoles (1.13 - 0.62) of M, respectively 5.6% and 22% of the 2.31 μmoles of infused adrenaline. The relative contribution of the other metabolites cannot be computed from these data, probably because infusion of an amount of adrenaline as in this study, only produces marginal and insignificant changes in the large amounts excreted of VMA and MHPG. Studies in the rat using radiolabelled catecholamines and MAO and COMT blockade have demonstrated that O-methylation is the major metabolic route of infused adrenaline. However, subsequently M is extensively deaminated (10).

Metabolism of endogenous noradrenaline is more complex and differs from that of exogenously administrated noradrenaline. Endogenous noradrenaline is synthesized and stored in the sympathetic nerves throughout the body and in the central nervous system. It may be metabolized before reaching the circulation. When ^{3}H-noradrenaline is infused, part of the radioactivity is rapidly excreted, either as unchanged catecholamine or as NM, which, however, can be further metabolized to VMA and MHPG. This indicates that O-methylation is the major primary route for metabolic inactivation of circulating noradrenaline (11). Part of the ^{3}H-noradrenaline will be taken up into the tissues. Examination of the metabolites found in the urine during the interval after this rapid excretion is completed, provides insight into the fate of intraneuronally bound noradrenaline (11). The proportion of deaminated catecholamines excreted in this phase increases markedly, suggesting that deamination is the important route of metabolic inactivation of noradrenaline, which had been retained in the nerves. Based on the above mentioned observations and on various pharmacological studies, which are reviewed elsewhere (3), it is now clear that the metabolic fate of noradrenaline depends upon the site of its release and uptake. When endogenous noradrenaline reaches the circulation it is mainly O-methylated in extraneuronal tissues. When uptake in the neurons occurs, storage in the vesicles prevents metabolism by MAO. Uptake in extraneuronal sites results in metabolism by COMT and if COMT is blocked deamination by MAO. Noradrenaline leaking from the storage granules is

destroyed by MAO.

Metabolism of endogenous noradrenaline is extensive before it reaches the circulation. This can be illustrated by the fact that to sustain a given increment in blood pressure in the pitched rats, noradrenaline infused intravenously must attain a steady-state level of noradrenaline in plasma about 10-fold greater than that attending sympathetic stimulation, which produces a similar rise in blood pressure (12). This suggest that the major part of noradrenaline is removed from the neuroeffector junction and does not reach the circulation. To some extent this is a desipramine-sensitive process. Hoeldtke et al (4) found that in normal humans, the apparent noradrenaline secretion rate (determined by isotope dilution of tritiated noradrenaline) is about 22% of the noradrenaline production rate (determined from the total of the urinary metabolites of noradrenaline). Thus, the major fraction of noradrenaline must have be metabolized before reaching the circulation.

Experiments of nature frequently provide insight in the normal processes of life. Observations in patients with idiopathic orthostatic hypotension and multiple system atrophy (syndrome of Shy and Drager) have led to a better understanding of noradrenaline metabolism and have allowed to collect evidence in humans for the above mentioned routes of noradrenaline metabolism. Idiopathic orthostatic hypotension is a syndrome in which patients have a loss of peripheral sympathetic nerves and it was firstly described in 1925 (13). These patients have defective vasoconstrictor responses to intra-arterially administrated tyramine, which acts indirectly by releasing noradrenaline from sympathetic nerves (14). Multiple system atrophy is a distinct syndrome of orthostatic hypotension accompanying central neurological deficits, which appear to be a result of degenerative changes in several parts of the central nervous system, but largely spares the peripheral sympathetic nerves, although peripheral sympathetic nerves cannot be appropriately stimulated (15). Basal levels of noradrenaline are low in patients with idiopathic orthostatic hypotension and normal in multiple system atrophy. In both syndromes there is orthostatic hypotension and a lack of normal posturally induced increments in plasma noradrenaline (16).

Comparing the urinary excretions of metabolites in these patients have contributed to the understanding of noradrenaline metabolism (17). Excretion of total amounts of metabolites (MHPG, VMA and NM) were low in idiopathic orthostatic hypotension as compared to normals and patients with multiple system atrophy. In multiple system atrophy patients VMA was normal and MHPG and NM were slightly decreased. So this confirms the inability of idiopathic orthostatic hypotension patients to synthesise and release noradrenaline. Patients with multiple system atrophy have almost normal or slightly decreased amounts of noradrenaline

metabolites in urine. Since the deficit in these patients appears to be the inability to appropriately release noradrenaline, this results in a proportionately greater decrease in NM as compared to VMA and MHPG. This disproportionate decrease in NM in patients with multiple system atrophy support the observations done in experimental animals, which are briefly outlined above, showing that intraneuronally metabolized noradrenaline is mainly deaminated, whereas after its release from the nerve endings into the tissue or reaching the circulation, noradrenaline is mainly O-methylated to form NM.

Our data demonstrate a significant increase in excretion in noradrenaline and NM during the infusion period of noradrenaline and of NM in the postinfusion period. The increase in noradrenaline and NM excretion in the total infusion period was only 0.47 μmoles ([0.45-0.24] + [1.23-0.97]), which is approximately 9% of the noradrenaline infused. We found no differences in excretion rates of VMA and MHPG, but small alterations in the amounts excreted may be unnoticeable in this study.

These data provide some valuable information on the supposed mechanism of the pressor response observed in the postinfusion period of adrenaline. Since NM is formed primarily from released noradrenaline, its proportion of the total amount of noradrenaline metabolites excreted, is sensitive to sympathetic neuronal activity (3). In the postinfusion urine of the adrenaline study a significant increase in NM excretion as compared to dextrose was found (Table 1), also NM excretion during the total collection period was higher (Table 2). These results might be taken as an indication for the existence of increased sympathetic nervous system activity in this postinfusion period, which is hypothesized to be the cause of the observed increase in blood pressure in this period. However, no relation was found between the mean differences in mean blood pressure during the postinfusion period of the adrenaline study as compared to corresponding values of the dextrose study and the mean of the increases in NM excretion during the adrenaline study as compared to the dextrose study ($r=-0.06$, $n=10$, $p=0.87$).

The results of the measurements of urinary excretion rates of noradrenaline and adrenaline and their metabolites presented in this chapter, allow the following conclusions: 1. Adrenaline and noradrenaline excreted in the urine are the most sensitive indicators of changes in circulating adrenaline and noradrenaline during their respective infusions. 2. Changes in circulating plasma adrenaline and noradrenaline are not readily reflected in changes in urinary VMA and MHPG, probably because these changes are quantitatively unimportant as compared to other metabolic origins of VMA and MHPG. 3. Changes in NM excretion rate can be due not only to changes in circulating noradrenaline, but also indirectly to those

in circulating adrenaline. 5. The increased NM excretion after the adrenaline infusion might be taken as an indication that the hypothesized mechanism of the observed pressor effect in this period as discussed in chapter 3, is indeed true.

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Chapter 6

METABOLIC AND HORMONAL EFFECTS OF INFUSED NORADRENALINE AND ADRENALINE IN NORMAL MAN.

Based on: PJ Blankestijn, AJ Man in 't Veld, JHM Tulen, AH van den Meiracker, FHM Derkx, F Boomsma, P Moleman, SWJ Lamberts, MADH Schalekamp. Cardiovascular, hormonal and metabolic effects of a 6 hours infusion of adrenaline and noradrenaline in normal man. In preparation.

INTRODUCTION

It was probably Claude Bernard who first described a metabolic effect mediated by catecholamine, although it could not have been recognized as such at that time. He observed a transitory outpouring of sugar in the urine after he punctured the forth ventricle of rabbits. It is now clear that "puncture hyperglycaemia" is the result of release of adrenaline from the adrenals in response to sympathetic activation (1). The precise roles of the catecholamines in metabolic (patho) physiologic processes have only begun to emerge in more recent years. It is now appreciated that catecholamines play an important role in the regulation of carbohydrate, lipid, ketone and protein metabolism, the release of several hormones and the regulation of the plasma concentrations of certain electrolytes as well as thermogenesis (2). It is also clear that their role is changed in certain disease states such as in insulin-dependent diabetes mellitus (3). Much of the insight in the role of catecholamines has been gained by infusion experiments with relatively high loads of catecholamines. Furthermore, the effects of adrenaline has been studied in much more detail than those of noradrenaline.

In this chapter we describe the results of experiments in which low doses of adrenaline and noradrenaline were infused for 6 hours in normal volunteers. The effects on carbohydrate metabolism, growth hormone, prolactin, cortisol, renin, aldosterone and potassium are reported.

SUBJECTS AND METHODS

See chapter 2.

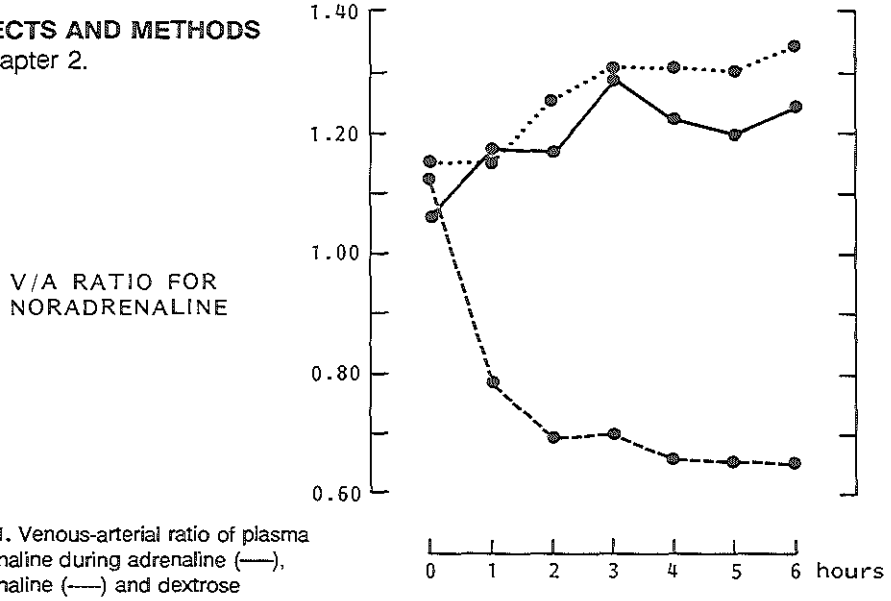

Figure 1. Venous-arterial ratio of plasma noradrenaline during adrenaline (——), noradrenaline (– – –) and dextrose (•••••) infusion.

RESULTS

Effects on adrenaline and noradrenaline

Baseline values of arterial and venous adrenaline and noradrenaline did not differ between the three study days. During the infusion of adrenaline venous adrenaline rose from 21 ± 4 to a mean of 230 ± 28 pg/ml and arterial adrenaline rose from 34 ± 6 to a mean of 387 ± 15 pg/ml (both $p < 0.001$). Both venous and arterial plasma levels during infusion of adrenaline were higher than during dextrose infusion (ANOVA, both $p<0.001$). Neither venous nor arterial plasma noradrenaline changed during adrenaline infusion.

During noradrenaline infusion venous noradrenaline rose from 207 ± 17 to a mean of 705 ± 58 pg/ml and arterial noradrenaline from 183 ± 15 pg/ml to a mean of 1025 ± 38 pg/ml. Both venous and arterial plasma concentrations were higher than during dextrose infusion (ANOVA, both $p<0.001$). Venous and arterial plasma adrenaline were little, although significantly, lower during noradrenaline infusion as compared to corresponding values during dextrose infusion (both $p<0.001$, ANOVA).

The mean (SEM) ratios of venous/arterial noradrenaline before administration of the infusions were not different on the three study occasions; before dextrose 1.17 (0.08), before noradrenaline 1.14 (0.05) and before adrenaline 1.06 (0.09). The mean ratio during adrenaline and dextrose infusion were not different, respectively 1.22 (0.08) and 1.29 (0.05). The mean ratio during noradrenaline was 0.69 (0.04) and was significantly lower than during dextrose (see Figure 1). This decrease was caused by the fact that arterial noradrenaline was more markedly increased (mean 560%) than venous noradrenaline (mean 341%).

Effects on glucose and insulin

Baseline glucose levels were not different between the three study occasions (Table 1, Figure 2). During adrenaline infusion glucose rose from 4.5 ± 0.2 mmol/l to 5.5 ± 0.2 mmol/l after one hour and was still increased with 5.5 ± 0.1 mmol/l after 6 hours ($p < 0.001$ as compared to corresponding values during dextrose infusion). Glucose remained unchanged during dextrose infusion; baseline 4.3 ± 0.1 mmol/l to 4.5 ± 0.1 after one hour and 4.4 ± 0.1 after 6 hours of infusion. During noradrenaline infusion glucose rose from 4.5 ± 0.2 mmol/l to 4.9 ± 0.2 mmol/l after one hour and 5.0 ± 0.1 mmol/l after 6 hours ($p < 0.002$ as compared to the dextrose study).

Basal insulin levels were identical during the three study days. During all 3 infusions insulin decreased during the day. Insulin levels during adrenaline infusion were higher than in the control study (ANOVA, $p < 0.02$). Insulin during noradrenaline tended to be higher, but this difference was not significant from the dextrose study.

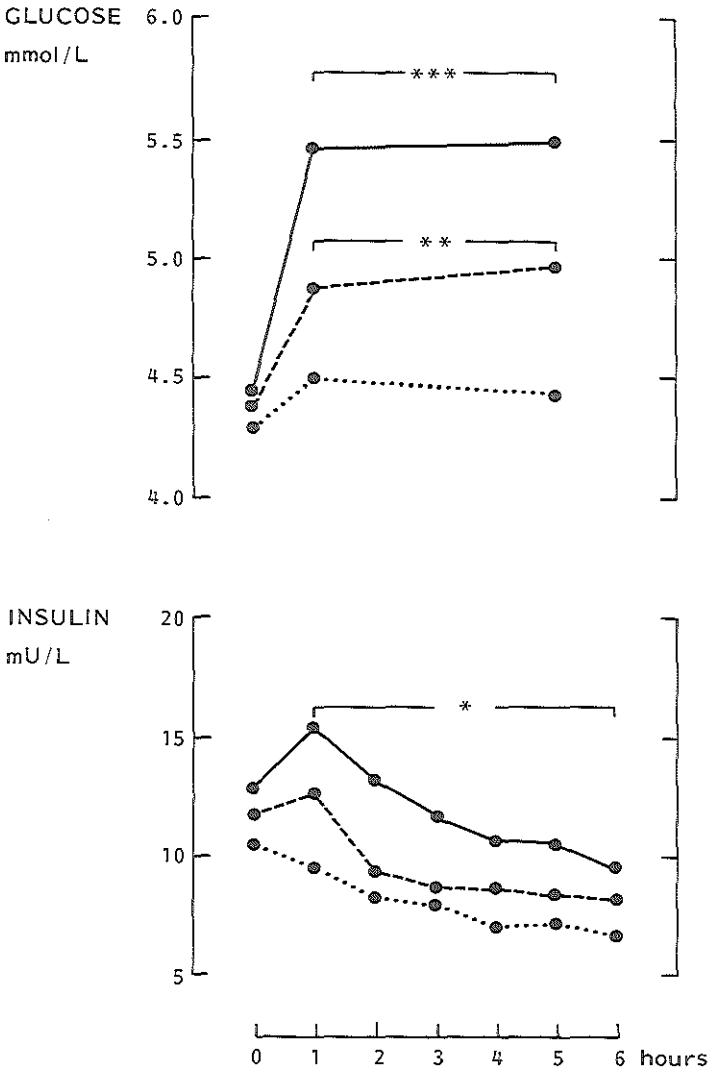

Figure 2. Plasma glucose and insulin during infusion of adrenaline (——), noradrenaline (– – –) and dextrose (•••••).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to dextrose infusion

TABLE 1 CONCENTRATIONS OF PLASMA INSULIN AND BLOOD GLUCOSE BEFORE AND DURING INFUSIONS OF NORADRENALINE AND ADRENALINE

	INSULIN (mU/L)			GLUCOSE (mmol/L)		
time (h)	dex	nor	adr	dex	nor	adr
0	10.5(1.3)	12.1(1.7)	13.4(1.5)	4.3(0.1)	4.5(0.2)	4.5(0.1)
1	9.4 (1.3)	12.6(1.9)	15.4(2.6)	4.5(0.2)	4.9(0.2)	5.5(0.2)
2	8.1 (1.0)	9.4 (1.5)	13.0(2.0)			
3	7.8 (1.1)	8.7 (1.1)	11.5(1.4)			
4	6.9 (0.9)	8.4 (1.0)	10.6(1.7)			
5	7.1 (0.6)	8.2 (0.1)	10.4(1.5)			
6	7.1 (0.6)	7.9 (0.8)	9.3 (1.5)	4.4(0.1)	4.9(0.2)	5.5(0.1)

Data are mean (SEM)
dex = dextrose infusion, nor = noradrenaline infusion, adr = adrenaline infusion

TABLE 2 PLASMA CONCENTRATIONS OF GROWTH HORMONE, PROLACTIN AND CORTISOL BEFORE, DURING AND AFTER INFUSIONS.

	GROWTH HORMONE (μg/L)			PROLACTIN (μg/L)			CORTISOL (nmol/L)		
time (h)	dex	nor	adr	dex	nor	adr	dex	nor	adr
0	1.4(0.6)	0.7(0.1)	0.6(0.1)	4.6(0.2)	4.4(0.2)	4.2(0.3)	425(31)	416(31)	437(53)
1	1.1(0.2)	2.0(0.9)	1.8(0.9)	4.3(0.2)	3.9(0.2)	4.1(0.1)	334(28)	341(38)	311(41)
2	1.5(0.5)	1.3(0.3)	1.1(0.3)	4.6(0.5)	3.9(0.1)	4.0(0.2)	330(46)	308(39)	317(30)
3	1.0(0.2)	0.7(0.1)	0.9(0.2)	4.4(0.4)	3.8(0.1)	4.1(0.2)	318(41)	281(28)	290(31)
4	1.9(0.7)	0.7(0.1)	0.7(0.1)	4.2(0.2)	4.0(0.2)	4.2(0.2)	326(25)	305(30)	313(33)
5	3.1(1.6)	0.8(0.2)	1.2(0.3)	4.6(0.1)	4.1(0.2)	4.8(0.3)	347(31)	343(33)	391(50)
6	3.0(0.9)	1.9(0.8)	1.9(0.7)	4.8(0.3)	4.5(0.2)	4.8(0.3)	356(34)	287(20)	282(36)
7	3.0(0.9)	1.5(0.4)	1.0(0.2)	5.0(0.4)	5.0(0.4)	5.4(0.7)	260(64)	268(32)	350(30)

Data are mean (SEM).
Dex = dextrose infusion, nor = noradrenaline infusion, adr = adrenaline infusion

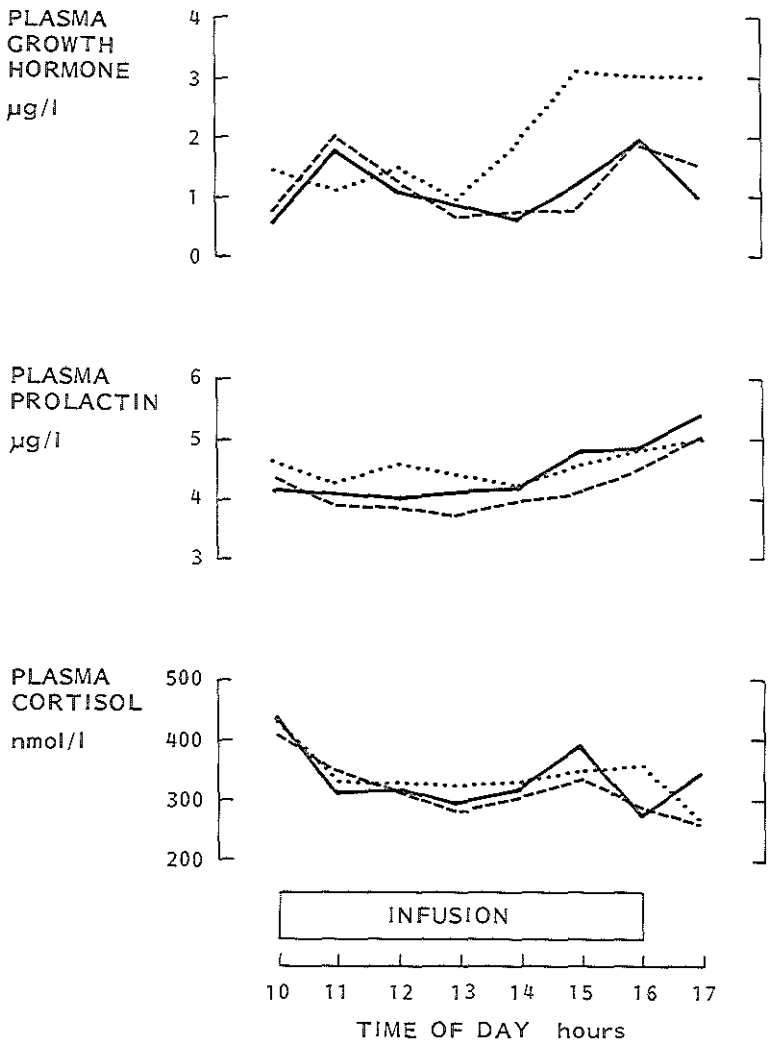

Figure 3. Effects of adrenaline (——), noradrenaline (– – –) and dextrose (•••••) on plasma concentrations of growth hormone, prolactin and cortisol.

Effects on cortisol, growth hormone and prolactin

Basal plasma concentrations of cortisol, growth hormone and prolactin during the three study occasions did not differ. The normal fall in cortisol between 9.00 h and 17.00 h ($p < 0.01$, t-test) was seen on each of the infusion days. There were no differences in plasma cortisol, growth hormone or prolactin during the three infusion experiments (Table 2, Figure 3).

Effect on potassium

Baseline serum potassium levels were not different on the study days of adrenaline and dextrose infusion (Table 3). During the adrenaline infusion a small but significant decrease in potassium concentration as compared to corresponding values during dextrose infusion was detected after 6 hours of infusion; after one hour of adrenaline respectively dextrose infusion potassium concentrations were 3.95 ± 0.06 and 4.01 ± 0.07 mmol/l respectively (not different), and after 6 hours 3.80 ± 0.04 respectively 3.97 ± 0.07 mmol/l ($p=0.016$) (Table 3, Figure 4). Baseline values on the noradrenaline study occasion were significantly lower ($p=0.034$, t-test) than corresponding values of the dextrose study. During noradrenaline infusion plasma concentrations were not different from those in the dextrose study; after one hour 3.97 ± 0.07 and after 6 hours 3.91 ± 0.05 mmol/l. Also no significant differences existed between values after one hour of infusion as compared to baseline and after 6 hours of infusion as compared to baseline.

TABLE 3 PLASMA CONCENTRATIONS OF POTASSIUM BEFORE AND DURING INFUSIONS OF NORADRENALINE AND ADRENALINE

	POTASSIUM (mmol/L)		
time(h)	dex	nor	adr
0	3.97(0.09)	3.83(0.05)	3.94(0.06)
1	4.01(0.07)	3.97(0.07)	3.95(0.06)
6	3.97(0.07)	3.91(0.05)	3.80(0.04)

Data are means (SEM)
dex = dextrose, nor = noradrenaline, adr = adrenaline

Urinary excretion of potassium was measured during the infusions and in the postinfusion period. No differences in urinary potassium excretion between adrenaline or noradrenaline versus dextrose were detected (Table 4)

Table 4 URINARY POTASSIUM EXCRETION DURING AND AFTER INFUSION OF ADRENALINE, NORADRENALINE OR DEXTROSE

	ADRENALINE	NORADRENALINE	DEXTROSE
infusion period			
mmol	37 (6)	34 (3)	36 (4)
mmol/h	4.1 (0.6)	3.8 (0.4)	4.0 (0.5)
mmol/mmol creatinine	5.4 (0.7)	4.9 (0.5)	5.4 (0.7)
post-infusion period			
mmol	37 (5)	34 (5)	33 (4)
mmol/h	2.2 (0.3)	2.0 (0.3)	1.9 (0.3)
mmol/mmol creatinine	5.6 (1.5)	3.7 (0.5)	4.6 (0.6)
total period			
mmol	74 (9)	68 (7)	69 (8)
mmol/h	3.0 (0.4)	2.7 (0.3)	2.8 (0.3)
mmol/mmol creatinine	5.2 (0.7)	4.2 (0.3)	4.7 (0.5)

data are means (SEM)
infusion period = 8.30 - 17.30 h, postinfusion period = 17.30 - 8.30 h next morning,
total period = infusion period + postinfusion period
Differences between the adrenaline and noradrenaline condition versus dextrose were not significant.

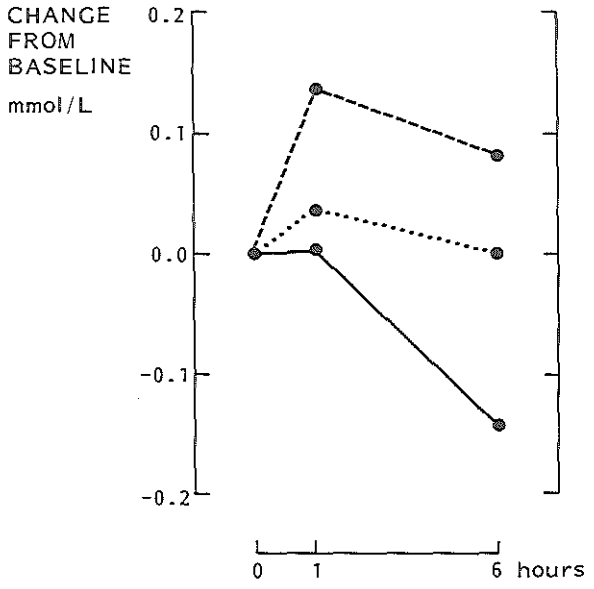

Figure 4. Change from baseline in plasma potassium during adrenaline (——), noradrenaline (- - - -) and dextrose (••••) infusion.

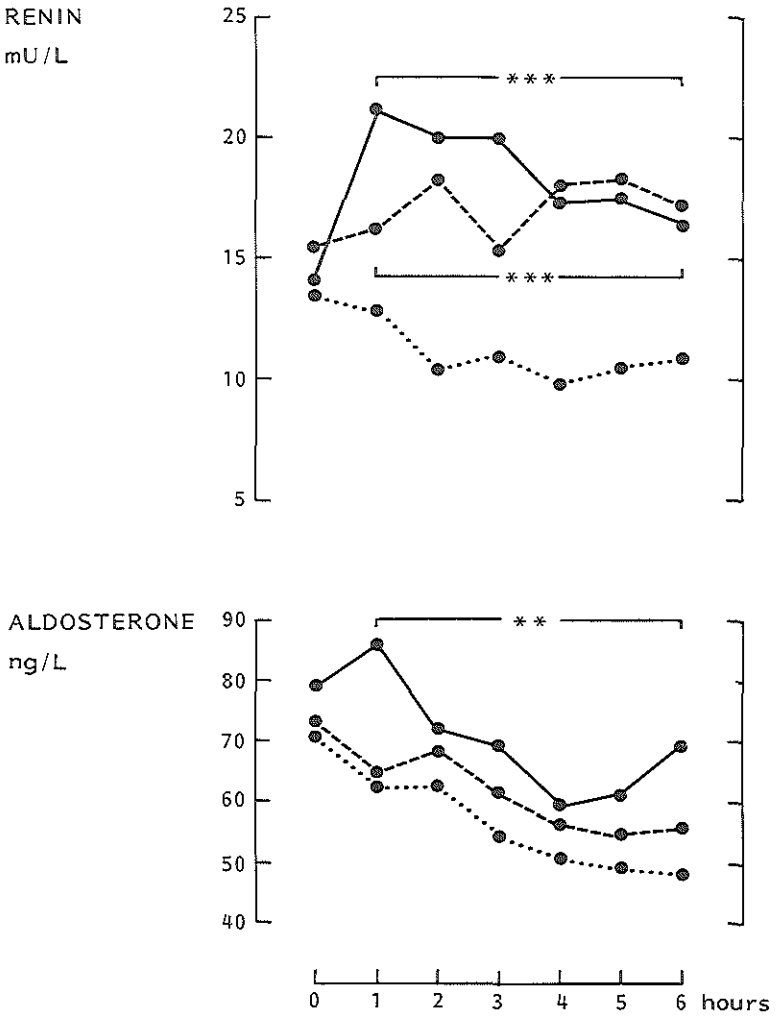

Figure 5. Plasma renin and aldosterone before and during infusion of adrenaline (——), noradrenaline (— —) and dextrose (•••••).
** $p < 0.01$, *** $p < 0.001$ as compared to dextrose infusion

Effects on renin and aldosterone

Baseline concentrations of renin were similar on the three study days. Renin values rose during the adrenaline and noradrenaline infusions and they were slightly, although significantly higher as compared to dextrose study; mean (range) renin concentrations during adrenaline and noradrenaline were 17.9 (14.1-24.8) and 17.3 (12.0-24.8) mU/L respectively, both significantly different (ANOVA, $p<0.001$) as compared to dextrose, 11.6 (8.4-15.6) mU/L.

Baseline plasma aldosterone levels were not different on the three study days; before adrenaline, noradrenaline and dextrose 80 ± 6, 72 ± 4 and 72 ± 5 ng/L respectively. During adrenaline infusion mean plasma aldosterone was slightly although significantly higher than during dextrose (70 ± 4 vs 52 ± 4 ng/L, ANOVA $p<0.01$). No difference existed between the mean values during noradrenaline and dextrose (61 ± 6 vs 52 ± 4 ng/L).

TABLE 5 PLASMA CONCENTRATIONS OF RENIN AND ALDOSTERONE BEFORE, DURING AND AFTER INFUSIONS.

	RENIN (mU/L)			ALDOSTERONE (ng/L)		
time(h)	dex	nor	adr	dex	nor	adr
0	13.4(12.0-14.9)	15.5(14.6-21.0)	14.4(11.4-18.1)	72(5)	72(4)	80(6)
1	12.8(10.5-15.6)	15.6(12.0-20.3)	21.5(18.9-24.4)	62(5)	62(6)	88(6)
2	10.7(8.7-13.2)	18.2(13.9-23.9)	19.9(16.6-23.9)	62(5)	69(8)	71(5)
3	11.7(10.0-13.6)	15.7(12.4-19.8)	20.1(16.3-24.8)	54(6)	63(8)	69(7)
4	9.9 (8.6-11.4)	18.4(13.9-24.3)	17.1(14.7-20.0)	51(5)	58(7)	60(4)
5	10.5(8.4-13.0)	18.7(14.1-24.8)	17.2(14.8-19.9)	50(5)	56(4)	62(6)
6	11.1(8.8-14.1)	17.1(13.2-22.2)	16.4(14.1-19.2)	46(5)	58(6)	70(8)
7	10.0(8.2-12.3)	19.7(14.9-26.1)	15.3(12.9-18.0)	51(6)	62(9)	75(8)

Data of aldosterone are mean (SEM) and of renin are geometric means (range).
Dex = dextrose infusion, nor = noradrenaline infusion, adr = adrenaline infusion

DISCUSSION

In this study we have demonstrated that moderately increased plasma catecholamines concentrations after infusion of adrenaline or noradrenaline elicit some metabolic effects besides the haemodynamic effects as described in chapter 3. The plasma concentrations of catecholamines achieved by the infusions in this study are well within the physiological range. Venous concentrations of noradrenaline of approximately 700 pg/ml can be seen during quiet standing and mild physical exercise (4). Plasma concentrations of adrenaline of approximately 200 - 300 pg/ml have been reported during surgery, during moderate to severe physical exercise and during public speaking (4,5). Plasma concentrations achieved during the infusions in this study were comparable with those in studies in which approximately the same amounts were infused (6,7,8). The ratio venous/arterial noradrenaline, as a crude measure for net local noradrenaline production was not different during adrenaline and dextrose infusion, whereas it was suppressed during noradrenaline infusion. This seemed to be a result of a more marked increase in arterial noradrenaline than in venous noradrenaline during noradrenaline infusion. Venous noradrenaline is mainly derived from local production (9). Whether the relatively less increased venous plasma concentration of noradrenaline as compared to the arterial plasma concentration is the result of activation of prejunctional α_2-adrenoceptors can not be determined from this study. Stimulation of these adrenoceptors leads to inhibition of noradrenaline release (10).

Effects on glucose and insulin

The primary controllers of glucose metabolism are insulin and glucagon released by the endocrine pancreas in response to neural, humoral and local factors. However, it has became apparent that the catecholamines and in particular adrenaline, have clear effects on glucose metabolism, i.e. they increase blood glucose levels. The mechanism of this hyperglycaemic effect is complex. Catecholamines alter glucose metabolism directly, by affecting liver and skeletal muscle metabolism and indirectly by an effect on the function of the endocrine pancreas. In this hyperglycaemic process both α- and ß-adrenoceptor mediated actions play a role. This study provides a further contribution to the understanding of the different parts of each of these processes in the hyperglycaemic effect of catecholamines.

In the early phase of the rise of blood glucose during adrenaline infusion an indirect effect on the endocrine pancreas seems to be important, because this rise is associated with an decrease in insulin concentrations. This effect is observed by some (7), but not by other investigators (11). However, within 30-40 minutes of the

adrenaline induced hyperglycaemia, a rise in plasma insulin levels occurs. The initial limitation of insulin secretion seems to be mediated by α_2-adrenoceptors in the mouse and the rat and probably also in man. It is an important indirect hyperglycaemic mechanism (7,12,13). This is evidenced for instance by the sharp increase in plasma insulin levels in response to elevated glucose concentrations, that follows discontinuation of adrenaline infusion (7). Another indication of the importance of this insulin suppressor mechanism is apparent when one considers that isoprenaline, a non-selective ß-adrenoceptor agonist devoid of α-adrenergic agonistic properties, produces minimal increments in plasma glucose, despite its prominent direct (ß-adrenergic) glycaemic actions (11,14). Despite this inhibitory effect of adrenaline on insulin release, we found slightly higher insulin levels during adrenaline infusion than during the control study. This finding is in accordance with several other studies (7,15,16) and is probably a response to the increase in blood glucose concentration. This small increase in insulin is a critical regulatory event to limit the glycaemic response to adrenaline, since this glycaemic response is increased substantially when insulin levels are kept "constant" experimentally with somatostatin, or when insulin secretion can not vary as in insulin dependent diabetes mellitus (16). The difference in insulin levels during adrenaline and noradrenaline infusion in our study can be explained by the difference in the magnitude of the rise in blood glucose. So the α-adrenoceptor mediated inhibition of insulin release is counterbalanced by the stimulation of insulin secretion by the rise in blood glucose.

In this study we did not measure glucagon, the other important pancreatic blood glucose regulating hormone. Increments in plasma glucagon during adrenaline infusion have been observed in some studies in normal humans but not in others (12,15,17). Of course it can be argued that since an increase in plasma glucose would be expected to suppress glucagon secretion, the absence of this decrease during adrenaline induced increments in blood glucose in fact implies stimulation of glucagon secretion (2). However, adrenaline induced increments in blood glucose clearly occur in the absence of glucagon release (17-19). Furthermore, the finding that the glycaemic response to adrenaline is increased substantially despite prevention of any increase in glucagon, when an increase in insulin secretion is prevented in normal humans (16), all suggest that glucagon is not a major factor in the hyperglycaemic response to adrenaline.

What remains to be discussed is the quantitatively different glycaemic responses to infused noradrenaline and adrenaline. The direct hyperglycaemic action of catecholamines include stimulation of hepatic glucose production and limitation of glucose utilization. The enhanced glucose production by the liver is a result of a transient rise in glycogenolysis and gluconeogenesis. Several studies have

indicated that hepatic glucose production have returned to basal levels after 60 to 120 minutes of adrenaline infusion (11,12). However, the maintenance of basal rates of glucose production during prolonged infusion of adrenaline in the presence of a degree of hyperglycaemia, that had completely suppressed glucose production when produced by an exogenous glucose infusion, indicates that prolonged infusion of adrenaline continues to have a stimulating effect on the hepatic glucose output. This stimulating effect is predominantly mediated through a β_2-adrenergic mechanism (18). Several studies (13,18) demonstrated that the effects of adrenaline on glucose production were not completely blocked by propranolol, which could be an indication for a small, although definite α-adrenoceptor mediated contribution in the adrenaline stimulated glucose production. Indeed it has been shown that the human livercell contains both β_2- and α-adrenergic receptors (20).

The limitation of glucose utilization is a sustained effect of adrenaline infusion (21). This effect is ß-adrenoceptor mediated. Indirect evidence suggests that the subtype of β_2-adrenoceptors is involved (22). So, the sustained rise of blood glucose during a 6 hours infusion of adrenaline as demonstrated in this study is an effect of decreased glucose utilization and to some extent to the absence of suppression of hepatic glucose production.

Another interesting finding of this study was the fact that blood glucose after 6 hours of noradrenaline infusion was still higher than during control study. As stated before it has been suggested that the sustained rise in glucose after prolonged adrenaline infusion is mainly the result of the limitation of glucose utilization, which is probably β_2-adrenoceptor mediated. Also the effect of adrenaline on hepatic glucose production appears to be predominantly β_2-adrenoceptor mediated. However, noradrenaline infusion for 2 hours also gives a transient rise in liver glucose production, which is much less than the one induced by adrenaline and which returns to basal levels after approximately 60 minutes of infusion (11). Our finding of the maintained increase in blood glucose after 6 hours of noradrenaline infusion supports 2 conclusions: 1, Since noradrenaline has no effect on glucose utilization (11), the fact that glucose production during sustained infusion of noradrenaline returns to basal and not below that level indicates that also noradrenaline has a prolonged effect on hepatic glucose output. 2, Besides β_2-adrenoceptor mediated mechanisms of stimulation of liver glucose output, also an α-adrenoceptor mediated effect is operational.

In conclusion, the integrated mechanisms of the hyperglycaemic effect of catecholamines, which are outlined in Table 6, differ considerably. This is a consequence of the different affinities of the catecholamines to the adrenergic receptor subtypes, which are involved in regulating the glucose homeostasis. The results confirm the fact that adrenaline in contrast to noradrenaline is man's

TABLE 6 EFFECTS OF DIFFERENT CATECHOLAMINES ON GLUCOSE METABOLISM

	GLUCOSE			INSULIN
	production	utilization	concentration	concentration
Adrenaline (α, $\beta_2 > \beta_1$)	↑↑↑↑	0 - ↑	↑↑↑	↑
Noradrenaline (α, $\beta_1 > \beta_2$)	↑↑	0	↑↑	0
Isoprenaline (β_1 + β_2)	↑	↑↑	≈ 0	↑↑↑

endogenous β_2-adrenoceptor stimulant. Whether or not the current topic of insuline-resistence in essential hypertension (23) can be (partly) explained by the adrenaline-hypertension hypothesis, leading to intermittently elevated insulin levels, merits further investigation

Effect on growth hormone, prolactin and cortisol

The anterior pituitary lacks direct neuronal innervation, however, its hormonal secretions are controlled precisely by the overlying brain. Regulation of the anterior pituitary hormone secretion is achieved with specific hypothalamic releasing hormones and for some pituitary hormones by feedback mechanisms. These releasing hormones are, among others, somatostatin, growth hormone releasing hormone and corticotropin releasing factor. The control of pituitary function by the central nervous system is provided by neuronal afferents, which synapse on hypothalamic releasing factor cells. The releasing factor cells have been termed "neuro-endocrine transmitters", since they translate a neuronal (neurotransmitter) input into a hormonal output. Neurons may either stimulate or inhibit these cells through interactions in the median eminence or at other brain sites. Several neurotransmitter substances such as noradrenaline, adrenaline, dopamine and 5-hydroxytryptamine have been investigated for their role in the regulation of the anterior pituitary. Circulating adrenaline and noradrenaline are hydrophylic substances and therefor they do not cross the blood-brain barrier (24,25), but they do reach the pituitary gland and to a certain extend the median eminence.

Growth hormone

The release of growth hormone from the pituitary is regulated by inhibitory (somatostatin) and excitatory (such as growth hormone releasing hormone) hypothalamic hormones. Extensive animal research (for review see reference 26) has provided evidence that the release of catecholamines from nerve terminals in the hypothalamus modifies growth hormone release. It is likely, that α-

adrenoceptors mediate the stimulating effect on growth hormone secretion induced by noradrenaline. Noradrenaline injected into the hypothalamus of conscious baboons stimulates growth hormone secretion and phentolamine, an α-adrenoceptor antagonist, reduces it. The ß-adrenoceptor involved in the regulation of growth hormone release is probably of the $ß_2$-subtype. Salbutamol, a selective $ß_2$-adrenergic receptor agonist, lowers growth hormone levels. Conversely ß-adrenergic receptor antagonism by propranolol or the selective $ß_2$-adrenoceptor blocker ICI-118551, markedly elevated plasma growth hormone levels. Practolol, a ß,-selective adrenoceptor antagonist, does not alter basal growth hormone levels (27). Elevation of growth hormone by ß-adrenoceptor antagonist may be due to inhibition of somatostatin release, since isoprenaline applied to rat brain slices stimulated the release of somatostatin.

Also in man adrenergic receptors mediates modulation of growth hormone secretion. L-dopa, the precursor of dopamine and noradrenaline, stimulates growth hormone release in man, an effect, which is blocked by phentholamine (28). Systemic administration of α-adrenoceptor antagonists inhibits growth hormone secretion (29), whereas the α-adrenoceptor agonist clonidine stimulates growth hormone release (30). Since clonidine is a relative selective $α_2$-adrenoceptor agonist, this modulation of growth hormone secretion seems to be $α_2$-adrenoceptor mediated. Supporting this view is the finding that yohimbine, a selective $α_2$-adrenoceptor blocker, blunted the rise of growth hormone induced by insulin-induced hypoglycaemia (31). *In vitro* experiments have disclosed that clonidine is able to release growth hormone releasing hormone from the perifused hypothalamus (32). Therefore the growth hormone releasing effect of clonidine probably acts via increased endogenous growth hormone releasing hormone release. Recently it was suggested that clonidine acts by inhibiting somatostatin release (33). The ability of $α_2$-adrenoceptor agonists to trigger growth hormone synthesis may have clinical impact in certain growth disorders (defective release but intact synthesis of growth hormone releasing hormone). Indeed, it has been shown that clonidine is able to accelerate growth velocity in some children with constitutional growth delay (34).

Activation of ß-adrenergic receptors exerts an inhibitory action on growth hormone release. An increase in circulating growth hormone levels was present during combined administration of propranolol and adrenaline (35), whereas inhibition occurred by concomitant infusion of phentholamine (36). An explanation which has been suggested for these findings with adrenaline in the presence of propranolol was that these were the results of an unopposed α-effect. Also a marked enhancing effect of propranolol on the growth hormone releasing hormone induced growth hormone rise was demonstrated in children (37), whereas it is

ineffective in altering basal levels per se.

The above mentioned results indicate that both α- and ß-adrenoceptor mediated mechanisms are involved in the regulation of growth hormone release. The effect on the growth hormone release seems to be a result of alteration of the release of somatostatin and/or growth hormone releasing hormone from the hypothalamus to the hypophysis.

In this study, in which adrenaline and noradrenaline were infused intravenously leading to high physiological plasma concentrations no effect on growth hormone levels were detected. This is in accordance with other studies in which adrenaline (7) or noradrenaline (6,38) were infused intravenously, generally however in much higher doses. In none of these studies an effect on growth hormone levels was reported. These results, however, do not rule out that circulating catecholamines even in concentrations achieved in this study can have an inhibitory or stimulating effect on growth hormone secretion. Further research, for instance measurements of growth hormone response to stimuli during catecholamine infusion leading to physiological plasma concentrations is needed to clarify in more detail the role of catecholamines on growth hormone secretion.

Cortisol

Studies of the effects of adrenaline and noradrenaline on ACTH and cortisol release *in vitro* and *in vivo* have produced conflicting results in animals (35,36). Some studies describe the absence of effects of adrenaline and noradrenaline, whereas others show a direct stimulating effect on ACTH secretion. Probably the discrepancies are explicable on the basis of interspecies and methodological differences (39,40).

In man, intravenous infusion of the lipid soluble α_1-adrenoceptor agonist methoxamine, which passes the blood-brain barrier, stimulates ACTH secretion and this effect is abolished by concomitant administration of the α_1-adrenoceptor antagonist thymoxamine. Hydrophylic noradrenaline in equipotent doses with respect to cardiovascular responses, did not produce this effect (41). Further studies with α_2- and ß-adrenoceptor agonists and antagonists demonstrated that the additional (i.e. α_2- and ß-agonistic) properties of noradrenaline do not account for this different response to methoxamine.

In man, intravenous infusion of adrenaline which elevates plasma adrenaline concentrations to 600-700 pg/ml (41) or to about 350 pg/ml (42) had no effect on basal levels of cortisol or on the stimulating effect of corticotrophin releasing factor. Also, several studies in man indicate that circulating noradrenaline and adrenaline, administrated exogenously leading to high physiological plasma concentrations, do not alter plasma ACTH and cortisol (6,7,44,45). Clutter et al (7) infused adrenaline

in normal man resulting in plasma levels of around 1000 pg/ml and Silverberg et al (6) infused noradrenaline (plasma concentrations approximately 2100 pg/ml). In both studies no changes in plasma cortisol were detected. The results of the last mentioned study in which systolic arterial pressure increased from 107 to 130 mmHg, seems to be in contrast with 2 other studies, in which very high doses of noradrenaline were infused in order to increase systolic arterial pressure by 24% (41) and 30% (45). In the latter two studies cortisol (41,45) and ACTH (45) decreased. It was argued that high doses of noradrenaline might be able to inhibit vasopressin release and vasopressin may act as or augment the actions of corticotrophin releasing factor (45).

Our data show that infusions of neither noradrenaline nor adrenaline leading to plasma concentrations in physiological range, exert an effect on plasma cortisol. These results are in accordance with the above mentioned results in the literature.

Prolactin

Prolactin is unique in that its secretion is tonically inhibited by the hypothalamus, while other adrenohypophysial hormones are mainly regulated by releasing hormones. This inhibitory action is mediated by prolactin inhibiting factors, of which dopamine seems to be the most important one (46). Experimental data also suggest the existence of prolactin releasing factors. Serotonin (47), TRH (48,49) and several other peptides have been proposed as prolactin releasing factors. The stimulation of prolactin release by various forms of stress (hypoglycaemia, exercise, surgery) is considered to arise from stimulation of prolactin releasing factors rather than removal of dopaminergic inhibition (48,49). Psychological stress is not accompanied by increases in prolactin, except for "stresses" directly connected with reproduction (50).

In animal studies it remains controversial whether there is a role for circulating noradrenaline and adrenaline in the control of prolactin release, with reports of a stimulating or an inhibitory effect or no effect at all on prolactin secretion (46). This, again, might be caused by interspecies differences.

In man, it seems unlikely that circulating adrenaline and noradrenaline are important in stimulating prolactin release. Woolf et al (51) studied the prolactin response to insulin induced hypoglycaemia in patients with absent adrenaline (bilateral adrenalectomy) or impaired sympathetic nervous function (complete C5-6 cervical cord transection). The adrenalectomized patients had normal prolactin responses while the patients with spinal cord transection had exaggerated responses. It was concluded that neither adrenaline nor noradrenaline were important in stimulating prolactin release, although an inhibitory action of noradrenaline cannot be ruled out.

Our data demonstrate that circulating adrenaline and noradrenaline in physiological concentrations do not affect plasma prolactin levels. So, whereas it is established that central catecholaminergic (mainly dopaminergic) systems participate in the regulation of prolactin release, there is not much evidence to suggest that circulating adrenaline and noradrenaline have any contribution in this process.

Effect on potassium

More than 50 years ago D'Silva (52) showed that adrenaline might play a role in potassium homeostasis. He found that an intravenous infusion of adrenaline into anaethetised cats resulted in an initial transient rise in plasma potassium followed by a prolonged decline. Only the last decade, the clinical significance of the regulatory function of catecholamines, in particular adrenaline, in the potassium homeostasis has been recognized (53-55).

It is now appreciated that renal excretion is the dominant factor in determining the potassium balance (for review reference 53), however, cellular uptake of potassium is of great importance in the maintenance of the plasma potassium within narrow limits. There is now much evidence that catecholamines have considerable influence on extrarenal potassium regulation (54,55).

Intravenous administration of adrenaline leads to an initial increase followed by a decrease in potassium. The initial rise is the result of potassium released by the liver (56,57) and the subsequent decrease in potassium reflects an accelerated uptake in potassium, primarily in the skeletal muscles, but probably also in the heart and liver (54,58) The hyperkalaemic response is inhibited by α-adrenoceptor blockade (59). The decline in potassium has been found to be independent of insulin, aldosterone and renal excretion of potassium (54). In previous studies from our group the effects on plasma potassium of graded infusions of ß-adrenoceptor agonists isoprenaline (non-selective), prenalterol ($ß_1$-adrenoceptor selective) and salbutamol ($ß_2$-adrenoceptor selective) were studied (60). All agonists induced a decrease in potassium but the magnitude of the hypokalaemia-inducing effect was in the order: salbutamol > isoprenaline > prenalterol. These results confirmed other reports indicating that this effect is mediated by $ß_2$- rather than $ß_1$ adrenoceptors (61). This conclusion was further substantiated by the observation that propranolol, but not atenolol, was able to antagonize the hypokalaemic effect of isoprenaline, when the latter was given in doses high enough to overcome the blockade of the heart rate response. In the study of Brown et al (61) it was demonstrated that an adrenaline infusion that produced plasma levels of approximately 1700 pg/ml resulted in a significant decrease in potassium, an effect

that could be prevented by prior administration of the $ß_2$-adrenoceptor antagonist ICI-118551. So the hypokalaemic effect appeared indeed to be $ß_2$-adrenoceptor mediated.

In the current study we found that only moderately increased plasma adrenaline concentrations already induce a small although significant decrease in plasma potassium. These alterations occurred without changes in the urinary excretion rate of potassium. These observations are of clinical importance since adrenaline levels which have been observed in patients during various forms of stress, such as myocardial infarction, appear to be able to reduce plasma potassium. In patients with myocardial infarction this hypokalaemic effect can predispose these patients to cardiac arrhythmias, particularly when they are more susceptible because of concomitant therapy with digitalis or because of diuretic induced potassium depletion (62). Hypokalaemia is clearly associated with an increased incidence and severity of arrhythmias.

Another area where this $ß_2$-adrenoceptor mediated effect is suspected to be of clinical relevance is in patients who used $ß_2$-agonists because of asthma. Workers from Utrecht showed that inhalation of fenoterol, a $ß_2$-agonist, in doses frequently used by asthma patients, was able to induce marked a decrease in plasma potassium (63). In asthma patients this effect might even be more pronounced when the phosphodiesterase inhibitor theofylline is used concomitantly. The combination of aminophylline and the $ß_2$-adrenoceptor agonist hexopreline, both given intravenously, has been shown to have a greater effect on plasma potassium than either drug given alone (64). So it was suggested (63) that the hypokalaemia induced by inhalation therapy increases the risk of cardiac dysrhythmias, which might account for a proportion of the sudden deaths seen in asthma patients.

While $ß_2$-adrenergic stimulation enhances extrarenal potassium uptake, there are indications that α-adrenoceptor stimulation has the opposite effect. In man, Williams et al (65) showed that the rise in potassium after a potassium load was augmented by phenylephrine. This effect could be blocked by phentholamine. Urinary potassium excretion did not change during these studies. In an other set of experiments (66) the same group studied the effects of α- and ß-adrenoceptor blockade on exercise induced hyperkalaemia. Plasma noradrenaline and adrenaline increased to around 1600 and 340 pg/ml respectively during exercise. They showed that propranolol increased the exercise-induced rise in potassium, whereas phentholamine inhibited the response. The effect appeared to be independent of venous pH, bicarbonate, glucose and insulin concentrations, whereas also potassium excretion was not altered. So, this α-adrenoceptor mediated mechanism is likely to inhibit cellular uptake of potassium. Although, in our study the plasma potassium during noradrenaline tended to be higher than during control, the

differences were not statistically significant.

All these results indicate that catecholamines exert an effect on potassium homeostasis, which can be of great clinical relevance. Our study shows that only mildly increased plasma adrenaline already has a lowering effect on plasma potassium.

Effects on renin and aldosterone

The kidney is by far most, if not the only, source of renin in plasma, since in bilateral nephrectomized patients plasma renin is very low or below the detection limit (67,68). Extensive research has demonstrated that adrenergic receptor-mediated processes are important in controlling renin release (69). The release of renin is governed by both intra- and extrarenal mechanisms. Intrarenal baroreceptors are influenced by "stretch" changes in the afferent arteriole: increased perfusion pressure increases stretch which somehow reduces renin secretion. Reduced "stretch" increases renin release. The second intrarenal mechanism is the "macula densa" chemoreceptor mechanism. The macula densa is stimulated by a decrease in NaCl delivery in the distal tubule, which then leads to an increase in renin release. The extrarenal mechanisms comprise the feedback by plasma angiotensin II and the renal nerves and plasma catecholamines.

β_1-Adrenoceptor agonists have been shown to induce renin release from the juxtaglomerular cells, in which granulae renin is produced, stored and excreted (69). Also renal nerve stimulation was demonstrated to increase renin secretion (70). In these earlier studies there were gross (renal) haemodynamic changes, which might have accounted for a stimulation of renin release. However, later Osborn et al (71) showed that low frequency renal nerve stimulation, which did not affect renal haemodynamics or sodium excretion, still induced a considerable increase in renin secretion.

Also in man plasma renin is increased during infusion of β_1-adrenoceptor agonists. There are, however, also reports which suggest a β_2-adrenoceptor mediated enhancement of renin release. This is probably an indirect effect. Kopp and DiBona infused a low dose of adrenaline bilaterally in dogs in an innervated and a denervated kidney and measured renin secretion rate (72). They showed that the enhancement of the renin secretion produced by adrenaline was much more pronounced on the innervated site. Both metoprolol (β_1-adrenoceptor selective antagonist) and ICI-118551 (β_2-adrenoceptor selective antagonist) abolished the increased renin secretion induced by adrenaline. These results support the hypothesis that adrenaline enhances renin secretion indirectly through activation of prejunctional β_2-adrenoceptors, which results in enhancement of noradrenaline

release which on its turn stimulates postjunctional β_1-adrenoceptors on the juxtaglomerular cells (72). *In vitro* experiments indeed have indicated that activation of prejunctional β_2-adrenoceptors in the kidney facilitate noradrenaline release (69) and it was also demonstrated that renal nerve stimulation results in an increase of noradrenaline outflow from the kidney (74).

Our group has provided data indicating that this process of renin secretion enhancement is also operational in humans (75). It was shown that prenalterol (β_1-adrenoceptor selective agonist), salbutamol (β_2-adrenoceptor selective agonist) and isoprenaline (non-selective agonist) all induced a rise in plasma renin. The β_2-adrenoceptor-agonist induced rise in renin was correlated with the rise in plasma noradrenaline. During treatment with atenolol (β_1-selective blockade), ICI-118551 (β_2-adrenoceptor selective blockade) and propranolol (non-selective) baseline plasma renin was decreased in all three cases. The antagonistic effect of atenolol and propranolol on the isoprenaline induced rise of renin were comparable, whereas ICI-118551 had only a minor inhibiting effect. These results are compatible with the hypothesis that the β_2-adrenoceptor agonist induced rise of plasma renin is a result of activation of prejunctional β_2-adrenoceptors, subsequent noradrenaline release, which then stimulates postjunctional β_1-adrenoceptor mediated renin release.

There are also indications that α-adrenoceptor mediated mechanisms are involved in the regulation of renin release (76,77). De Leeuw et al have studied the contribution of renal α-adrenoceptors (78-80). In a series of experiments they showed in untreated hypertensives that intrarenal administration of doxazosin (α_1-adrenoceptor antagonist) slightly, but phentholamine (non-selective α-adrenoceptor antagonist) markedly enhanced renin secretion during isometric exercise (78). In a second study they showed that intrarenal infusion of yohimbine (α_2 antagonist) was more effective in raising renin secretion than doxazosin (79). In a third set of experiments the same group evaluated whether α_2-adrenoceptors are able to modify the renin release on its own or whether it required the presence of functioning β_1-adrenoceptors (80). One could argue that the α_2-adrenoceptor mediated effect shown in the previous studies is a consequence of modulation of prejunctional α_2-adrenoceptors. Stimulation of these adrenoceptors leads to a reduction of noradrenaline release and subsequent decreases in activity of postjunctional β_1-adrenoceptors. Intrarenal infusion of yohimbine markedly increased renin release, however in the presence of atenolol this response was blunted. So experimental work (76,77) and studies in humans indicate that α_2- rather than α_1-adrenoceptors might be involved in inhibition of renin secretion and that, at least in part, these adrenoceptors are located prejunctionally.

In this study we have demonstrated that both adrenaline and noradrenaline had a

very small but significant effect on plasma renin concentration. Despite the fact that we did not find an increase in plasma noradrenaline during adrenaline infusion, (which then would have been an indication for the above mentioned "indirect" β_2-adrenoceptor mediated effect) renin was higher during adrenaline than during dextrose. However, blood pressure was lower during adrenaline than during dextrose and this might also have been responsible for the stimulation of renin secretion.

As a consequence of the activation of renin release also plasma aldosterone concentrations were little, but significantly, increased during adrenaline as compared to dextrose. Also during noradrenaline aldosterone tended to be higher, but this was not significant. The question, whether also a direct stimulating effect on aldosterone secretion through ß-adrenoceptors, as was demonstrated *in vitro* (81), is also present *in vivo*, can not be answered by our data.

So extensive research has established that the renal autonomic nervous system and circulating adrenaline and noradrenaline can have considerable effect on renin release. Our results support this conclusion for the effects of these plasma catecholamines in the high physiological range.

Conclusion

In the century after Claude Bernard it has been established that the circulating hormone adrenaline but also the neurotransmitter noradrenaline have considerable impact on various parts of metabolism in man. Our results confirm and extend this knowledge and they clearly demonstrate that adrenaline and noradrenaline in physiological plasma concentrations can elicit multiple metabolic effects.

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Chapter 7

EFFECTS OF "STRESS" LEVELS OF NORADRENALINE AND ADRENALINE ON URINARY SODIUM OUTPUT IN NORMAL SODIUM REPLETE MAN.

Based on: PJ Blankestijn, AJ Man in 't Veld, JH Tulen, F Boomsma, FHM Derkx, P Moleman, MADH Schalekamp. Effects of "stress" levels of noradrenaline and adrenaline on urinary sodium output in normal sodium replete man. Submitted.

INTRODUCTION

Evidence suggesting that the renal sympathetic nervous system may influence urinary sodium and water excretion was first presented by Claude Bernard more than 100 years ago (1). He observed an increase in urine flow following section of the splanchnic nerve in the anaesthetized animal. Since then a great number of studies have established that this system can contribute to the regulation of sodium excretion (for reviews 2-5). Renal denervation has provided an approach to examen the role of renal nerves. Studies in animals with pharmacologically and anatomically denervated kidneys and pharmacological interventions in man, have addressed this issue. Another approach to examine the role of renal nerves in sodium homeostasis, has been the use of renal nerve stimulation. More than 30 years ago is was noted that direct renal nerve stimulation results in an ipsilateral decrease in urinary sodium excretion (6). In those studies marked decreases in renal blood flow and glomerular filtration rate accompanied this antinatriuresis. More recent studies have demonstrated that low level electrical stimulation reduces sodium excretion without affecting glomerular filtration rate or renal blood flow (7). The primary site in the nephron influenced by catecholamines appears to be the proximal tubule.

Studies on the effects of circulating catecholamines in man have produced conflicting results, probably due to methodological differences such as variation in the doses used and the duration of the experiments. In this study we examined the effects of a 6 hours lasting intravenous infusions of noradrenaline and adrenaline resulting in plasma concentrations in the high physiological range, on renal sodium excretion for 24 hours.

SUBJECTS AND METHODS

See chapter 2.

RESULTS

Hormones

The mean baseline concentrations of plasma adrenaline and noradrenaline were similar under the three different conditions. During the infusion of adrenaline venous adrenaline rose from 21 ± 4 to a mean of 230 ± 28 pg/ml and during noradrenaline infusion venous noradrenaline rose from 207 ± 17 to a mean of 705 ± 58 pg/ml (Table 1). Plasma adrenaline during adrenaline and plasma noradrenaline during noradrenaline infusion were significantly higher than corresponding values during dextrose (ANOVA, both $p<0.001$). Less than 5 minutes after the infusions were stopped catecholamines concentrations had returned to baseline values (for a more detailed description see Chapter 3).

TABLE 1 MEAN VENOUS CONCENTRATIONS OF ADRENALINE, NORADRENALINE, RENIN AND ALDOSTERONE BEFORE AND DURING A 6 HOUR INFUSION OF ADRENALINE AND NORADRENALINE

	INFUSION CONDITION		
	dextrose	noradrenaline	adrenaline
adrenaline (pg/ml)			
- baseline	31(6)	22(4)	21(4)
- infusion period	30(3)	22(3)	230(28)***
noradrenaline (pg/ml)			
- baseline	204(20)	207(17)	203(21)
- infusion period	219(24)	705(58)***	197(17)
renin (mU/L)			
- baseline	13.4(12.0-14.9)	15.5(14.6-21.0)	14.4(11.4-18.1)
- infusion period	11.6(9.6 -13.9)	17.3(13.8-21.5)***	17.9(15.5-20.8)***
aldosterone (pg/ml)			
- baseline	72(5)	72(4)	80(6)
- infusion period	52(4)	61(6)	70(4)**

Data are mean (SEM), mean values of renin (range) were calculated after logarithmic transformation. Baseline period = 9.00 - 10.00 h, infusion period = 10.00 - 16.00 h.
** $p < 0.01$, *** $p < 0.001$ adrenaline or noradrenaline versus dextrose

Baseline concentrations of active renin and aldosterone were not different. During both the infusions of adrenaline and noradrenaline active renin increased and was significantly higher than during dextrose. There were no significant differences between the plasma renin values during adrenaline and noradrenaline. Also one hour after cessation of the infusions there was no significant difference. Aldosterone during adrenaline was slightly although significantly higher than during dextrose (for a more detailed description see Chapter 6).

Haemodynamics

The mean (SEM) baseline blood pressure values were not different (adrenaline study 103(3)/62(2), noradrenaline study 104(3)/64(2), dextrose study 106(3)/63(2) mmhg) (Table 1). Arterial pressure during adrenaline infusion was lower and during noradrenaline infusion higher than during dextrose (resp. 102(2)/57(3) and 109(2)/66(2) versus 106(2)/63(2) mmHg, both $p<0.001$ ANOVA). In the post-infusion period arterial pressure after adrenaline was higher than after dextrose (107(2)/66(2) vs 103(2)/64(1) mmHg, $p<0.001$ ANOVA). Blood pressure after noradrenaline was not different from the dextrose study (for more detailed description of haemodynamic results see Chapter 3).

TABLE 2 MEAN BLOOD PRESSURE BEFORE DURING AND AFTER A 6 HOUR INFUSION OF ADRENALINE AND NORADRENALINE

	BLOOD PRESSURE (mmHg)		
	dextrose	noradrenaline	adrenaline
baseline	106(3)/63(2)	104(3)/64(2)	103(3)/62(2)
infusion period	106(2)/63(2)	109(2)/66(2)***	102(2)/57(3)***
postinfusion period	103(2)/64(1)	104(3)/63(3)	107(2)/66(2)***

Data are mean (SEM)
Baseline period = 9.00-10.00 h, infusion period = 10.00-16.00 h, postinfusion period = 16.00-9.30 h.
*** $p < 0.001$ adrenaline or noradrenaline versus dextrose

Sodium excretion

During the infusion periods and during the postinfusion periods no differences in sodium excretion were detected (Table 3).

TABLE 3 URINARY SODIUM AND CREATININE EXCRETION DURING AND AFTER INFUSION OF ADRENALINE, NORADRENALINE OR DEXTROSE

SODIUM	ADRENALINE	NORADRENALINE	DEXTROSE
infusion period			
mmol	119 (14)	122 (13)	123 (14)
mmol/h	13 (1)	13 (1)	13 (2)
mmol/mmol creatinine	18 (1)	18 (2)	19 (3)
post-infusion period			
mmol	85 (12)	107 (22)	102 (19)
mmol/h	5 (1)	7 (1)	6 (1)
mmol/mmol creatinine	12 (3)	12 (2)	13 (2)
total period			
mmol	204 (21)	230 (27)	224 (26)
mmol/h	8 (1)	9 (1)	9 (1)
mmol/mmol creatinine	14 (2)	15 (2)	15 (1)

data are means (SEM)
infusion period = 8.30 - 17.30 h, postinfusion period = 17.30 - 9.30 h next morning,
total period = infusion period + postinfusion period
Differences between adrenaline and noradrenaline versus dextrose were not significant.

DISCUSSION

The mechanism by which catecholamines enhance the renal sodium reabsorption may be multifactorial. Firstly, catecholamines have the ability to influence renal blood flow and glomerular filtration rate and can thereby alter physical factors of the proximal tubular environment. In addition it has been shown that renal nerve activity can activate the renin-angiotensin system, which can influence sodium excretion. Thirdly, there are indications of a direct action of catecholamines on proximal tubular cells (3-5).

The evaluation of the individual contributions of the above mentioned mechanisms by which adrenergic mechanisms could modify renal sodium excretion, is not without difficulties. This appears to be due to the counteracting renal and extrarenal actions of the sympathetic nervous system. Activation of the autonomic nervous system may enhance sodium reabsorption (direct renal effect) whereas the systemic haemodynamic effect (increase in blood pressure) tend to reduce sodium reabsorption. This problem is illustrated in studies with the sympatholytic agent guanethidine. When given in doses not affecting the systemic pressure, the adrenergic effect on the kidney was blocked, resulting in an increase in sodium excretion (8). However, when the drug is used in higher doses which reduce blood pressure, the hypotensive effect predominates and sodium retention occurs.

There is abundant evidence that renal nerves and catecholamines are able to exert a sodium retaining action. Bello-Reuss (9) showed that renal nerve stimulation can increase sodium reabsorption without affecting glomerular filtration rate. She further demonstrated that the increased sodium reabsorption occurs at the proximal tubular site, but also in the ascending loop of Henle. Indeed sympathetic nervous system fibres were found to innervate, besides the juxtaglomerular apparatus and renal vessels, also the proximal tubule segments (10). In studies in rats, dogs and rabbits the sodium retaining effect seems to be α-adrenoceptor mediated, most likely through the postjunctional α_1-adrenoceptor subtype (5,11,12). Osborn et al demonstrated in a study in dogs using low-frequency renal nerve stimulation (so that renal blood flow and glomerular filtration rate remained unchanged), that intrarenal infusion of phentholamine (non-selective α-adrenoceptor blockade) and prazosin (selective α_1-adrenoceptor blockade), but not yohimbine (selective α_2-adrenoceptor blockade), propranolol or atenolol, were all able to inhibit the renal nerve stimulation induced antinatriuresis (11). So apparently α_2-adrenoceptors, but not α_1-, β_1- and β_2- play a role here.

Another system which has been utilized to assess the role of catecholamines on renal sodium excretion has been the isolated perfused kidney. This system allows for the evaluation of direct effects of various agents on renal function, since the kidney is denervated and perfused outside of the body with artificial medium devoid of renin substrate or other vasopressor substances. The concentrations of catecholamines can

be altered and renal perfusion pressure and renal flow can be controlled. Besarab et al (13) showed that both adrenaline and noradrenaline have a direct effect on sodium handling in the isolated perfused rat kidney. When perfusion pressure was kept constant, sodium excretion decreased markedly after addition of either of the agents. This effect was blocked by propranolol. Thus at least in isolated rat kidney studies, there seems to be a direct role for ß-adrenoceptor stimulation in the control of sodium excretion. Propranolol enhances renal excretion of sodium in the rat by a tubular mechanism. Since metoprolol causes a similar effect it is likely that the receptor involved is of the $ß_1$-subtype (14,15). In summary, the receptor involved in the tubular transport effect of low level renal nerve stimulation is an α_1-adrenoceptor. However, with other -primarily pharmacological- forms of stimulation α_2 and ß-adrenoceptors may also influence tubular transport function.

As stated before, interactions of the direct tubular effect with other mechanisms might be important. Sympathetic nervous system activation also leads to augmented renin release, especially during reduction of renal perfusion pressure or volume depletion. The precise role of angiotensin II in the antinatriuretic effect of renal nerve stimulation is not completely elucidated. Handa & Johns have used converting enzyme inhibition and angiotensin II antagonism in rats to demonstrate an angiotensin II requirement for renal nerve stimulation to increase renal sodium reabsorption (16). However, Zembraski and DiBona showed that angiotensin II antagonism did not modify the renal sodium conserving action of renal nerve input (17).

Renal sympathetic nerve activity is also a stimulus for prostaglandin production (18). Prostaglandins are known to inhibit sodium reabsorption in the thick ascending limb and collecting duct. Sodium reabsorption induced by renal nerve stimulation in the dog was not affected by prostaglandin synthesis inhibition (19), whereas it impairs acute denervation natriuresis in the rat (20).

So it has become clear from animal studies, that renal nerves and catecholamines are able to contribute to the regulation of renal sodium excretion.

In this study in sodium replete normal humans we found no differences in urinary sodium excretion during a 6 hours infusion of noradrenaline or adrenaline as compared to control. The infusions resulted in plasma concentrations in the high physiological range. These findings argue against a major role of circulating noradrenaline or adrenaline in renal salt handling. There are, however, indications that the adrenergically mediated sodium conserving effects are operational in man. Gill and Bartter (21) demonstrated in normal human subjects that when autonomic insufficiency is produced by guanethidine, urinary sodium excretion could not be sufficiently reduced to avoid negative sodium balance in response to reduction in dietary sodium

intake, despite decreases in glomerular filtration rate and marked increases in urinary aldosterone excretion. Also patients with idiopathic autonomic insufficiency could not lower their urinary sodium excretion below 50-60 mmol/day while consuming a diet with sodium intake of 17 mmol/day. Even mineralocorticoid drug substitution failed to correct the negative sodium balance (22). These clinical studies support the view that renal innervation appears to be essential for the kidney to express its full ability to maximally reabsorb sodium in response to reduction of dietary sodium intake.

Several studies have addressed the question of the possible effects of circulating adrenaline and noradrenaline on sodium excretion in humans. Older studies in which noradrenaline and adrenaline were infused in much higher doses than we did, inducing substantial rises in blood pressure and decreases in renal blood flow, generally show a marked sodium retention (23-25). Also in a recent study in normal man noradrenaline was infused leading to approximately the same plasma levels as in our study (26). A marked decrease (20%) in sodium excretion was found. Since creatinine clearance was not effected, but a decline in lithium clearance was found, the results were taken to indicate an increase in tubular sodium reabsorption. The investigators did not detect major blood pressure changes, although blood pressure was monitored indirectly, and small haemodynamic changes might have gone unnoticed.

In our study no effect of adrenaline or noradrenaline on natriuresis was noted during the infusions as compared to the control experiment, while blood pressure was higher during noradrenaline and lower during adrenaline as compared to control. It was in 1925, when Starling and Verney studied isolated perfused kidneys and reported that alterations in renal perfusion pressure were accompanied by parallel changes in urinary sodium excretion (27). It is now understood from both isolated kidney studies and *in vivo* experiments that a small elevation of blood pressure results in an increase in urinary sodium excretion (28,29). The mechanism by which increases in renal perfusion pressure cause natriuresis appears to involve small increases in glomerular filtration rate and filtered sodium load as well as reduction in tubular reabsorption (29). In the "autoregulatory" range, which is in the dog a renal perfusion pressure between 80-180 mmHg, the increase in urinary sodium excretion is largely a consequence of altered tubular behaviour, whereas glomerular filtration rate remains remarkably constant (30). In our study, however, there was no enhanced sodium excretion during the noradrenaline infusion, despite the fact that blood pressure during noradrenaline was higher than during dextrose and adrenaline infusion. Either the haemodynamic effects of noradrenaline were insufficient to cause natriuresis, or it might be possible that the pressor natriuresis was counteracted by the stimulating effect of noradrenaline on the proximal tubular reabsorption, which led to the zero net result in sodium excretion. In the post-infusion period after adrenaline infusion, in which blood pressure was higher than after noradrenaline and control infusion, we also did not detect an

enhanced sodium excretion. Since one might have expected increased noradrenaline levels in this period (see Chapter 3), these could have led to enhanced sodium reabsorption leading to the net result of a sodium output in balance. Thus, one could argue that adrenaline like noradrenaline is an antinatriuretic factor.

In our study blood pressure decreased during adrenaline infusion. A decrease in sodium excretion could have been expected. However, sodium loss during adrenaline was not significantly different from control infusion. Johnson et Barger found during intravenous administration of adrenaline (5, 25 and 125 ng/kg/min) in dogs a decrease in urinary sodium loss without affecting glomerular filtration rate and renal blood flow (31). Intrarenal infusion did not have the same effect. The authors concluded that the effect of adrenaline was a result of an extrarenal mechanism. The sustained reduction in blood pressure during the 25 ng/kg/min infusion and the transitory fall in blood pressure during 125 ng/kg/min might have been of importance. The increased renin secretion and/or an enhanced renal sympathetic nerve activity were hypothesized to be the cause of the antinatriuretic effect. In another study in dogs isoprenaline was infused in low dose without influencing blood pressure and increased sodium excretion was noted, which was suspected to be the result of the vasodilator response to isoprenaline (32). However, intravenous infusion of isoprenaline leading to substantial blood pressure reduction resulted in an overall decrease of sodium excretion, although the direct effect (increased sodium excretion) was still detectable during concomitant intrarenal administration of isoprenaline. Thus the systemic effect (blood pressure reduction → decrease in sodium excretion) antagonized the renal effect (enhanced sodium excretion). It was suggested that this antagonism was caused by an increased sympathetic nervous system stimulation to the kidneys during the blood pressure fall (32). In a more recent study in dogs Kopp and DiBona showed that renal intra-arterial infusion of adrenaline, not affecting systemic blood pressure, had no effect on urinary sodium loss (33). Finally the antinatriuretic response to low frequency electrical stimulation in dogs (without affecting renal blood flow) was not influenced by atenolol or propranolol (11). So, in dogs adrenaline in subpressor doses does not appear to have an effect on sodium excretion, whereas an enhancing effect of ß-adrenoceptor stimulation was documented in the rat (14,15). In our study in humans no significant effect on sodium excretion was found. Since blood pressure decreased, the $ß_2$-adrenoceptor mediated vasodilatory action of adrenaline must have predominated. The possible sodium excretion enhancing effect of this (renal) vasodilation as was observed in the dog (32) must have been counteracted by the systemic effect (lowering blood pressure → inhibiting sodium excretion). Since we have no data on renal haemodynamics, the exact mechanism of our findings is not elucidated by our study. Adrenaline infused in higher doses resulting in a rise in blood pressure, so when α-adrenoceptor mediated actions

predominate, results in a marked decrease in sodium excretion, by enhancing tubular reabsorption (23).

The abundant amount of data in the literature, which are briefly outlined in this paper, have clearly demonstrated that the adrenergic control of renal tubular sodium excretion is recognized as an important physiological mechanism in the overall regulation of sodium and water homeostasis in mammalian organisms. The antinatriuresis is mediated by renal α_1-adrenoceptors. Research in animals on the exact role of ß-adrenoceptors produced conflicting results, which might be caused by interspecies differences.

Our data show that circulating adrenaline and noradrenaline in physiological concentrations in plasma do not affect renal sodium excretion in normal sodium replete men. These results are in keeping with the assumption of DiBona (5) that the renal adrenergic system is not required for the renal regulation of sodium balance during normal or even moderate reduction in dietary sodium intake. This conclusion is also supported by the above mentioned observations in humans with autonomic failure, in whom only very severe reduction in dietary sodium intake leads to negative sodium balance. Also patients with a kidney transplant appeared to be able to maintain sodium balance (34). In a study in 5 patients who had had their own kidneys removed previously and had received a kidney transplant 46 to 922 days earlier, it was demonstrated that the ability of the patients to conserve sodium on a dietary intake of 10 mmol/day appeared to be normal. The authors assumed that renal nerve regeneration did not occur. In rats, however, there is evidence for beginning functional renal nerve regeneration about 1 month after bilateral denervation (35,36).

Our results could be interpreted also in another way. The increased blood pressure during noradrenaline infusion did not result in an enhancement in natriuresis, which can be taken as an indication of increased sodium reabsorption. In the post-infusion period after adrenaline infusion, the observed rise in blood pressure was not accompanied by an increase in urinary sodium loss. Again, this might be a consequence of higher plasma noradrenaline which are suspected to occur in this period (Chapter 3). In both situations a higher blood pressure was needed to maintain natriuresis. It was Borst (37) and later Guyton (38) who suggested that "deficient" sodium excretion is the essential feature in essential hypertension. According to their theory the renal abnormality involves a change in the normal relationship between arterial pressure and sodium excretion, in such a way that a higher than normal pressure is required for sodium excretion to match sodium intake. It is now clear that this abnormality exists in many if not all types of experimental and clinical hypertension (39-41). The mechanism responsible for this resetting and the question whether it is a cause or consequence of hypertension is, however, still a matter of debate. Our

results would fit in an involvement of the catecholaminergic system. Indeed there is evidence from animal studies for the existence of an increased renal nerve activity in certain forms of experimental hypertension (for review 42). Workers from Maastricht also provided evidence for a causal relationship between efferent renal nerve activity and hypertension (43,44). Intrarenal infusion of noradrenaline in doses that were ineffective or much less effective when given intravenously, induced a sustained increase in blood pressure. The intrarenal mechanism which is triggered by the increased nerve activity and leads to an increase in blood pressure, might be the enhanced sodium resorption as was observed in dogs (45,46). These observations were, however, not confirmed in the rat (47).

Insulin, like aldosterone, can be regarded as a sodium-retaining hormone (48). The higher circulating insulin and aldosterone concentrations during the adrenaline infusion as compared to the other conditions, could also have contributed to the "inability" of the kidneys to excrete a sufficient amount of sodium.

Although difficult to obtain there is also evidence that efferent renal sympathetic nerve activity is increased in human essential hypertension. Hollenberg et al demonstrated that the increase in renal blood flow in response to the intra-renal administration of the α-antagonist phentholamine, was greater in humans with essential hypertension than in normotensive controls, suggesting an increased vasoconstrictor influence on renal vasculature (49). Esler et al have demonstrated that noradrenaline spillover to plasma, an index of total sympathetic nervous system activity, is increased in essential hypertension and that this derives in large part from the increase in renal noradrenaline spillover (50).

In conclusion, our results combined with the data in the literature, which are briefly summarized above, suggest an involvement of the renal adrenoceptor system in the pathogenesis of human hypertension and that the mechanism of this involvement might be its interaction with renal salt handling. At any rate, the potential role of adrenaline in the pathogenesis of hypertension might have two faces: the release of noradrenaline which causes vasoconstriction along with the prevention of pressure-natriuresis through an antinatriuretic action.

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Chapter 8

ADRENALINE-INDUCED ENHANCEMENT OF SYMPATHETIC ACTIVITY IN MAN; INHIBITION BY NON-SELECTIVE AS WELL AS ß1-SELECTIVE ß-ADRENOCEPTOR BLOCKADE.

Reprinted from: AH van den Meiracker, AJ Man in 't Veld, F Boomsma, PJ Blankestijn, MADH Schalekamp. Epinephrine-induced enhancement of sympathetic activity in humans: inhibition by non-selective as well as $ß_1$-selective ß-adrenoceptor blockade. J Cardiovasc Pharmacol 1990; 15: 610-617

INTRODUCTION

In numerous in-vitro studies using animal as well human tissues it has been well established that stimulation of prejunctional ß-adrenoceptors facilitates the neuronal release of noradrenaline (1-4). Adrenaline is likely to be the physiological agonist of the prejunctional ß-receptor (5-9). This has stimulated interest for a possible role of adrenaline in the pathogenesis of certain forms of hypertension (10-12).

Studies in man have shown that sympathetic activity, as assessed by plasma noradrenaline concentrations and as assessed by neurogenic vasoconstriction, is increased by low-dose infusions of adrenaline (13-19). Other studies, however, failed to demonstrate such an effect (20-23). In some instances these discrepant results could be related to the doses of adrenaline that have been used. Too high levels of adrenaline may not only activate the stimulatory ß-type prejunctional ß-adrenoceptors, but also the inhibitory α-type, thereby masking a facilitating effect of adrenaline on noradrenaline release (22). Furthermore, since noradrenaline is extensively metabolized in tissues, the site of noradrenaline sampling may also be important for the demonstration of adrenaline-induced increments in noradrenaline plasma levels. In fact, in one study arterial plasma levels of noradrenaline rose during intravenous infusion of adrenaline, whereas venous levels did not (24).

The present study was conducted in order to collect more information on the facilitating effect of adrenaline on noradrenaline release in man. Our aim was twofold. First, to confirm and extend earlier observations that adrenaline, in concentrations within the physiological range, enhances resting and activated sympathetic activity. Second, to study the effects of non-selective and $ß_1$-selective ß-adrenoceptor blockade on this adrenaline-induced enhancement in sympathetic activity. Arterial plasma levels of noradrenaline were used as an index of overall sympathetic activity, whereas the production of noradrenaline in the forearm, with exclusion of the hand, was used as an index of local muscle sympathetic activity.

SUBJECTS AND METHODS

Sixteen subjects with a mean age of 41 years, range 24 to 52 years, participated in the study after they had given informed consent. All subjects had mild hypertension. Their sitting diastolic blood pressure varied between 95 and 105 mm Hg, when untreated. A secondary cause of hypertension was excluded by routine clinical and laboratory evaluation. None of the subjects had a history or clinical signs of ischaemic heart disease, cardiac failure, cerebrovascular disease, chronic obstructive lung disease, diabetes mellitus or any other serious illness.

Study Protocol.

A washout phase of at least 2 weeks was followed by a single blind placebo period of 2 weeks. After this period 10 subjects were randomly allocated to either atenolol or bopindolol in a double-blind cross-over comparison. Atenolol and bopindolol were given for one week. To avoid a carry-over effect this active treatment period was interrupted by a second placebo period of two weeks. Studies were performed at the end of the first placebo period and at the end of both active treatment periods. Placebo capsules during the active treatment and placebo periods were matched. One capsule had to be taken every morning. Adherence to the medication was checked by pill counting. Atenolol was given in a once daily dose of 50 mg. Bopindolol, which is a long-acting non-selective ß-adrenoceptor antagonist with some degree of partial agonist activity (25), was given in a once daily dose of 1 mg.

Procedures and Tests.

Patients arrived at 8 A.M. in the laboratory after a light breakfast at home. While resting supine on a tilt-table the brachial artery of the non-dominant arm was cannulated with a 1.0 mm thick, 5 cm long, Teflon catheter after local anaesthesia with a 2% lidocaine solution. A deep antecubital vein was cannulated in the same arm for venous blood sampling. An indwelling catheter in a vein of the contralateral forearm was used for intravenous infusions. The flow in the non-dominant arm was measured semicontinuously by an ECG triggered venous occlusion plethysmograph (Janssen Scientific Instruments, Beerse, Belgium). A mercury-in-Silastic strain gauge was placed around the mid-forearm. The venous occlusion pressure of 50 mm Hg was intermittently applied for a period of three heart beats, with a recovery period of two heart beats. During flow measurements the arm was slightly elevated above heart level and handflow was excluded from the circulation by a small wrist cuff inflated 30 mm Hg above the systolic arterial pressure. Heart rate was derived from a continuously recorded ECG.

After completion of instrumentation an intravenous saline infusion (5.4 ml per hour) was started. Thirty minutes after start of this infusion baseline arterial and venous plasma catecholamines, active plasma renin and haemodynamics were measured. Ten minutes afterwards an isometric exercise test, cold pressor test, orthostatic stress test and a mental stress test (colour word stress test) were subsequently performed, with a period of 15 minutes supine rest between each test. After completion of the colour word test the saline-infusion was switched to an intravenous adrenaline infusion with an infusion rate of 20 ng/kg/min. The timing and sequence of measurements and tests during the adrenaline-infusion were the same as during infusion of saline. For evaluating possible sequence effects, 6

additional subjects only receiving placebo capsules (control group), were studied. In the control group, a second saline-infusion was given instead of the adrenaline-infusion.

Isometric exercise test was performed by lifting a weight of approximately 9% of body weight over a pulley by 90° flexion of the elbow of the dominant arm, while resting supine. This test lasted three minutes. For the cold pressor test the hand up to the wrist of the dominant arm was immersed in ice-cold water for one minute. Orthostatic stress test was performed by 60° head-up tilting for ten minutes by means of a tilt-table. For mental stress an audio-visual version of Stroop's colour word conflict test was used. This test consists of four different colour-words written in incongruent colours. The words are randomly shown on a television monitor with a frequency of 140 words per five minutes. The task of the subject is to ignore the written word and to mark the colour on an answer-sheet on which successive rows of randomly ordered abbreviations of the four colours are printed. The test lasts for 5 minutes.

Just before and immediately at the end of each test arterial and venous blood was simultaneously sampled for measurement of catecholamines. Forearm flow was measured during isometric exercise test, cold pressor test and colour word test, but for technical reasons, it was not measured during orthostatic stress test.

Analytical Procedures and Calculations.

For determination of catecholamines 10 ml of arterial and venous blood was collected in chilled tubes containing 12 mg gluthatione and 5 IE sodium heparin. After centrifugation at 0° C, plasma was removed and stored at -70° until assay. Plasma catecholamine concentrations were determined by high performance liquid chromatography (HPLC) with electrochemical detection (26).

The fractional extraction (%) of adrenaline in the forearm was calculated as (arterial adrenaline - venous adrenaline)/ arterial adrenaline x 100. The production of noradrenaline in the forearm was calculated as the product of forearm flow and the venous-arterial difference of noradrenaline plus the extraction of noradrenaline in the forearm. Noradrenaline extraction was calculated as arterial noradrenaline x fractional extraction adrenaline, assuming that the fractional extractions of noradrenaline and adrenaline in the forearm are similar (27).

Active plasma renin concentration was measured by RIA, as described previously (28).

Statistical Analysis.

Data are presented as mean values ± SEM. For active renin the mean was calculated after logarithmic transformation of the values, since values were not distributed normally. For statistical analysis Student's t-test for paired observations was used. p-Values < 0.05 were considered to indicate a statistically significant difference.

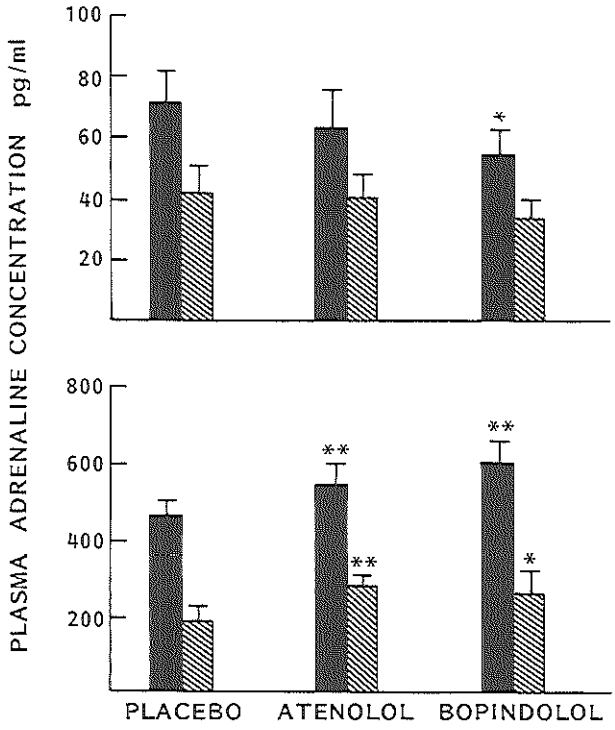

Figure 1. Arterial (▩) and venous (//) plasma levels of adrenaline during placebo, atenolol and bopindolol. Upper panel: infusion of saline, lower panel: infusion of adrenaline (20 ng/kg min).
* $p < 0.05$, ** $p < 0.01$ atenolol or bopindolol versus placebo

RESULTS

Resting Conditions.

Values of arterial and venous plasma levels of adrenaline 30 min after starting the infusions of saline and adrenaline are shown in Figure 1. During saline arterial adrenaline was lower with bopindolol ($p<0.05$) than with placebo. During infusion of adrenaline arterial adrenaline and venous adrenaline increased five- to tenfold. Compared to placebo the adrenaline-infusion induced increments in arterial and venous adrenaline were higher with the two ß-adrenoceptor antagonists (Figure 1). The fractional extraction of adrenaline in the forearm during saline-infusion on placebo was 40±5%. Comparable values were seen with atenolol (37±6%) and with bopindolol (34±6%). Infusion of adrenaline caused an increase in the fractional extraction of adrenaline in the forearm to respectively 49±5% ($p<0.01$) during placebo, to 44±2% during atenolol (n.s.) and to 50±8% ($p<0.05$) during bopindolol. In the control group resting values of arterial and venous adrenaline and the fractional extraction of adrenaline in the forearm 30 min after the first and second saline-infusion were not different. The duplicate values were 78±14 and 52±7 pg/ml for arterial adrenaline, 35±5 and 30±4 pg/ml for venous adrenaline and 46±6% and 40±9% for the fractional extraction of adrenaline.

Active renin decreased to a similar degree after atenolol and bopindolol. Values were 23.1±1.3 mU/L during placebo, 11.3±1.2 mU/L ($p<0.01$) during atenolol, and 10.9±1.2 mU/L ($p<0.01$) during bopindolol. Active renin did not change by infusion of adrenaline during any of the three treatments: the values during placebo, atenolol and bopindolol were 24.5±1.2, 12.9±1.2 mU/L and 8.3±1.2 mU/L respectively.

As compared to placebo resting blood pressure and heart rate were reduced by the ß-adrenoceptor antagonists (Table 1). Forearm flow was not affected by the two ß-adrenoceptor antagonists. Infusion of adrenaline caused a decrease in diastolic arterial pressure both during placebo and during ß,-selective ß-blockade with atenolol. In contrast, with bopindolol adrenaline-infusion led to a small increase in diastolic pressure (Table 1). Neither during placebo nor during the two ß-adrenoceptor antagonists did infusion of adrenaline cause any change in systolic arterial pressure. Forearm flow did not change significantly by adrenaline during any of the three treatments. Systolic and diastolic arterial pressure, heart rate and forearm flow in the control group 30 min after the first and second saline-infusion were not different (Table 1).

Resting values of arterial noradrenaline and the production of noradrenaline in the forearm 30 min after start of saline or adrenaline-infusion are shown in Figure 2. Although the values of these two parameters during placebo, atenolol or bopindolol, at the time of saline-infusion, did not differ, arterial noradrenaline tended

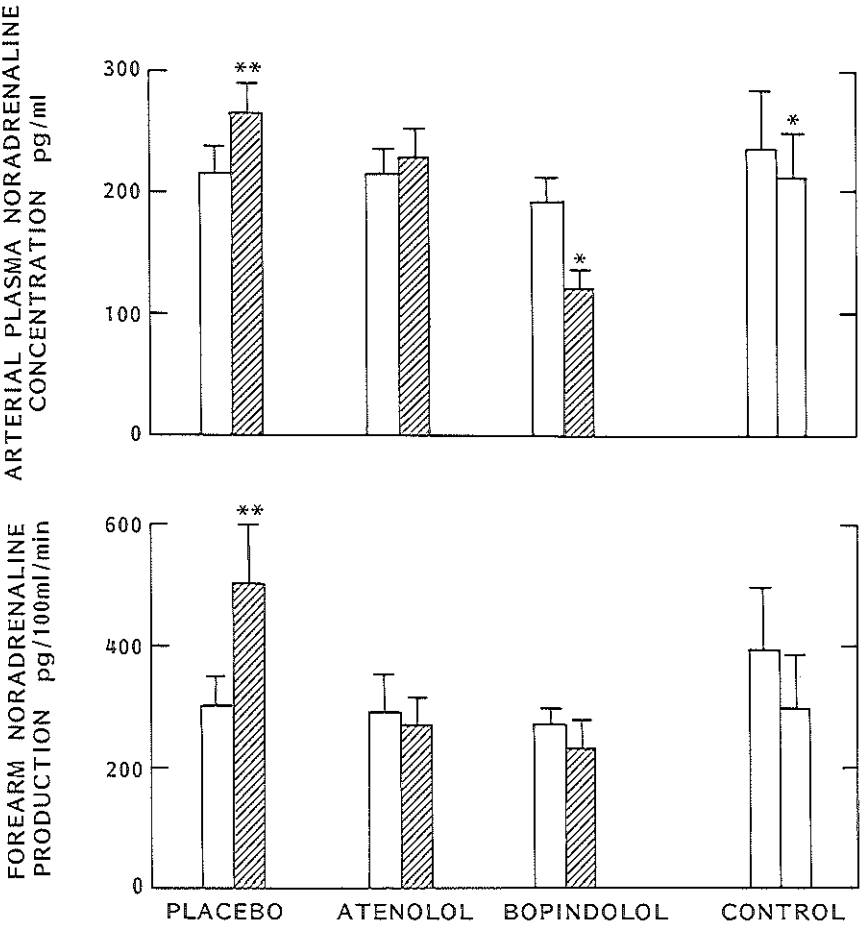

Figure 2. Effects of adrenaline on resting concentrations of noradrenaline and forearm noradrenaline production during placebo, atenolol and bopindolol (□ saline, /// adrenaline infusion)
* $p < 0.05$, ** $p < 0.01$ adrenaline versus saline or saline versus saline control group.

TABLE 1. EFFECTS OF ADRENALINE ON RESTING VALUES OF SYSTOLIC AND DIASTOLIC ARTERIAL PRESSURE, HEART RATE AND FOREARM FLOW DURING PLACEBO, ATENOLOL AND BOPINDOLOL

	PLACEBO		ATENOLOL		BOPINDOLOL		CONTROL#	
	SAL	ADR	SAL	ADR	SAL	ADR	SAL	SAL
SAP mmHg	154±5	151±6	130±5	128±5	140±5	143±5	147±4	145±4
DAP mmHg	89±2	78±3**	72±2	67±3*	79±2	82±3	86±3	84±4
HR bpm	71±3	78±2**	59±3	58±2	63±3	61±3	68±1	65±3
FAF ml/min/100ml	3.0±0.3	3.5±0.5	3.4±0.7	2.6±0.3	3.2±0.3	2.9±0.4	4.7±1.3	3.9±0.7

# Control group saline infusion only.
SAL = saline and ADR = adrenaline infusion. SAP and DAP systolic and diastolic arterial pressure respectively; HR = heart rate; FAF = forearm flow.
*$p<0.05$; ** $p<0.01$ adrenaline versus saline.

to be lower with bopindolol. Infusion of adrenaline during placebo caused an increase in arterial noradrenaline ($p<0.01$) and noradrenaline forearm production ($p<0.05$). No adrenaline-induced change in these parameters occurred with atenolol, whereas with bopindolol adrenaline-infusion led to a decrease in arterial noradrenaline ($p<0.05$), but not to a decrease in noradrenaline forearm production (Figure 2). In the control group arterial noradrenaline during the second saline-infusion was lower ($p<0.05$) than during the first infusion, but no difference in noradrenaline forearm production was seen during the first and second saline-infusion (Figure 2).

Stress Tests

The responses of systolic arterial pressure and diastolic arterial pressure to the four stress tests during placebo, atenolol and bopindolol were the same (Table 2). The increments in heart rate in response to the isometric exercise test, the colour word conflict test and orthostatic stress test during placebo were attenuated or abolished by the two ß-adrenoceptor antagonists. As compared with placebo, the two ß-adrenoceptor antagonists had no effect on the responses of forearm flow or forearm vascular resistance to the isometric exercise test or the cold pressor test. However, the increase in forearm flow during the colour word conflict test was diminished by atenolol. During placebo, but not during atenolol and bopindolol, the increase in systolic arterial pressure in response to isometric exercise test and the increase in diastolic arterial pressure in response to orthostatic stress were

TABLE 2. Effects of adrenaline on responses of SAP, DAP, HR, FAF and FVR to the four stress tests during placebo, atenolol and bopindolol.

	PLACEBO		ATENOLOL		BOPINDOLOL		CONTROL¶	
	SAL	ADR-SAL	SAL	ADR-SAL	SAL	ADR-SAL	SAL	SAL-SAL
IET								
SAP (mmHg)	29±5	4±2*	26±3	3±3	24±2	-1±3	27±8	-3±3
DAP (mmHg)	18±3	1±2	19±2	1±3	16±2	0±1	18±4	1±1
HR (bpm)	10±1	-4±1**	5±1#	1±1	6±1#	0±1	13±4	-4±4
FAF (ml/100ml/min)	0.9±0.4	-0.5±0.3*	1.1±0.5	0.2±0.5	0.5±0.4	0.6±0.3	1.4±0.5	-0.1±0.5
FVR (U)	0±4	19±13*	1±4	2±4	3±10	-2±8	-3±3	2±2
CPT								
SAP (mmHg)	12±3	3±2	11±3	3±2	15±3	1±3	13±2	0±2
DAP (mmHg)	9±3	0±2	9±2	0±1	11±2	-2±2	10±2	1±2
HR (bpm)	2±2	-1±2	0±2	1±2	1±3	1±4	5±2	1±2
FAF (ml/100ml/min)	-0.2±0.2	-0.5±0.3	0.2±0.3	-0.2±0.3	-0.2±0.2	0.0±0.4	-0.2±0.4	0.3±0.3
FVR (U)	6±3	4±3	13±8	-3±9	13±6	3±11	2±4	5±6
CWT								
SAP (mmHg)	24±3	7±4	21±5	-1±3	22±5	-1±3	21±4	-2±1
DAP (mmHg)	16±2	0±3	13±3	0±3	16±3	-2±2	14±4	-2±1
HR (bpm)	18±4	-2±3	7±1##	1±1	7±1##	2±12	13±2	3±2
FAF (ml/100ml/min)	2.6±0.6	-0.8±0.7	1.0±0.2#	0.0±0.6	1.0±0.5	-0.2±0.3	2.7±0.4	0.2±0.2
FVR (U)	-12±5	10±7	-10±6	13±8	10±7	-11±9	-13±5	1.0±1
OST								
SAP (mmHg)	-3±1	2±1	-3±1	2±2	-4±2	-3±1	-2±3	-2±3
DAP (mmHg)	2±2	5±2**	-2±1	2±1	4±1	-4±2	4±2	0±1
HR (bpm)	11±2	-1±2	0±1###	0±3	5±1##	3±1	9±3	2±4

IET, isometric exercise test; CPT, cold pressor test; CWT, colour word test (mental stress); OST, orthostatic stress test; ADR-SAL, difference in response between ADR and SAL; SAL-SAL, difference in response between the second and the first SAL infusion; FVR, forearm vascular resistance; other abbreviations as in Table 1

¶ control group, SAL infusion only

* $p < 0.05$, ** $p < 0.01$ ADR versus SAL

# $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, atenolol or bopindolol versus placebo

TABLE 3. Effects of adrenaline on arterial levels of noradrenaline (pg/ml) before and after isometric exercise, cold provocation, orthostatic stress and mental stress during placebo, atenolol and bopindolol.

		PLACEBO		ATENOLOL		BOPINDOLOL		CONTROL¶	
		SAL	ADR	SAL	ADR	SAL	ADR	SAL	SAL
IET	B	202±20	234±24	223±16	202±24	185±24	118±16	203±34	186±27
	A	256±22	314±27	326±19	305±26	269±28	200±26	273±20	261±34
CPT	B	208±23	231±21	229±18	196±24	189±23	121±18	207±37	212±39
	A	235±30	278±26	263±19	232±27	228±27	154±26	240±41	255±43
OST	B	194±22	237±22	221±17	189±21	192±22	119±18	199±36	214±37
	A	350±35	405±38	380±27	361±38	375±33	285±50	353±41	384±45
CWT	B	238±22	270±22	274±2	231±24	236±19	162±21	233±45	254±49
	A	299±43	366±38	329±29	296±32	261±21	209±34	291±51	286±40

¶ Control group saline infusion only.
IET: isometric exercise test; CPT: cold pressor test; OST: orthostatic stress test; CWT: colour word test.

TABLE 4 Effects of adrenaline on forearm noradrenaline production (pg ml^{-1} min^{-1}) before (B) and after (A) isometric exercise, cold provocation, orthostatic stress and mental stress during placebo, atenolol and bopindolol.

		PLACEBO		ATENOLOL		BOPINDOLOL		CONTROL#	
		SAL	ADR	SAL	ADR	SAL	ADR	SAL	SAL
IET	B	263±55	661±117	308±82	310±67	273±92	233±54	468±127	362±95
	A	526±79	938±219	747±168	785±131	416±226	505±92	919±279	672±343
	Δ	263±81	277±155	439±122	474±132	143±69	272±63	451±157	309±272
CPT	B	267±66	558±110	267±43	312±73	253±69	251±43	470±166	285±80
	A	352±57	590±135	463±89	499±109	334±78	363±73	795±180	556±136
	Δ	85±60	32±55	196±79	187±80	80±57	112±57	325±154	271±97
CWT	B	401±67	787±157	295±62	395±73	320±76	279±80	396±109	441±181
	A	733±170	1014±296	524±113	502±96	554±114	517±114	619±111	701±131
	Δ	332±147	227±239	229±64	106±63	223±72	237±73	223±167	259±110

Control group saline infusion only.
IET: isometric exercise test; CPT: cold pressor test; CWT: colour word test.

augmented by adrenaline infusion. In the control group, the haemodynamic responses to the first and second series of tests were not different.

All four tests caused an increase in arterial noradrenaline (Table 3). This increment was highest during orthostatic stress test and lowest during cold provocation (Figure 3). As compared to placebo the response of arterial noradrenaline to isometric exercise test, but not to the other three tests was higher with atenolol ($p<0.05$) and with bopindolol ($p<0.05$). During placebo, infusion of adrenaline enhanced the responses of arterial noradrenaline to isometric exercise test ($p<0.05$), cold pressor test ($p<0.05$) and colour word test ($p<0.01$), but not to orthostatic stress test (Figure 3). This enhancement was abolished by bopindolol as well as by atenolol. In the control group there was no difference in the responses of arterial noradrenaline to the respective tests during the first and second saline-infusion (Figure 3). The responses of noradrenaline forearm production to isometric exercise test, cold pressor test and orthostatic stress test are summarized in Table 4. With the three different treatments (placebo, atenolol and bopindolol) the responses of noradrenaline forearm production to the stress tests showed a considerable variation, but differences were never significant. Unlike to what was observed for the responses of arterial noradrenaline, the noradrenaline forearm production in response to the stress tests was not enhanced by adrenaline-infusion during placebo. Adrenaline had also no effect on the responses of noradrenaline forearm production during administration of atenolol or bopindolol. In the control group changes in noradrenaline forearm production in response to the first and second series of tests did not differ (Table 4).

DISCUSSION

Effects of ß-Adrenoceptor blockade on plasma adrenaline.

With the infusion rate of adrenaline currently used venous levels of adrenaline increased to approximately 200 pg/ml during placebo. These levels are within the high physiological range (29). It is well known that adrenaline is cleared by ß-adrenergic mechanisms and that ß-adrenoceptor antagonists diminish the clearance of adrenaline (30). This explains why both arterial and venous levels of adrenaline were higher during administration of the two ß-adrenoceptor antagonists. During infusion of adrenaline arterial adrenaline rose relatively more than venous adrenaline. The fractional extraction of adrenaline in the forearm was therefore increased. An adrenaline-induced increase in the fractional extraction of adrenaline was also observed during administration of atenolol and bopindolol, which is at variance with a report of Best and Halter (24). In their study an acute intravenous dose of propranolol during adrenaline-infusion undid the adrenaline-induced increase in forearm fractional extraction of adrenaline. Differences in the

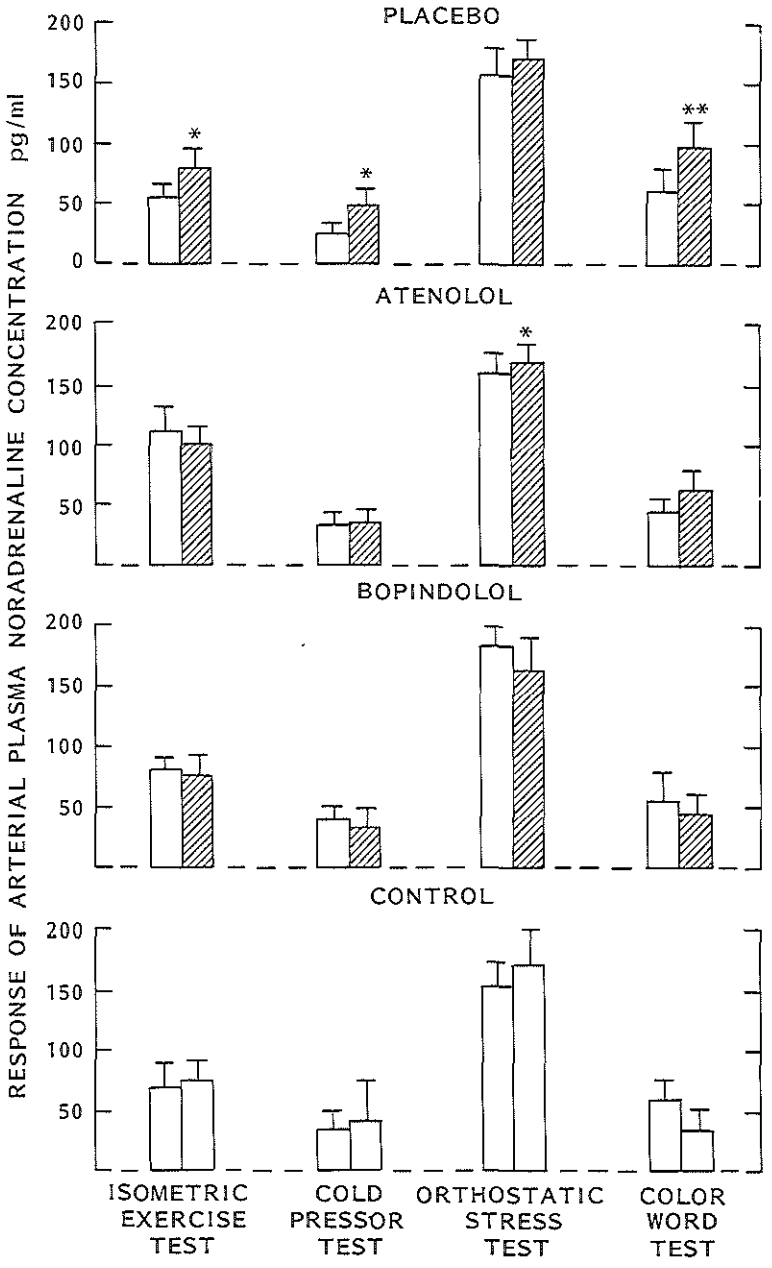

Figure 3. Effect of adrenaline on delta responses of arterial plasma levels of noradrenaline to isometric exercise (IET), cold provocation (CPT), orthostatic stress (OST) and mental stress (CWT) during placebo, atenolol and bopindolol. □ saline, /// adrenaline infusion.
* $p < 0.05$, ** $p < 0.01$ adrenaline versus saline.

haemodynamic effects of ß-adrenoceptor antagonists, when administrated acutely or for a more prolonged period, might account for the difference between Best and Halter's findings and our study.

Effects of adrenaline on plasma noradrenaline.

An increase in plasma noradrenaline during relatively low-dose intravenous infusions of adrenaline has been demonstrated (13-18). In the present study these observations could be confirmed. In addition, the responses of arterial plasma noradrenaline to three of the four stress-tests were also augmented by adrenaline. That these increments in plasma noradrenaline were indeed an effect of adrenalineis stressed by the absence of such an effect in the control group to which only repeated saline infusions were given.

Effect of adrenaline on local noradrenaline production.

In an attempt to collect also information on local sympathetic activity, predominantly in the muscles, the production of noradrenaline in the forearm (excluding the hand) was calculated. The production at rest was markedly increased by adrenaline but, unlike the responses of arterial plasma noradrenaline, the responses of noradrenaline forearm production to the stress tests were not increased by adrenaline. For these differential effects of adrenaline on arterial plasma noradrenaline and on noradrenaline forearm production during stress we have no easy explanation. One possibility is that changes in local muscle sympathetic activity do not run in parallel with changes in overall sympathetic activity, a phenomenon that might be related to regional differences in sympathetic activation during different forms of stresses (31). Another possibility to consider is that during stress the changes in the fractional extraction of adrenaline are not representative for the changes in the fractional extraction of noradrenaline, so that under these circumstances the formula by which we calculated noradrenaline production was not valid.

Effects of adrenaline on baroreflex activity.

The haemodynamic effects of adrenaline might have influenced sympathetic discharge by changing afferent arterial baroreflex activity. In this regard, the influence of changes in pulse pressure appear to outweigh the influence of changes in mean arterial pressure (32). It would therefore be expected that the haemodynamic effects of adrenaline will stimulate rather than suppress baroreceptors, and as a consequence, will diminish sympathetic activity. This is supported by a recent study of Arnold et al (33), showing that a constant infusion of the non-selective ß-adrenoceptor agonist isoprenaline, with haemodynamic

effects comparable to that of adrenaline, caused a reflex-increase rather than a reflex-withdrawal of vagal cardiac tone. Furthermore, in the present study, although adrenaline-induced changes in arterial pressure during placebo and atenolol were comparable, noradrenaline increased only during placebo, suggesting that other than haemodynamic changes underlie the alterations in noradrenaline.

Effect of adrenaline on noradrenaline clearance.

For interpretation of plasma concentrations of noradrenaline not only changes in its release into plasma, but also changes in its clearance rate from the plasma have to be considered. The clearance of noradrenaline appears not to be influenced by relatively low concentrations of adrenaline (16). On the other hand ß-adrenoceptor antagonists tend to diminish the clearance of noradrenaline (34). In light of this knowledge it is unlikely that alterations in the clearance of noradrenaline are responsible for the observed adrenaline-induced changes in plasma noradrenaline either during placebo or during administration of the two ß-adrenoceptor antagonists.

Role of prejunctional ß-adrenoceptors.

The demonstration that the adrenaline-induced elevations in plasma noradrenaline and noradrenaline forearm production is abolished by bopindolol as well as by atenolol, indicates the involvement of ß-adrenoceptors. Similar observations were reported previously (13,14,17,18). The present study adds to this that in this regard $ß_1$-selective blockade with atenolol is equally effective. This finding seems to contradict some pharmacological studies that suggest that prejunctional ß-adrenoceptors are of a $ß_2$ subtype (3,4). However, in other experiments it has been shown that facilitation of noradrenaline outflow by stimulation of prejunctional ß-adrenoceptors can indeed be abolished by the $ß_1$-selective ß-adrenoceptor antagonist metoprolol (5,7,35). Therefore, some caution is necessary, when the *in vitro* classification of the prejunctional ß-adrenoceptor is extrapolated to the *in vivo* situation.

The sympathetic nervous system and the renin angiotensin system are closely interrelated. Angiotensin II enhances sympathetic activity in several ways (36). However, active renin was not increased by infusion of adrenaline, which makes it unlikely that the adrenaline-induced elevations in noradrenaline were mediated by the renin-angiotensin system.

In conclusion, the present results provide evidence for the hypothesis that elevated levels of adrenaline are capable of enhancing sympathetic activity in man. This enhancement is mediated by ß-adrenoceptors and can be antagonized not only by non-selective, but apparently also by $ß_1$-selective ß-adrenoceptor blocking agents.

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Chapter 9

SUMMARY AND CONCLUSIONS

That stress can be detrimental for man's health, is widely accepted. However, the mechanisms through which stress could have such a deleterious effect has long been a matter of debate. In this thesis we describe studies exploring the possibility of adrenaline playing a role in the pathogenesis of a rise in blood pressure that could lead to the development of primary hypertension.

The relationships between stress and the release of adrenaline and between stress and the elevation of blood pressure are well recognized. However, since infused adrenaline leading to plasma concentrations in the high physiological range, rather decrease than increase blood pressure, the mechanism through which adrenaline would be able to lead to an increase in blood pressure therefore remained to be elucidated. It was some 10 years ago that an hypothesis was put forward indicating a possible way through which adrenaline-mediated increases in blood pressure could occur (1-3). At that time it was clear from experimental studies that adrenaline could enhance noradrenaline release through stimulation of prejunctional ß-adrenoceptors, thereby increasing vasoconstrictor tone (4). This effect was thought to be mediated by circulating adrenaline. Experimental studies also indicated that adrenaline could be taken up in sympathetic neurons and be "co-released" along with noradrenaline during sympathetic stimulation and then exerts its noradrenaline release enhancing effect (4). In that way adrenaline could mediate its blood pressure increasing effect also after plasma adrenaline concentrations had returned to normal again. It was hypothesized that these adrenaline mediated effects on blood pressure would also be operational in man and thereby suggesting that adrenaline could play a role in the pathogenesis of hypertension (1-3). Indeed our group demonstrated that infusion of ß-adrenoceptor agonists resulted in an increase in plasma noradrenaline (5). Furthermore it was shown that infusion of adrenaline leading to plasma concentrations into the high physiological range, augmented the pressor responses to reflex sympathetic nervous system stimulation by standardized cold pressor and isometric exercise testing (3,6,7).

At the time we started our experiments (1986), two studies were available on the haemodynamic effects of adrenaline during, but also after its administration. Brown and Dollery studied 6 normotensive volunteers and infused adrenaline over 2 hours (8). Indirectly measured blood pressure was higher upto 2½ hours after cessation of the infusion, however, the authors reported an absence of statistical significance. Nezu et al (9) showed that 30 minutes of adrenaline infusion resulted in a significant rise in blood pressure after cessation of the infusions, which lasted at least for 90 minutes. Pretreatment with propranolol completely abolished this effect. No data were available on more long term (several hours) pressor effects of adrenaline and no studies existed examining the question whether the effect observed in the two

studies just mentioned (8,9), was unique for adrenaline. So we formulated questions addressing these issues and we hoped they could be answered by the studies presented in this thesis.

The questions, which are described in Chapter 1, were:

1 Does adrenaline, when infused intravenously in normotensive subjects leading to plasma levels in the high physiological range, cause a sustained and protracted rise in blood pressure, which outlasts the duration of the increments in circulating adrenaline? And if so, does this effect on blood pressure occur at rest or during periods of activation of the sympathetic nervous system?

2 If the questions under number 1 are positively answered, does intravenously administered noradrenaline have the same effect? When the effect of adrenaline is indeed mediated through prejunctional β_2-adrenoceptors, we hypothesized that this would not be the case.

3 Do the pressor responses to standardized sympathetic nervous system stimulation by cold pressor and isometric exercise testing before, during and 18 hours after cessation of infusions of adrenaline, noradrenaline or dextrose 5% differ? Again we hypothesized that infusion of adrenaline but not noradrenaline would lead to an amplification of the blood pressure responses.

4 Are the changes in plasma concentrations of adrenaline and noradrenaline during the infusions of adrenaline and noradrenaline also detectable in alterations in the amounts of catecholamines and their metabolites excreted in the urine?

5 Do the infusions of catecholamines, which lead to alterations in plasma concentrations within the physiological range, have any effect on urinary sodium excretion or on plasma levels of several hormones, potassium or glucose?

6 What is the effect of non-selective and β_1-selective ß-blockade on the adrenaline mediated facilitation of noradrenaline release and the adrenaline induced enhancement of reflex sympathetic nervous system activity?

In Chapter 2 an outline is given of the study protocol, the subjects, the methods and the statistical tests that were used.

In Chapter 3 we addressed question 1 and 2, as mentioned above. We studied the effects of intravenously administered adrenaline in normotensive subjects. Since it was hypothesized, that the blood pressure increasing effect of adrenaline would be

mediated through β_2-adrenoceptors, we compared the results not only with those of placebo infusion (dextrose 5%), but also with those of noradrenaline infusion. It was found that, as could be expected from the literature, during the infusions, while the subjects rested supine in a quiet and half-dark room (in order to achieve a condition of low-grade sympathetic nervous system activity), blood pressure decreased during adrenaline and increased during noradrenaline, whereas heart rate increased during adrenaline and decreased during noradrenaline. However, during the 18 hours postinfusion period a significantly higher blood pressure was found during the adrenaline study as compared to the dextrose study, whereas noradrenaline did not exert a similar pressor effect. The effects on blood pressure were especially seen in periods of (assumed) increased sympathetic nervous system activity, that is during ambulation and in the second part of the night (in which more frequent periods of REM sleep, which are accompanied by increased activity of the sympathetic nervous system, are known to occur). The blood pressure effects were less clear during the first part of the night and the next morning, while the subjects were lying in supine position for 1 hour, both periods in which sympathetic nervous system activity is low.

Question 3 was addressed in Chapter 4. The blood pressure response during cold exposure and isometric exercise testing is a result of sympathetic nervous system activation. The blood pressure responses during the tests performed before starting the infusions, were similar. The blood pressure values obtained during the tests during the infusions, showed an enhanced response during adrenaline, whereas results during noradrenaline and dextrose infusion were not different. The tests done approximately 18 hours after cessation of the infusions showed similar results, that is an enhanced response during the adrenaline study as compared to the noradrenaline and dextrose study and no differences between the noradrenaline and dextrose studies.

In Chapter 5 question 4 was addressed. It is clear from the results presented in this chapter, that the major urinary metabolites of adrenaline and noradrenaline are vanillyl mandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). Relatively small amounts of unmetabolized adrenaline and noradrenaline and metanephrine and normetanephrine are excreted. The amounts of adrenaline infused during our studies induced a detectable increase in the amounts of adrenaline and metanephrine excreted. However, only about 28% of the infused adrenaline could be "traced" back by increased excretions of these metabolites. In the amounts of VMA and MHPG excreted no changes were detected. Significantly enhanced excretion of noradrenaline and normetanephrine was observed during

the noradrenaline study, however, only approximately 9% of the amount of noradrenaline infused could be "traced" in this way. Again no changes in the amounts of VMA and MHPG excreted were found. During the postinfusion period of the adrenaline study, we also found an increased excretion of normetanephrine. Since normetanephrine is primarily formed from released noradrenaline, these results might be taken as an indication of increased noradrenaline release in this period, which is hypothesized to be the explanation of the observed pressor effect (see discussion of Chapter 3).

Question 5 was explored in Chapter 6. Small although significant changes in blood glucose and plasma concentrations of potassium and some hormones like renin, aldosterone and insulin were found, both during adrenaline and noradrenaline infusion. These results extend the knowledge on the so-called "metabolic" effects of circulating catecholamines and they clearly show that adrenaline and noradrenaline have besides haemodynamic effects, definitely also "metabolic" effects, which could be of clinical relevance.

In Chapter 7 the results of measurements of sodium excretion are presented (question 5). Although there are several possible mechanisms through which catecholamines can influence renal sodium excretion, we did not find any difference between the three infusion conditions during, but also not in the 18 hour period after the three infusions, despite the fact that the infusions had marked haemodynamic effects. The possible explanations for these findings are discussed in Chapter 7. At any rate the results are compatible with the suggestion of Borst and others (10), that "deficient" sodium excretion is an essential feature in the development and maintenance of hypertension. They further suggest a role for catecholamines mediated mechanisms in establishing this "deficient" sodium excretion.

Question 6 was addressed in Chapter 8. As was shown in previous studies of our group, low dose intravenous infusion of adrenaline increased arterial levels of noradrenaline as an index of overall sympathetic activity. Infusion of adrenaline also increased the production of noradrenaline in the forearm as an index of local muscle sympathetic activity. This facilitating effect of adrenaline was abolished by both β_1-selective and non-selective ß-adrenoceptor antagonists.

Final conclusions

Considering the above mentioned findings, it can be concluded that the

"adrenaline-hypertension" hypothesis might indeed be true in man. The studies clearly show that the adrenaline mediated effect can occur while plasma concentrations are increased (during infusion), since we observed an amplification of the blood pressure response during cold exposure and isometric exercise testing. But adrenaline can also exert a pressor effect after plasma concentrations had been returned to normal. We observed an increased blood pressure during the 18 hour postinfusion period and also an amplification of the blood pressure response to cold exposure and isometric exercise testing done 18 hours after cessation of the adrenaline infusion. Since plasma half-live of adrenaline is only a few minutes, this effect is probably a result of adrenaline being taken up in sympathetic neurons and being co-released along with noradrenaline during sympathetic stimulation. Subsequently adrenaline can facilitate noradrenaline release and in that way increase vasoconstrictor tone. Since noradrenaline is devoid of this facilitating effect, the adrenoceptors involved are hypothesized to be of β_2-subtype. The finding that the adrenaline mediated enhancement of noradrenaline release and its pressor effects can be antagonized by ß-adrenoceptor blockade, further support the view that ß-adrenoceptors are involved in this mechanism.

The results described in this thesis also present an explanation for the observed "vasodilatory" action of ß-adrenoceptor antagonists, in that they interfere with the adrenaline-induced amplification of sympathetic activity (11,12). Indeed, it has been shown that a β_2-selective adrenoceptor antagonist lowers blood pressure (13). We have, however, to conclude that the results of these pharmacological studies indicate, that *in vivo* the prejunctional ß-adrenoceptors involved are not of an absolute β_2-selective subtype, since atenolol (β_1-adrenoceptor selective) besides bopindolol (non-selective) antagonized the effects of adrenaline. Another possibility might be that atenolol in the dosage used in this study was able to antagonize the prejunctional β_2-adrenoceptor, despite its proven selectivity for postjunctional β_1-adrenoceptors at the dose used.

That the observed sustained and protracted pressor effect of adrenaline in the period after the infusion had been stopped, is indeed a result of enhanced noradrenaline release, is supported by the finding of increased urinary normetanephrine excretion in that period. Because normetanephrine is formed primarily from released noradrenaline, it can be taken as a measure for increased sympathetic neuronal activity.

A potential additional role of adrenaline in the pathogenesis of hypertension is its interaction with renal sodium excretion in such a way, that it counteracts pressure natriuresis. We observed no increased sodium excretion despite of the fact that blood pressure was higher in the postinfusion period of adrenaline.

Finally, we have demonstrated that both adrenaline and noradrenaline, besides having haemodynamic effects, also induce some "metabolic" changes and can influence the plasma concentrations of some hormones at plasma concentrations of these catecholamines well within the physiological range.

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Chapter 10

SAMENVATTING EN CONCLUSIES

De stelling, dat stress slecht is voor de gezondheid, is algemeen aanvaard. Echter hoe stress zijn nadelige invloed kan uitoefenen is niet geheel opgehelderd. In dit proefschrift worden studies beschreven naar de mogelijke rol van het bijniermerghormoon adrenaline bij het ontstaan van hypertensie.

Dat stress leidt tot een toename van de adrenaline secretie door de bijnier is al lang bekend. Echter hoe adrenaline een bloeddrukverhogend effect zou moeten hebben was minder duidelijk. Wanneer men een zodanige hoeveelheid adrenaline per infuus toedient, dat de plasmaspiegel van adrenaline hoog fysiologische concentraties bereikt, zoals die in het dagelijks leven ook voorkomen, blijft de bloeddruk gelijk of neemt zelfs iets af. Circa 10 jaar geleden werd een hypothese geformuleerd omtrent het mechanisme van het bloeddrukverhogende effect van adrenaline (1-3). Deze hypothese was gebaseerd op uitgebreid *in vitro* en dierexperimenteel onderzoek (4). Volgens deze hypothese zou adrenaline, wanneer het in verhoogde plasmaconcentraties voorkomt, de vrijmaking van noradrenaline kunnen laten toenemen door stimulatie van prejunctionele ß-adrenoceptoren. Noradrenaline is een sterk vaatvernauwende stof en leidt op die wijze tot bloeddrukverhoging. Ook zou het mogelijk zijn dat adrenaline wordt opgenomen in het sympatisch neuron. Adrenaline zou dan kunnen worden vrijgemaakt tezamen met noradrenaline tijdens activatie van het sympatische zenuwstelsel en vervolgens de vrijmaking van noradrenaline verder kunnen stimuleren via activatie van de prejunctionele ß-adrenoceptoren. Op deze wijze zouden ook tijdelijke verhogingen van de plasmaconcentratie van adrenaline kunnen leiden tot dit bloeddruk verhogende effect. Resultaten van experimenteel onderzoek bevestigden het bestaan van dit mechanisme. Bij de mens was dit nog niet bewezen. Wel had onze groep reeds aangetoond dat ß-adrenoceptor agonisten de plasma noradrenaline concentratie konden doen toenemen (5). Voorts was inmiddels aangetoond dat de bloeddrukrespons op reflex sympatische stimulatie (onderdompeling van één hand in ijswater en isometrische inspanning) tijdens de infusie van adrenaline was toegenomen (3,6,7).

Rond de tijd dat wij onze onderzoekingen begonnen (1986) waren slechts 2 studies gepubliceerd die de hemodynamische veranderingen beschreven tijdens doch vooral ook na adrenaline infusie. Brown en Dollery infundeerden adrenaline bij 6 vrijwilligers gedurende 2 uur en registreerden de bloeddruk gedurende 2½ uur (8). Zij vonden ten opzichte van een controle experiment een verhoogde bloeddruk na beëindiging van het adrenaline infuus, doch de verschillen waren niet significant. Nezu en medewerkers (9) vonden een significant verhoogde bloeddruk na adrenaline infusie ten opzichte van een placebo studie gedurende 1½ uur na beëindiging van het adrenaline infuus. Dit effect werd tenietgedaan door

propranolol.

Het doel van de onderzoekingen, die zijn beschreven in dit proefschrift, was dus: het nagaan of het bestaan van bovengeschets bloeddrukverhogend mechanisme ook bij de mens waarschijnlijk kon worden gemaakt. De vragen die wij ons daartoe hebben gesteld, staan geformuleerd in Hoofdstuk 1.

De vragen waren:

1 Geeft adrenaline, wanneer het intraveneus geïnfundeerd wordt in zodanige hoeveelheden dat plasmaconcentraties worden bereikt binnen het fysiologisch bereik, een verhoging van de bloeddruk welke langer duurt dan de duur van het infuus? Wanneer dit inderdaad het geval is, treedt dit effect dan op in rust of tijdens sympatische activatie?

2 Indien de eerste vraag onder 1 bevestigend was beantwoord, heeft dan intraveneuse toediening van noradrenaline hetzelfde effect op de bloeddruk?

3 Zijn de veranderingen in de bloeddruk, welke optreden tijdens op gestandaardiseerde wijze uitgevoerde stimulatietesten van het sympatisch zenuwstelsel (onderdompeling van een hand in ijswater en isometrische inspanning), voor, tijdens en 18 uur na beëindiging van infusies van adrenaline, noradrenaline en placebo (glucose 5%) verschillend?

4 Zijn de veranderingen in de plasmaconcentraties van adrenaline en noradrenaline tijdens en na de infusen van adrenaline en noradrenaline ook te herkennen in veranderingen in de hoeveelheden van deze catecholamines en van de catecholamine metabolieten, die in de urine worden uitgescheiden?

5 Leiden de infusen van adrenaline en noradrenaline tot veranderingen in de plasmaconcentraties van hormonen als renine, aldosteron, groeihormoon, prolactine en cortisol, kalium en glucose en wat is het effect op de natriumuitscheiding in de urine?

6 Wat is het effect van non-selectieve en β_1-selectieve adrenoceptor blokkade op de door adrenaline gemedieerde stimulatie van noradrenaline vrijmaking en de door adrenaline gemedieerde versterking van de sympaticus activiteit?

In Hoofdstuk 2 worden het studieprotokol, de proefpersonen, de methoden van onderzoek en de procedures voor statistische bewerking beschreven.

In Hoofdstuk 3 beschrijven wij de effecten op de bloeddruk en hartfrequentie van de infusies van adrenaline, noradrenaline en placebo. Tijdens de infusen van adrenaline neemt de bloeddruk af en tijdens die van noradrenaline toe, terwijl de

hartfrequentie toeneemt tijdens adrenaline en afneemt tijdens noradrenaline. In de ongeveer 18 uur durende periode, dat de bloeddruk nog geregistreerd werd na beëindiging van de infusen, vonden wij dat de bloeddruk na adrenaline hoger was dan na noradrenaline of placebo. Dit effect van adrenaline op de bloeddruk in deze postinfusie periode was het meest uitgesproken in perioden van (verondersteIde) toegenomen sympatische activiteit d.w.z. tijdens mobilisatie en tijdens het tweede deel van de nacht (wanneer REM slaap vaker voorkomt en de sympatische activiteit hoger is) en het minst duidelijk in perioden van lage sympatische activiteit, d.w.z. in het eerste deel van de nacht en gedurende de 1 uur durende periode van bedrust de volgende ochtend voor het beëindigen van de bloeddrukregistratie. De bloeddruk na noradrenaline infusie was niet verschillend van die na placebo toediening. Verschillen in hartfrequentie werden niet gevonden in de postinfusie perioden.

In Hoofdstuk 4 beschrijven wij de effecten op de bloeddruk en hartfrequentie tijdens de koude expositie en isometrische inspanning. De veranderingen in de bloeddruk tijdens de testen gedaan voorafgaand aan de 3 infuus condities, vertoonden geen significante verschillen. De bloeddruk tijdens de testen, welke werden uitgevoerd tijdens de infusies en 18 uur na beëindiging hiervan, vertoonde een sterkere stijging bij de adrenaline studie dan bij de placebo studie. Bij de noradrenaline studie werd dit versterkende effect niet waargenomen. Veranderingen in de hartfrequentie zowel voor, tijdens als na de infusen vertoonden geen significante verschillen tussen de 3 infuus condities.

In Hoofdstuk 5 worden de meetresultaten van de urine-uitscheiding van de hoeveelheden adrenaline, noradrenaline en metabolieten beschreven. Vanillylamandelzuur (VMA) en 3-hydroxy-4-hydroxyfenylglycol (MHPG) blijken kwantitatief de belangrijkste metabolieten van adrenaline en noradrenaline te zijn, terwijl een geringe hoeveelheid van de twee catecholamines ongemetabolizeerd of als normetanefrine of metanefrine in de urine verschijnt. Infusie van adrenaline leidt tot een toename van de urine-uitscheiding van adrenaline en metanefrine, terwijl infusie van noradrenaline leidt tot een toename van noradrenaline en normetanefrine uitscheiding. Aangezien de totaal geïnfundeerde hoeveelheden adrenaline en noradrenaline slechts gering waren, konden geen veranderingen in de uitscheiding van VMA en MHPG worden vastgesteld. Opvallend en mogelijk van groot belang was de bevinding dat tijdens de periode na de adrenaline infusie een verhoogde uitscheiding van normetanefrine werd gevonden. Daar verondersteld wordt dat dit metaboliet met name afkomstig is van neuronaal vrijgemaakt noradrenaline kan deze bevinding worden beschouwd als een aanwijzing dat in

deze periode inderdaad een toegenomen hoeveelheid noradrenaline is vrijgemaakt. Zoals gesteld is Hoofdstuk 3, veronderstellen wij dat het bloeddrukeffect van de adrenaline infusie tijdens deze postinfusie periode te verklaren is door een toegenomen noradrenaline vrijmaking.

In Hoofdstuk 6 wordt beschreven, dat adrenaline en noradrenaline, behalve hemodynamische effecten ook invloed hebben op de plasmaspiegel van kalium en de bloedspiegel van glucose. Tevens bleken de plasmaconcentraties van renine, aldosteron en insuline te veranderen onder invloed van infusies van zowel adrenaline als noradrenaline.

In Hoofdstuk 7 worden de meetresultaten van de natriumuitscheiding in de urine beschreven. Zowel tijdens de infusen als gedurende de 18 uur durende postinfusie periode, werden geen verschillen in natriumuitscheiding gevonden. Dit ondanks het feit dat toch duidelijk waarneembare hemodynamische veranderingen optraden. De mogelijke mechanismen van de catecholamine gemedieerde effecten op de natriumuitscheiding worden besproken. In ieder geval moet worden vastgesteld dat deze waarneming past in de door Borst (10) in de begin zestiger jaren geformuleerde hypothese. Hij veronderstelde dat een "ontoereikende" natriumuitscheiding een centrale "afwijking" was bij hypertensie, in die zin dat bij hypertensie een hogere bloeddruk noodzakelijk is om de natriumuitscheiding op peil te houden. Onze resultaten suggereren een rol voor een catecholamine gemedieerd mechanisme in deze "afwijkende" natriumuitscheiding, zowel voor adrenaline als noradrenaline.

In Hoofdstuk 8 bevestigen wij eerdere bevindingen van onze groep dat lage doseringen adrenaline intraveneus toegediend leiden tot een toename van de plasmaconcentratie van noradrenaline. Adrenaline induceert ook een toename van de noradrenaline productie in de arm. Dit faciliterende effect van adrenaline kan worden tegengegaan door ß-adrenoceptor blokkade.

Eindconclusies

Bovengenoemde resultaten steunen de "adrenaline-hypertensie" hypothese. De studies tonen aan dat infusie van adrenaline, waardoor plasmaconcentraties worden bereikt welke in het hoog fysiologische bereik liggen, leidt tot amplificatie van de bloeddrukresponse tijdens koude expositie en isometrische inspanning. Beide testen veroorzaken een stimulatie van het sympatisch zenuwstelsel en infusie van adrenaline leidt tot versterking daarvan.

Adrenaline blijkt echter ook een effect te hebben nadat het infuus was beëindigd

en de plasmaconcentraties van adrenaline weer op uitgangsniveau waren teruggekeerd. Wij vonden namelijk een hogere bloeddruk in de 18 uur durende postinfusie periode van adrenaline in vergelijking met de bloeddruk in die periode na placebo infusie. Tevens is de bloeddruk response tijdens koude expositie en tijdens isometrische inspanning 18 uur na beëindiging van het adrenaline infuus groter dan de responsen tijdens de testen na noradrenaline en placebo infusie. Aangezien de halfwaarde tijd van plasma adrenaline niet meer is dan enkele minuten, zijn deze resultaten het best als volgt te verklaren. Circulerend adrenaline kan worden opgenomen in sympatische neuronen en kan worden vrijgemaakt tezamen met noradrenaline tijdens sympatische activatie. Vervolgens kan adrenaline de prejunctionele ß-adrenoceptoren stimuleren, wat leidt tot verdere toename van de noradrenaline vrijmaking, hetgeen vasoconstrictie veroorzaakt en stijging van de bloeddruk tot gevolg heeft. Aangezien noradrenaline de bovengeschetste effecten niet heeft, wordt verondersteld dat de verantwoordelijke adrenoceptor van het $ß_2$-subtype is. De bevinding dat de adrenaline gemedieerde toename van noradrenaline vrijmaking en de toegenomen bloeddrukstijging tijdens de testen die leiden tot toegenomen sympatische activatie, kunnen worden tegengegaan door ß-adrenoceptor blokkade, is een extra aanwijzing dat inderdaad ß-adrenoceptoren een rol spelen in dit proces. Het te-niet-doen van de adrenaline geïnduceerde toename van sympatische activiteit door ß-adrenoceptor blokkers kan ook worden beschouwd als een mogelijke verklaring voor de beschreven vaatverwijdende werking van deze medicamenten (11,12). Onze groep toonde aan in een voorgaande studie, dat een $ß_2$-selectieve adrenoceptor blokker een bloeddrukverlagend effect bleek te hebben (13). Dat de adrenaline gemedieerde potentiëring van de sympatische activiteit kan worden tegengegaan door zowel een $ß_1$-selectieve (atenolol) als een niet-selectieve ß-blokker (bopindolol), suggereert dat *in vivo* de prejunctionele adrenoceptoren niet strikt $ß_2$-selectief zijn. Een andere verklaring zou kunnen zijn dat atenolol in de dosering zoals die in deze studie werd gebruikt, ook in staat is prejunctionele $ß_2$-adrenoceptoren te blokkeren, ondanks de gebleken selectiviteit van atenolol voor postjunctionele $ß_1$-adrenoceptors in de gebruikte dosering.

In de postinfuus periode van de adrenaline studie vonden wij een verhoogde uitscheiding in de urine van normetanefrine. Aangezien van normetanefrine wordt verondersteld dat deze metaboliet met name wordt gevormd uit neuronaal vrijgemaakt noradrenaline kan deze bevinding worden gezien als een aanwijzing dat er in deze periode inderdaad sprake was van een toegenomen noradrenaline produktie. Zoals beschreven in Hoofdstuk 3 vermoeden wij dat deze toegenomen noradrenaline vrijmaking de verklaring is van het gevonden effect op de bloeddruk in de postinfusie periode van adrenaline.

Een andere mogelijke bijdrage van adrenaline in de pathogenese van hypertensie is het effect op de natriumuitscheiding in de urine. Het bleek namelijk dat "druk-natriurese" werd voorkomen, daar wij geen toegenomen natriumuitscheiding vonden in de periode na het adrenaline infuus, terwijl in deze periode de bloeddruk verhoogd was.

Tenslotte bleek dat zowel adrenaline als noradrenaline toegediend in een zodanige dosering dat plasmaconcentraties worden bereikt in het hoog fysiologische bereik, nog andere dan hemodynamische effecten hadden. Veranderingen werden geconstateerd in plasma kalium en bloed glucose terwijl ook de plasmaconcentraties van renine, aldosteron en insuline werden beïnvloed.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 7 juni 1956 geboren in 's-Gravenhage. Na het behalen van het atheneum B diploma in 1974, werd in datzelfde jaar met de studie geneeskunde begonnen aan de Medische Faculteit van de Erasmus Universiteit te Rotterdam. Na het artsexamen in 1981 werkte hij voor 6 maanden als "vakantie-assistent" op de afdeling heelkunde van het Zuiderziekenhuis te Rotterdam (hoofd: Dr. G.A.A. Olthuis). In de periode april 1982 tot april 1987 volgde hij de opleiding interne geneeskunde op de afdeling Interne Geneeskunde 1 van het Academisch Ziekenhuis Rotterdam-Dijkzigt (hoofd: Prof. Dr. M.A.D.H. Schalekamp). Op 1 april 1987 werd hij geregistreerd als internist. Tot en met maart 1991 bleef hij werkzaam op deze afdeling. Vanaf 1 april 1991 is hij werkzaam op de afdeling Nefrologie en Hypertensie van het Academisch Ziekenhuis Utrecht (hoofd: Prof. Dr. H.A. Koomans).

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