BETA-2 ADRENERGIC EFFECTS ON THE SYMPATHETIC NERVOUS SYSTEM.

Beta-2 Adrenerge Effecten op het Sympathische Zenuwstelsel

PROEFSCHRIFT

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Chapter 1

INTRODUCTION

The origin of the studies, described in this thesis, dates back to 1979. In that year my colleague, R.P. Verhoeven, studied the responsiveness of hyperthyroid patients to beta adrenoceptor activation [1]. In his experiments he made the serendipitous observation that the plasma levels of the sympathetic transmitter, noradrenaline, increased during infusion of the beta adrenoceptor agonist isoprenaline. At the time most of us felt that this could be explained by reflex increase in sympathetic nervous activity, due to the vasodilatation caused by isoprenaline, my promotor, Prof. Schalekamp, took a different view. He suggested that the increase in noradrenaline during infusion of isoprenaline could be mediated by presynaptic beta adrenoceptors, which would serve to facilitate the release of noradrenaline [2,3,4]. He gave the impetus to the studies that followed.

THE SYMPATHO-ADRENAL SYSTEM

The autonomic nervous system consists of nerves controlling the functions of the heart, vascular and gut smooth muscle cells and several glands. Its efferent part can be divided in the cranio-sacral outflow or parasympathetic nervous system and the thoraco-lumbar outflow or sympathetic nervous system. The preganglionic sympathetic nerves run a short course from the cell bodies in the spinal cord to the ganglia in the sympathetic trunk, close to the vertebral column. Their neurotransmitter is acetylcholine. The postganglionic nerves run from the sympathetic trunk to the various effector organs. Their neurotransmitter is noradrenaline. Noradrenaline exerts its effects through receptors, which are designated 'alpha' or 'beta' adrenoceptors. Vasoconstriction is mediated by alpha receptors. The increase in heart rate and contractility and the stimulation of renin release are mediated by beta receptors.

The medulla of the adrenal gland can be regarded as a homologue of the sympathetic ganglion. It is innervated by nerves that have acetylcholine as the neurotransmitter. When stimulated, the adrenomedullary cells liberate the hormone adrenaline, which differs from noradrenaline only by one additional methyl group (Fig. 1). Adrenaline, like noradrenaline, exerts its effects on the target organs through alpha or beta adrenoceptors. Therefore, adrenaline and noradrenaline have many effects in common. Different affinities for a number of pharmacological compounds allowed these receptors to be further divided into the alpha-1, alpha-2, beta-1 and beta-2 subtypes. Alpha-1, alpha-2 and beta-1 receptors are activated both by adrenaline and noradrenaline. Beta-2 receptors are only activated by adrenaline. Beta-2 receptor stimulation causes vasodilatation, bronchodilatation and a number of metabolic effects.

The sympathetic nervous system is tonically active, but its influence becomes greater with physical activity. Under resting

conditions circulating levels of adrenaline are very low, probably sub-threshold for most of its effects. Adrenaline rises to high levels under conditions of physical activity or "stress", like pain, shock, hypothermia, hypoglycemia, etc. Also mental stress raises adrenaline levels. [5].

Since the sympathetic nervous system and the adrenal medulla usually act in concert, and have many effects in common, the combination is often referred to as the sympatho-adrenal system.

The studies presented in this thesis are concerned with the possible influence of the adrenomedullary hormone, adrenaline, on the sympathetic nervous system.

PRESYNAPTIC BETA ADRENOCEPTORS

A sympathetic neuro-effector junction or synapse consists of (1) the sympathetic nerve ending or terminal varicosity which contains vesicles that store the transmitter noradrenaline, (2) the synaptic cleft, into which noradrenaline is released, and (3) the effector organ with its alpha and/or beta adrenoceptors, which, when activated by noradrenaline, cause that organ to respond (see Fig. 2). The propagation of an electrical pulse into the nerve terminal leads to noradrenaline release. After its release, most noradrenaline is recaptured by the nerve ending into storage vesicles, from which it can be released again. Part of the noradrenaline is degraded in the surrounding tissue and a few percent leaks away and ultimately enters the blood [6].

The presence of several 'presynaptic' receptors, located on the nerve terminal itself, has been postulated. They are likely to exert a modulating influence on the amount of transmitter released in response to nerve stimulation. The best characterised are the presynaptic alpha adrenoceptors. It has been demonstrated that they can be activated by transmitter noradrenaline and mediate an inhibitory effect (negative feedback) on further noradrenaline release [7].

In 1975 Adler-Graschinski and Langer, who studied noradrenaline release from isolated guinea pig atria, were the first to report an increased noradrenaline release in response to nerve stimulation in the presence of the beta adrenoceptor agonist isoprenaline [2]. Since then, several beta agonists, including the hormone adrenaline, have been shown to facilitate the release of noradrenaline from a variety of tissues, irrespective of the type of postsynaptic receptor present. This facilitating effect has been shown to be reversible by beta antagonist drugs. Especially in blood vessels, presynaptic beta adrenergic facilitation of noradrenaline release has also been shown to augment the effector organ response. Thus, the existence of presynaptic beta adrenoceptors, which serve to facilitate the release of noradrenaline in response to nerve stimulation, is well established [for review see ref. 4].

Possible function of presynaptic beta adrenoceptors

It was originally proposed that transmitter noradrenaline in low concentrations facilitates its own release through activation of presynaptic beta receptors. At higher concentration activation of

presynaptic alpha receptors would lead to the opposite effect [2]. This view has now been abandoned.

Based on the order of potency of different beta agonists on presynaptic receptors, the latter may be classified as being of the beta-2 subtype. Noradrenaline is not a beta-2 agonist. Beta-2 receptors are activated by adrenaline. In 1976 Stjärne and Brundin showed that adrenaline, in physiological concentrations, increased noradrenaline release from human omental arteries and veins in response to electrical stimulation. They were the first to suggest that presynaptic beta receptors serve to augment sympathetic transmitter release in situations of increased secretion of adrenaline [3]. In this way adrenaline would increase the 'gain' of the sympathetic nervous system.

In 1981 this hypothesis was somewhat modified by Majewski et al who showed that adrenaline, after intravenous infusion, was taken up in rat atria and retained for several hours. After electrical stimulation of the isolated atria adrenaline was 'co-released' together with noradrenaline. Thus, neuronal adrenaline could still act on presynaptic beta receptors at a time when blood levels are no longer elevated [8].

In vivo evidence of presynaptic beta adrenoceptors facilitating noradrenaline release

At the time of Verhoeven's observation, that isoprenaline infusions in humans induce an increase in plasma noradrenaline, there was little in vivo evidence for a role of presynaptic beta adrenoceptors in noradrenaline release. In 1977 Yamaguchi et al, in experiments using electrical stimulation of the cardioaccelerator nerve in dogs, had shown that isoprenaline augmented the rise in coronary sinus plasma levels of noradrenaline. The betablocker sotalol had the opposite effect [9]. This evidence has recently been criticized because of the possible influence of altered noradrenaline kinetics resulting from changes in coronary blood flow [10]. In 1981 Dahlöf had shown that both noradrenaline release from cat hindlimb mucle and vasoconstriction in response to lumbar nerve stimulation were reduced by beta adrenoceptor blockade [11]. In 1982 Majewski et al provided evidence for an increased noradrenaline release rate in rabbits after intravenous administration of adrenaline, which could be prevented by a blocker of the neuronal uptake as well as by beta adrenoceptor blockade [12]. In vivo evidence for presynaptic beta adrenoceptors in man was entirely lacking when we started our studies on the effect of beta agonists on plasma noradrenaline concentration (see Chapters 2 and 3).

Relevance to physiology and pathophysiology

Presynaptic facilitation of noradrenaline release by adrenaline will enhance alpha and beta-1 receptor responses, for instance vasoconstriction, increased cardiac output and renin release. One may wonder about the physiological significance of this mechanism. In the classical 'fright and flight' reaction adrenaline might prepare for an optimal response of cardiac output when muscular activity commences. It could further serve to counteract the fall in blood pressure that results from

vasodilitation in the active muscle groups. It is possible that under pathological conditions the implications of presynaptic effects of adrenaline are of greater importance than in normal circumstances. Majewski et al have shown that a slow release depot implantation containing adrenaline caused hypertension in rats. Since heart rate remained unchanged, the rise in blood pressure was likely to be due to a rise in peripheral vascular resistance. Blood pressure remained increased when blood levels of adrenaline had returned to normal. After pretreatment with a betablocker, adrenaline had no such effect [13]. Patients with essential hypertension often have higher blood levels of adrenaline and noradrenaline than normotensive controls [14-17]. In such patients a positive correlation has been found between the level of adrenaline and the vasodilitation that could be achieved by alpha adrenoceptor blockade [18]. These data suggest that adrenaline, presumably through presynaptic beta receptor activation, may increase vascular resistance and lead to hypertension. Indeed it is conceivable that increased secretion of adrenaline is the primary cause of essential hypertension [19].

Implications for betablocker therapy

The mechanism by which betablockers lower blood pressur is unknown. Lowering of cardiac output, inhibition of renin production, resetting of the arterial baroreflex and central nervous system effects have all been put forward as the main mechanism underlying their antihypertensive effect. In 1976 Stjärne and Brundin suggested the possibililty that betablockers exert their antihypertensive effect through blockade of presynaptic beta receptors. Their hypothesis was never widely accepted. There may be several reasons for this. First, evidence that presynaptic beta receptors are functionally active in man has long been lacking. Second, as presynaptic beta receptors are supposed to be of the beta-2 subtype, the hypothesis would predict that non-selective betablockers have a better antihypertensive effect than beta-1 selective blockers. In fact, both seem to be equally effective. Moreover, the antihypertensive potential of beta-2 selective blockade had not been studied. Third, one might expect presynaptic beta blockade to cause a fall in plasma noradrenaline, as is observed with clonidine, a centrally acting blocker of sympathetic nervous activity. After betablockade, however, plasma noradrenaline is usually somewhat increased. This is difficult to reconcile with the view that betablockers inhibit noradrenaline release.

AIMS OF THIS THESIS

The main questions addressed in this thesis are:

1) Can presynaptic beta-2 receptors be shown to be functionally active in intact man.
2) What, if any, is their physiological role.
3) Are these receptors involved in the antihypertensive effect of betablocker therapy.

Chapter 1

Our studies were performed in patients with untreated essential hypertension and in normotensive volunteers. First we studied the changes in plasma noradrenaline after administering beta receptor agonists and antagonists with different beta-1 / beta-2 selectivity. The effects on plasma noradrenaline were analysed in relation to several beta receptor mediated responses. Changes in heart rate, systolic time intervals and renin were taken as beta-1 receptor mediated responses. Changes in diastolic pressure and plasma potassium were taken as beta-2 mediated responses. This analysis allowed us to examine which beta receptor subtype was involved in the rise in noradrenaline and wether this rise was a direct effect of beta receptor activation or the result of reflex sympathetic stimulation.

Second, we studied the effects of adrenaline, infused in physiological doses, on plasma adrenaline and noradrenaline and on the cardiovascular responses to cold exposure and isometric exercise. These studies were performed before and after betablockade.

Third, we carried out a double blind placebo controlled crossover trial in patients with essential hypertension to examine wether a selective beta-2 receptor antagonist is capable of lowering blood pressure.

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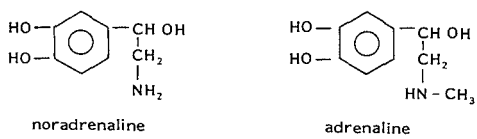

Fig.1. Molecular struction of noradrenaline and adrenaline

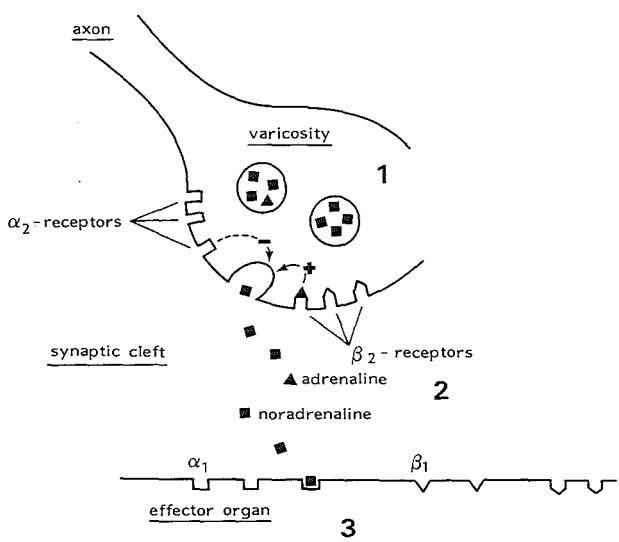

Fig.2. Schematic representation of the sympathetic synapse.

Chapter 2

Br. J. clin. Pharmac. (1982), **13**, 717–721

ELEVATED PLASMA NORADRENALINE IN RESPONSE TO β-ADRENOCEPTOR STIMULATION IN MAN

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1 Dose-dependent increments of plasma noradrenaline were observed during graded infusions of (±)isoprenaline (3.5–35 ng kg^{-1} min^{-1} i.v.) in seven normal subjects and in ten subjects with borderline hypertension. At the highest dose of isoprenaline, noradrenaline rose by 166 ± 16 pg/ml in normals and by 169 ± 34 pg/ml in hypertensives (mean ± s.e. mean).

2 In the subjects with borderline hypertension isoprenaline infusions were repeated after 7 days of treatment with (±)propranolol (320 mg/day, divided into 4 doses) and subsequently after 7 days of treatment with (±)atenolol (100 mg/day) 2–3 h after the morning dose of β-adrenoceptor blocker. The dose-response curve for plasma noradrenaline was shifted to higher doses of isoprenaline by a factor of 4 by atenolol and the heart rate response was similarly shifted. The heart rate response was shifted by a factor of 16 by propranolol, but plasma noradrenaline did not change after isoprenaline under propranolol treatment, even when isoprenaline was given at doses high enough to induce increments of heart rate similar to those without β-adrenoceptor blocker treatment.

3 In the subjects with borderline hypertension mean and diastolic intra-arterial pressures fell at the highest dose of isoprenaline by 9 ± 2 and 13 ± 2 mm Hg respectively. These effects were antagonized by propranolol and not by atenolol.

4 The observed rise in plasma noradrenaline after isoprenaline might have been caused by baroreflex-stimulation of central sympathetic outflow. The isoprenaline-induced decrease in mean arterial pressure, however, was small. Moreover pulse pressure rose and this tends to suppress rather than stimulate baroreflex-mediated sympathetic activity. Activation of presynaptic β-adrenoceptors, allegedly of the β_2-subtype, is known to facilitate noradrenaline release upon nerve stimulation of isolated tissues. Our results lend support to the hypothesis that such a facilitatory mechanism is also operative in intact man.

Introduction

Experimental studies have demonstrated that the release of noradrenaline from postganglionic sympathetic nerve fibres can be modulated by α- and β-adrenoceptors, which are located on the nerve endings (Langer, 1977; Starke, 1977). Activation of these so-called presynaptic α-adrenoceptors by noradrenaline triggers a negative feedback mechanism, by which the transmitter inhibits its own release. Measurements of noradrenaline overflow from isolated tissues have shown that activation of presynaptic β-adrenoceptors has the opposite effect; through these receptors the release of the sympathetic transmitter is facilitated during nerve stimulation (Langer, 1977). Such a mechanism may contribute to the cardio-vascular actions of β-adrenoceptor stimulants and blockers. It may also be important in the physiological control of sympathetic activity and in the pathogenesis of hypertension.

It remains to be proved that β-adrenoceptor-mediated facilitation of noradrenaline release is operative in intact man. In the context of a study on the mechanism of β-adrenoceptor blocker treatment of hypertension we have given graded infusions of isoprenaline before and after treatment with propranolol or atenolol and have measured the effects on plasma noradrenaline. The results are at least com-

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patible with the hypothesis that presynaptic β-adrenoceptors are important for the physiological regulation of noradrenaline release.

Methods

Seven normal subjects, aged 21–43 years (5 males) and ten subjects with hypertension, aged 21–40 years (all males) were studied. All subjects had given their informed consent. The hypertensive subjects were previously untreated, and blood pressure in the outpatient clinic was 140–160 mm Hg systolic and 90–105 mm Hg diastolic. They showed no signs of cardiac or renal disease and results of routine investigations, including intravenous urography, were normal.

The subjects were lying in bed. An indwelling cannula was placed in an arm vein for drug infusion. A catheter was placed in the radial artery for pressure measurement and blood sampling. First, NaCl (0.15 mol/l) was infused with a calibrated infusion pump at a rate of 22 ml/h. After 90 min the infusion pump was switched from saline to a solution of isoprenaline in saline. The dose was increased stepwise as indicated under **Results**. Each step lasted for 30 min. The total amount of fluid that was given was less than 100 ml. Arterial pressure and ECG were recorded continuously. The hypertensive subjects were studied on three occasions: (1) while untreated, (2) after 7 days on propranolol, 80 mg orally, 4 times daily, and (3) after 7 days on atenolol, 100 mg orally, once daily. The isoprenaline infusions were started 2–3 h after the subjects had taken their morning dose of β-adrenoceptor blocker.

Blood samples for measurement of plasma noradrenaline were drawn 30 and 1 min before starting isoprenaline infusion and at the end of each infusion step. Blood was collected in chilled heparinized 10 ml tubes containing 15 mg glutathione. Samples were centrifuged within 15 min at 4°C. Under vigorous stirring 0.5 ml 3 M trichloroacetic acid was added to 4.5 ml plasma. The deproteinized plasma was separated by centrifugation at 4°C and stored at −20°C. The samples were assayed within 2 weeks. Noradrenaline was measured by a radio-enzymatic method (Henry *et al.*, 1975) in which noradrenaline is converted to tritiated adrenaline by [^{3}H]-S-adenosyl-L-methionine and the enzyme phenylethanol-*N*-methyltransferase. This method is highly specific for noradrenaline; adrenaline, dopamine and isoprenaline do not interfere. Measurements were made in duplicate both with and without addition of noradrenaline as internal standard. Intra- and inter-assay variation coefficients are less than 10%. All samples of one infusion experiment were run in the same assay batch.

Data are presented as means ± s.e. mean.

Results

Plasma noradrenaline was 219 ± 18 pg/ml in the normal subjects and it rose by 19 ± 7, 46 ± 11, 134 ± 14 and 166 ± 16 pg/ml after isoprenaline at infusion rates of 3.5, 7.0, 14 and 35 ng kg^{-1} min^{-1} respectively. It had returned to 249 ± 22 pg/ml 15 min after the infusion of isoprenaline had been stopped. Five of the normal subjects had a control infusion of saline before active drug was given. With saline alone plasma noradrenaline had changed by −6 ± 4 pg/ml at the last infusion step after 2 h, as compared with +159 ± 14 pg/ml after active drug. In the hypertensive subjects the increase in noradrenaline was also dose-related and the response curve was similar to the curve in the normal subjects (Figure 1). The effects of 7 days treatment with propranolol or atenolol on resting levels of noradrenaline, heart rate and arterial pressure are presented in Table 1. Noradrenaline had not significantly changed and the effects on heart rate and arterial pressure were not different for the two β-adrenoceptor blockers.

The dose-response curve for noradrenaline was shifted to higher doses of isoprenaline by a factor of 4 by atenolol and the heart response was similarly shifted. The heart rate response was shifted by a factor of 16 by propranolol but plasma noradrenaline did not change after isoprenaline under propranolol

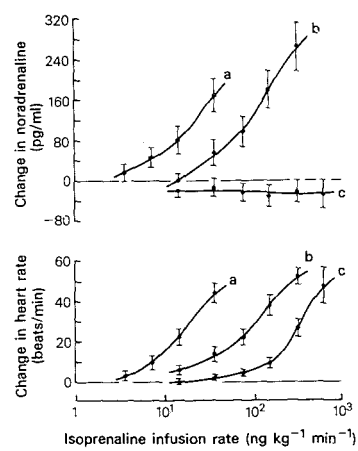

Figure 1 Changes in plasma noradrenaline and heart rate during isoprenaline infusion in ten subjects with borderline hypertension before β-adrenoceptor blocker treatment (a) and after treatment with atenolol (b) or propranolol (c). The means of the two pre-infusion values at −30 and −1 min were taken as control values.

Table 1 Basal values of plasma noradrenaline, heart rate and arterial pressure in the hypertensive subjects before and after β-adrenoceptor blocker treatment

Treatment	*Noradrenaline (pg/ml)*	*Heart rate (beats/min)*	*Arterial pressure*		
			systolic (mmHg)	*diastolic (mmHg)*	*mean (mmHg)*
None	224 ± 27	73 ± 3	152 ± 4	77 ± 3	99 ± 4
Atenolol 100 mg daily	252 ± 28	54 ± 2	130 ± 5	62 ± 2	82 ± 3
Propranolol 320 mg daily	256 ± 24	52 ± 2	130 ± 5	66 ± 2	84 ± 2

Basal values in each subject are the means of the two values determined 30 and 1 min before isoprenaline infusion was started.

treatment, even when isoprenaline was given at doses high enough to induce increments of heart rate similar to those without β-adrenoceptor blocker treatment.

Mean and diastolic intra-arterial pressure fell by 9 ± 2 and 13 ± 2 mm Hg respectively after isoprenaline, 35 ng kg^{-1} min^{-1}, without β-adrenoceptor blocker treatment. They fell by 13 ± 3 and 21 ± 3 mm Hg after isoprenaline, 280 ng kg^{-1} min^{-1} with atenolol, and did not change after isoprenaline, 560 ng kg^{-1} min^{-1}, with propranolol, despite similar increments of heart rate, i.e. 51 ± 4 beats/min under atenolol and 48 ± 9 beats/min under propranolol as compared with 44 ± 5 beats/min without β-adrenoceptor blocker treatment.

Systolic intra-arterial pressure and pulse pressure rose with doses of isoprenaline that increased heart rate both before β-adrenoceptor blocker treatment and during treatment with atenolol or propranolol.

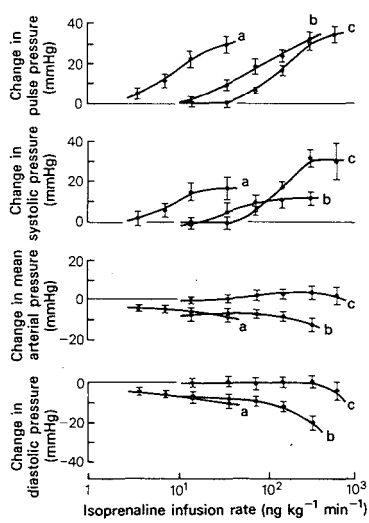

Figure 2 Changes in arterial pulse pressure and in systolic, mean and diastolic arterial pressures during isoprenaline infusion in ten subjects with borderline hypertension before β-adrenoceptor blocker treatment (a) and after treatment with atenolol (b) or propranolol (c). The means of the two pre-infusion values at −30 and −1 min were taken as control values.

Discussion

It is generally accepted that short-term changes in plasma noradrenaline during various forms of physical stress are an index of sympathetic activity (Lake *et al.*, 1976; Watson *et al.*, 1979). Most likely, the dose-dependent increments of noradrenaline we have observed during isoprenaline infusion are also caused by increased release of the transmitter.

One could argue that changes in the metabolic clearance rate of plasma noradrenaline have influenced our results. However, adrenaline and noradrenaline have been shown to accelerate their own clearance rate (Silverberg *et al.*, 1978; Clutter *et al.*, 1980), whereas propranolol is known to reduce the clearance of noradrenaline (Cryer *et al.*, 1980). In view of these findings isoprenaline would be expected to accelerate the metabolic clearance of noradrenaline. This strengthens our conclusion that the rise in plasma noradrenaline in response to isoprenaline infusion is caused by increased transmitter release and that this effect on transmitter release is blocked by propranolol.

Reflex stimulation of central sympathetic outflow through the carotico-aortic baroreceptor system might have caused the rise in noradrenaline we observed. However, there are reasons to assume that this is not the full explanation. Firstly, the fall in mean arterial pressure after isoprenaline was small and it is questionable whether the induced changes in baro-

reflex activity were sufficient to be reflected by changes in plasma noradrenaline. It has been reported that changes in transmural carotid sinus pressure elicited by means of a neck pressure chamber had no significant effect on plasma noradrenaline, even when pressure increments in the neck chamber had led to opposite changes in mean arterial pressure in the range of 10–15 mm Hg (Mancia *et al.*, 1979). Secondly, withdrawal of vagal tone through the baroreflex would perhaps increase the slope of the heart rate dose-response curve during isoprenaline infusion. The curve, however, was not steeper before β-adrenoceptor blocker treatment and with atenolol than with propranolol, whereas the hypotensive effect of isoprenaline was antagonized by propranolol and not by atenolol. Thirdly, pulse pressure was increased by isoprenaline. This causes suppression rather than stimulation of baroreflex-mediated sympathetic activity (Angell James & De Burgh Daly, 1970).

Although a role for the baroreflex in the observed changes in plasma noradrenaline cannot be disproved, our results are compatible with the hypothesis that presynaptic β-receptor-mediated facilitation of noradrenaline release, which has been shown during nerve stimulation of tissues *in vitro* (Langer, 1977; Stjärne & Brundin, 1976), is also operative in intact man. Such a facilitatory mechanism has also been demonstrated *in vivo* during cardioaccelerator nerve stimulation in the anaesthetized dog (Yamaguchi *et al.*, 1977).

In our study the non-selective β-adrenoceptor blocker, propranolol, prevented the effect of isoprenaline on noradrenaline even at doses of isoprenaline that caused tachycardia, whereas the effect of the selective β_1-adrenoceptor blocker, atenolol, on the noradrenaline response was overcome by such high doses of isoprenaline. This suggests that the facilitatory receptors are of the β_2-subtype. It should be kept in mind however, that the β_1–β_2-subdivision is based on the relative efficacies of β-adrenoceptor drugs on postsynaptic receptors and not on presynaptic receptors.

The role of this facilitatory mechanism in the cardiovascular effects of β-adrenoceptor stimulants and blockers remains to be clarified. Possibly the heart rate response to activation of postsynaptic cardiac β-adrenoceptors by isoprenaline is enhanced by presynaptic facilitation of noradrenaline release, whereas facilitated transmitter release could activate postsynaptic vascular α-adrenoceptors thereby counteracting the β-adrenoceptor-mediated vasodilatory effect of isoprenaline. Antagonism of presynaptic β-adrenoceptor stimulation could be a component of the hypotensive action of β-adrenoceptor blockers; diminished release of noradrenaline reduces the sympathetic tone on both heart and blood vessels.

In most hypertensive patients whose blood pressure is lowered by propranolol, plasma noradrenaline is either unchanged by the β-adrenoceptor blocker or slightly increased (Rahn *et al.*, 1978). However, the absence of a fall in circulating noradrenaline after propranolol is not incompatible with diminished release of the transmitter since the metabolic clearance of plasma noradrenaline is reduced by this β-adrenoceptor blocker (Cryer *et al.*, 1980).

Experiments with isolated tissues have indicated that adrenaline, in physiological concentrations, can act as an appropriate stimulus for presynaptic β-adrenoceptors to enhance the nerve stimulation-induced release of noradrenaline (Stjärne & Brundin, 1975; Majewski *et al.*, 1980). Continuous infusion of a so-called subpressor dose of adrenaline causes hypertension in rats (Majewski *et al.*, 1981). Increased plasma levels of adrenaline have been observed in patients with essential hypertension (De Champlain *et al.*, 1976; Franco-Morselli *et al.*, 1977). Such patients also showed exaggerated reflex increases in plasma catecholamines, and these responses were normalized under propranolol treatment (De Champlain *et al.*, 1977). Facilitated noradrenaline release through activation of β-adrenoceptors by adrenaline may therefore be important in the aetiology and maintenance of high blood pressure in patients with essential hypertension.

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Effects of Selective and Nonselective β-Agonists on Plasma Potassium and Norepinephrine

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Summary: The effects of graded infusions of the β-agonists isoproterenol (nonselective), *l*-prenalterol (β_1-selective), and salbutamol (β_2-selective) on plasma potassium and norepinephrine were compared in subjects with borderline hypertension. Potassium levels fell with all three agonists, and norepinephrine levels rose with isoproterenol and salbutamol. These effects on potassium and norepinephrine were closely correlated and occurred at the same dose ranges as the cardiovascular responses. The fall in plasma potassium was probably caused by activation of β-receptors, mainly on skeletal muscle, with subsequent stimulation of active sodium-potassium transport across the cellular membrane. The rise in plasma norepinephrine may have been due to activation of β-receptors on sympathetic nerve endings. Activation of these presynaptic receptors is known to enhance the release of norepinephrine during nerve stimulation. For a given increase in heart rate and cardiac contractility, as measured by the heart rate–corrected duration of total electromechanical systole, which are mainly β_1-responses, the effects on potassium and norepinephrine were in the order: salbutamol > isoproterenol > prenalterol. β-Blockade with propranolol (nonselective), 80 mg four times a day, or atenolol (β_1-selective), 100 mg once a day, antagonized the hypokalemic effect of isoproterenol as well as the rise in norepinephrine, but when isoproterenol was infused in doses high enough to overcome the blockade of the heart rate response, the effects on norepinephrine and potassium were abolished by propranolol and not by atenolol. Thus, the receptors in question appear to be of the β_2-subtype. Epinephrine, which is known to circulate in high concentrations under stressful conditions, is generally considered to be the endogenous activator of these receptors. β-Blockers may prevent hypokalemia and may suppress sympathetic activity, which could contribute to their so-called cardioprotective action. The evidence presented here and in other studies that β_2-type receptors are involved in stress-induced hypokalemia and in presynaptic facilitation of norepinephrine release warrants further consideration of the clinical significance of β-blocker selectivity. **Key Words:** β_1-Adrenoceptor—β_2-Adrenoceptor—Norepinephrine — Potassium — Presynaptic β-adrenoceptor.

There is growing evidence that the sympathoadrenal system is important for potassium homeostasis and that some drugs acting on adrenoceptors have a profound effect on the extracellular potassium concentration (1,2). Experiments with isolated tissues, skeletal muscle in particular, have demonstrated that active sodium-potassium transport is stimulated when the tissue is exposed to epinephrine and β-agonist drugs (3). Potassium is shifted by these agents to the intracellular compartment. The skeletal muscles contain the largest single pool of potassium in the body, and the rate of potassium exchange across the muscle plasma membrane is therefore of great importance for the minute-to-minute regulation of the extracellular potassium concentration.

It is also known that epinephrine and β-agonist drugs act on sympathetic nerve endings to facilitate the release of norepinephrine (4–7). Recently, we observed a dose-dependent rise in plasma norepinephrine during infusion of the nonselective β-agonist isoproterenol in healthy volunteers and in subjects with borderline hypertension. Presynaptic facilitation of transmitter release could be the underlying mechanism (8).

Stimulation of norepinephrine release together with a decrease in extracellular potassium may contribute to the high incidence of cardiac arrhythmias in patients on β-agonist drugs. High plasma levels of epinephrine and norepinephrine are known to occur in patients with myocardial infarction. In such cases epinephrine levels are high enough to

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induce hypokalemia (9). The nonselective β-blocker timolol prevents the hypokalemic response to epinephrine infusion (9). Propranolol, which is also a nonselective β-blocker, diminishes the rise in plasma norepinephrine in patients with myocardial infarction (10). Prevention of β-receptor-mediated hypokalemia and suppression of norepinephrine release may therefore contribute to the so-called cardioprotective action of β-blockers.

In this context we have addressed the following questions. Does the hypokalemic response to β-agonist drugs occur in the same dose range as their effect on plasma norepinephrine? How do these effects relate to the cardiovascular responses? Which β-receptor subtype is involved?

METHODS

Patients

Thirty male subjects, aged 21–40 years, participated in the study after they had given their informed consent. They had borderline hypertension and were previously untreated. Blood pressure in the outpatient clinic was 140–160 mm Hg systolic and 90–105 mm Hg diastolic. There were no signs of cardiac or renal disease, and results of routine investigations, including intravenous urography, were normal.

The β-agonists isoproterenol sulfate (nonselective), *l*-prenalterol (H133/22, Hässle; β_1-selective), and salbutamol (Ventolin℠, Glaxo; β_2-selective) were each given to 10 subjects. The subjects who received isoproterenol were studied on three occasions, that is, before β-blocker treatment, after 7 days of taking propranolol (Inderal℠, ICI; nonselective β-blocker) 80 mg four times a day, and finally after 7 days of taking atenolol (Tenormin℠, ICI; β_1-selective blocker) 100 mg once daily.

Infusions

The subjects were lying in bed. A cannula was placed in an arm vein for drug infusions. A 20-gauge cannula (Quik-Cath, Travenol) was placed in the radial artery for pressure measurement and blood sampling.

Isoproterenol and salbutamol were given via a calibrated infusion pump. First, 0.15 *M* NaCl was infused at a rate of 22 ml/h. After 1 h the infusion was switched from saline to active drug. Concentrations of active drug were chosen in such a way that the total quantity of fluid infused was less than 200 ml. The infusion rate of isoproterenol ranged from 3.5 to 35 ng $kg^{-1}min^{-1}$ in the absence of β-blockade (n = 10), from 14 to 140 (n = 10) or 280 (n = 4) ng $kg^{-1}min^{-1}$ with atenolol, and from 14 to 280 (n = 10) or 560 (n = 5) ng $kg^{-1}min^{-1}$ with propranolol. The infusions in the subjects who were receiving β-blockers were started 2–3 h after the morning dose of β-blocker. The infusion rate of isoproterenol was increased stepwise, each step lasting 30 min. The infusion rate of salbutamol was increased from 17.5 to 175 ng $kg^{-1}min^{-1}$ in steps lasting 15 min. Because the half-life of the chronotropic effect of salbutamol is 20–60 min as opposed to about 5 min for isoproterenol, a plateau state is not reached for salbutamol within 15 min. The maximum cumulative dose was 4.5 μg/kg. Blood samples were taken 15 min before starting active drug infusion, just before the infusion, and at the end of each infusion step. In five subjects who received isoproterenol and were not taking β-blocker and in five subjects who received salbutamol, blood samples were also taken 15, 30, 45, and 60 min after the infusions had been stopped.

Prenalterol was injected intravenously by hand. Each injection lasted 5 min, and the interval between successive injections was 15 min. Doses were increased from 1 to 32 μg/kg. As the half-life of the chronotropic effect of prenalterol is about 2 h, a plateau state is not reached in 15 min. The maximum cumulative dose was 63 μg/kg. Blood samples were taken 15 min before starting the injections, just before the injections, and 14–15 min after the start of each injection.

Heart rate remained below 140 beats/min at maximum doses of β-agonists.

Measurements

Intraarterial pressure and electrocardiographs (ECG) were continuously monitored. Systolic time intervals were recorded using the method described by Lewis et al. (11). Changes in the duration of total electromechanical systole, measured as the time interval between the onset of the Q wave of the ECG and the initial high-frequency vibrations of the aortic closure sound on the phonocardiogram corrected for heart rate (QS_2I), were taken as an index of β-adrenergic stimulation of cardiac contractility. Blood for norepinephrine measurement was collected in chilled, heparinized 10-ml tubes containing 15 mg glutathione. The samples were centrifuged at 4°C within 15 min after collection. Under vigorous stirring, 0.5 ml 3 *M* trichloroacetic acid was added to 4.5 ml plasma and the mixture was centrifuged. The deproteinized supernatant was stored at −20°C and assayed within 2 weeks. A radioenzymatic method was used in which norepinephrine is converted to tritiated epinephrine by phenylethanol-*N*-methyltransferase in the presence of [^{3}H]*S*-adenosyl-*l*-methionine (8). This method is highly specific; the β-agonists and blockers that were given to our subjects did not interfere. Measurements were made in duplicate both with and without addition of norepinephrine as internal standard. Intra- and interassay variation coefficients were less than 10%. All samples from one infusion experiment were assayed in the same batch.

Blood for potassium and sodium measurements was also collected in heparinized tubes and immediately centrifuged. Plasma was stored at −20°C until assayed in triplicate by flame photometry.

Statistics

Results are reported as means ± SEM and were analyzed by Student's *t* tests for paired and unpaired data.

RESULTS

Baseline measurements

Data on blood pressure, heart rate, and plasma concentrations of norepinephrine and potassium before β-agonists were given are summarized in Table 1. There were no significant differences between the groups of subjects when untreated.

Effects of β-agonists

Figures 1–4 illustrate the effects of isoproterenol, salbutamol, and prenalterol. All three agonists in-

TABLE 1. *Blood pressure, heart rate, plasma norepinephrine, and plasma potassium in three groups of subjects before β-agonist infusion*

Subjects	Arterial pressure			Heart rate (beats/min)	Plasma norepinephrine (pg/ml)	Plasma potassium (mEq/L)
	Systolic (mm Hg)	Mean (mm Hg)	Diastolic (mm Hg)			
Subjects to receive salbutamol (n = 10) without pretreatment	136 ± 5	95 ± 4	74 ± 3	70 ± 3	226 ± 28	3.93 ± 0.06
Subjects to receive prenalterol (n = 10) without pretreatment	142 ± 4	95 ± 3	73 ± 2	64 ± 4	204 ± 21	3.81 ± 0.06
Subjects to receive isoproterenol (n = 10)						
Without pretreatment	152 ± 4	99 ± 4	77 ± 3	73 ± 3	224 ± 27	3.82 ± 0.09
With propranolol	130 ± 5	84 ± 2	66 ± 2	52 ± 2	256 ± 24	3.83 ± 0.07
With atenolol	130 ± 5	82 ± 3	62 ± 2	54 ± 2	252 ± 28	3.81 ± 0.08

creased heart rate and systolic pressure and shortened the total electromechanical systole (QS_2I). Plasma norepinephrine rose with isoproterenol and salbutamol but was not significantly altered by prenalterol, whereas plasma potassium fell with all three drugs. There were no significant changes in plasma sodium. With isoproterenol 35 ng $kg^{-1}min^{-1}$, potassium fell below 3.40 mEq/L in 5 of 10 subjects. Both potassium and norepinephrine returned to the initial level 30–45 min after isoproterenol was stopped. With salbutamol, potassium became less than 3.40 mEq/L in 4 of 10 subjects

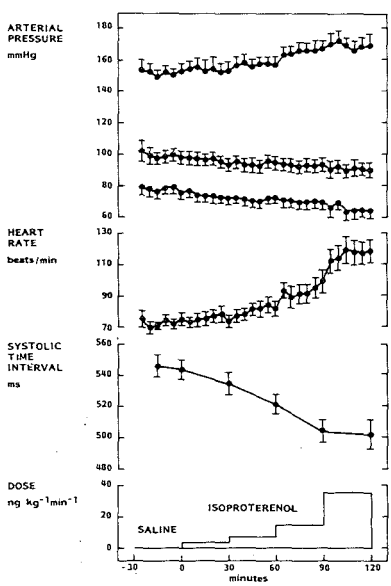

FIG. 1. Cardiovascular effects of intravenous isoproterenol (n = 10). Doses were 3.5, 7, 14, and 35 ng $kg^{-1}min^{-1}$. Systolic time interval refers to the duration of total electromechanical systole corrected for heart rate (QS_2I) and expressed in milliseconds.

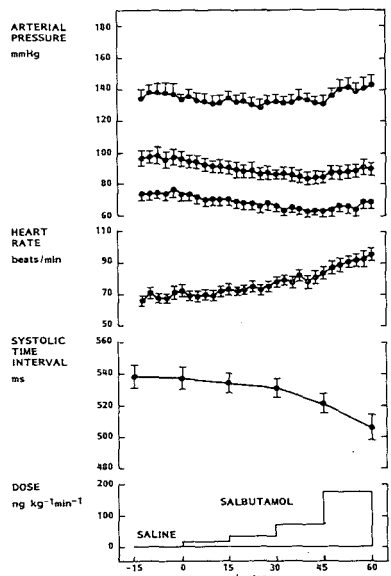

FIG. 2. Cardiovascular effects of intravenous salbutamol (n = 10). Doses were 17.5, 35, 70, and 175 ng $kg^{-1}min^{-1}$. Systolic time interval refers to the duration of total electromechanical systole corrected for heart rate (QS_2I) and expressed in milliseconds.

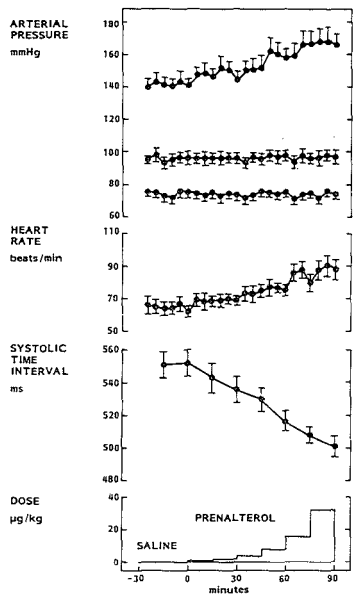

FIG. 3. Cardiovascular effects of intravenous prenalterol (n = 10). Doses were 1, 2, 4, 8, 16, and 32 μg/kg. Systolic time interval refers to the duration of total electromechanical systole corrected for heart rate (QS_2I) and expressed in milliseconds.

after a cumulative dose of 4.5 μg/kg. Both potassium and norepinephrine did not change for 30–45 min after salbutamol was stopped.

For a given increment in heart rate and for a given shortening of the QS_2I, the effects on norepinephrine and potassium were in the order: salbutamol > isoproterenol > prenalterol.

Effects of β-blockers

Neither atenolol nor propranolol had a significant effect on the plasma concentrations of norepinephrine, potassium, and sodium. The rise of norepinephrine and the decrease in potassium induced by isoproterenol were, however, antagonized by these β-blockers (Fig. 5). The heart rate response was shifted to the right by a dose factor of 5 with atenolol and by a factor of 16 with propranolol. The shortening of QS_2I, which is perhaps a more specific β_1-response, was shifted to the right by a factor of 9 with both antagonists. When isoproterenol was given in quantities large enough to overcome the blockade of the heart rate response, the effects on norepinephrine and potassium were abolished by propranolol but not by atenolol.

With isoproterenol 140 ng $kg^{-1}min^{-1}$ under atenolol, potassium fell below 3.40 mEq/L in 5 of 10 subjects, and it was less than 3.00 mEq/L in 2. In four subjects on atenolol, the rate of infusion of isoproterenol was increased to 280 ng $kg^{-1}min^{-1}$. Potassium fell below 3.40 mEq/L in all four, and it was less than 3.00 mEq/L in two.

DISCUSSION

β_1 versus β_2 effects

This study confirms and extends reports that β-receptor stimulation can induce hypokalemia. With isoproterenol, a short-acting β-agonist drug, the half-life of the hypokalemic effect was 30 min or less. The change in potassium was too rapid to be explained by changes in urinary potassium excretion. A rapid redistribution of potassium between the intracellular and extracellular compartments is the most likely explanation. Skeletal muscle is probably the main site of this potassium shift (12,13).

Hypokalemic responses to the β_2-selective agonist salbutamol have been reported in the clinical literature (14–19), but it is generally believed that such responses occur only at very high doses. However, the highest cumulative dose of 4.5 μg/kg we have given is not above the range that is given to patients with an attack of asthma. A significant fall in plasma potassium occurred with doses that increased heart rate by no more than 5 beats/min. With isoproterenol, which is a nonselective β-agonist, and with prenalterol, which is β_1-selective, the hypokalemic effect was also seen over the same dose range as the chronotropic and inotropic responses, although for doses that were equipotent in raising heart rate or in shortening QS_2I, the effect on potassium was less than that with salbutamol. The latter finding indicates that the decrease in plasma potassium is mediated by β_2- rather than β_1-receptors. This is further supported by our observation that the nonselective β-blocker propranolol, but not the β_1-selective blocker atenolol, was able to antagonize the hypokalemic effect of isoproterenol, when the latter was given in doses high enough to overcome the blockade of the heart rate response. In experiments with isolated skeletal muscle, it is also the agents with β_2-agonist activity that promote active sodium-potassium transport; β_1-selective agonists have little effect (20).

The hypokalemic responses to salbutamol and isoproterenol were accompanied by a rise in plasma norepinephrine. There was a fixed ratio between the rise in norepinephrine and the fall in potassium; this ratio was the same for isoproterenol, both without pretreatment and after atenolol, as for sal-

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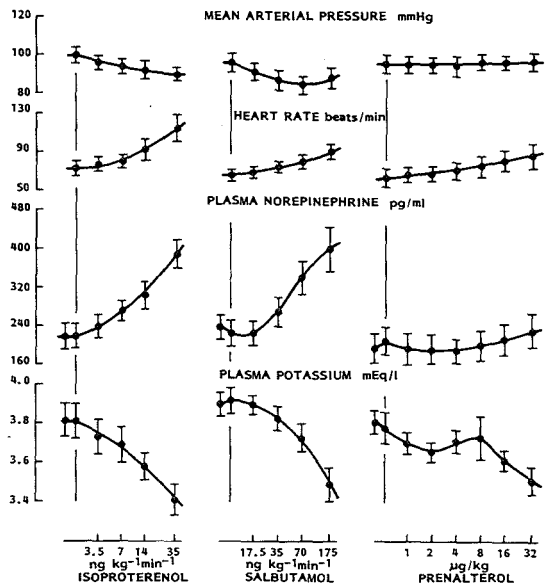

FIG. 4. Effects of isoproterenol, salbutamol, and prenalterol on the plasma concentrations of norepinephrine and potassium. The drugs were administered as indicated for Figs. 1–3.

butamol (Fig. 6). A similar correlation between the changes in norepinephrine and potassium was observed with prenalterol (Fig. 6, left). The effects of isoproterenol on plasma potassium and norepinephrine were equally blocked by propranolol (Fig. 6, right). This is an indication that the rise in norepinephrine is also a β_2-receptor-mediated response.

Theoretically, the rise in plasma norepinephrine may be caused by a diminished neuronal or extraneuronal uptake of the transmitter. Isoproterenol has little inhibitory effect on these uptake processes (21). Moreover, studies in man have shown that the clearance rate of plasma norepinephrine is increased rather than decreased by β-adrenoceptor stimulation (22). This strengthens the conclusion that the rise in plasma norepinephrine we have observed is caused by an increased release of the transmitter.

Presynaptic versus postsynaptic effects

β-Agonists are known to facilitate norepinephrine release from sympathetic nerve endings through activation of receptors located on them (4–6). This could be the mechanism by which plasma norepinephrine is raised by isoproterenol and salbutamol. An alternative explanation could be that β-receptor-mediated vasodilatation activates the arterial baroreflex. The vascular receptors involved in this dilatational effect are of the β_2-subtype, and there is evidence from *in vitro* experiments to suggest that the abovementioned presynaptic receptors are also of the β_2-subtype (23–25). Our results are therefore in accord with both explanations insofar as β_2- rather than β_1-receptors seem to be involved in the changes in norepinephrine levels we have observed.

Several lines of evidence argue against a dominant role for the baroreflex in the observed effects on norepinephrine. The decrease in mean arterial pressure in our experiments was small. Moreover, pulse pressure increased, which would suppress rather than stimulate baroreflex-mediated sympathetic outflow (26). Sympathetic activity was reflexly increased by a neck pressure chamber by Mancia et al. (27). This caused a 20 mm Hg rise in mean arterial pressure, but plasma norepinephrine did not change. On the other hand, Grossman et al. (28) measured a 20 pg/ml rise in plasma norepinephrine for every 1 mm Hg drop in mean arterial pressure during graded infusions of the vasodilator nitroprusside. Although we found a similar pressure-norepinephrine relationship with isoproterenol, it should be emphasized that Grossman et al.

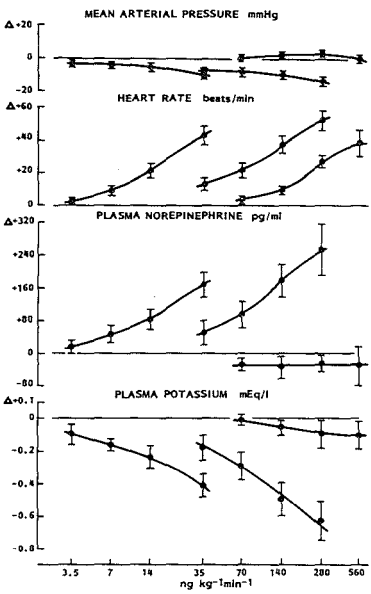

FIG. 5. Antagonistic effects of β-blockers on isoproterenol-induced changes in arterial pressure, heart rate, and plasma norepinephrine and potassium ($n = 10$). **Left:** Dose-response curve in the absence of β-blockade. **Center:** Dose-response curve with atenolol pretreatment. **Right:** Dose-response curve with propranolol pretreatment.

(28) used an indirect method for measuring arterial pressure, whereas we measured it directly. More importantly, the blood pressure-norepinephrine relationship was quite different during salbutamol than during isoproterenol infusion. The drop in mean arterial pressure with low doses of salbutamol was not much different from that after high doses of isoproterenol, but norepinephrine did not rise. In contrast, at higher doses of salbutamol, mean arterial pressure did not fall further, whereas norepinephrine rose to levels as high as or higher than those with isoproterenol. Perhaps the strongest argument against the dominant influence of the arterial baroreflex on the observed changes in plasma norepinephrine comes from the work of Arnold and McDevitt (29), who found that the chronotropic effect of isoproterenol was not modified by pretreatment with atropine. As withdrawal of parasympathetic tone through the baroreflex is the main mechanism underlying the increase in heart rate after the administration of vasodilator drugs (30), these findings cast doubt on an important contribution of the baroreflex.

Pathophysiologic implications

The pharmacologic evidence discussed indicates that activation of β-receptors lowers plasma potassium and stimulates norepinephrine release. The receptors in question appear to be of the β_2-subtype. Because the hormone epinephrine is a more potent stimulator of β_2-receptors than the neurotransmitter norepinephrine, it is likely that the endogenous catecholamine that produces these effects is epinephrine rather than norepinephrine. Epinephrine in the extracellular fluid is taken up by sympathetic nerve endings, and following nerve stimulation, it is "co-released" with norepinephrine. It has been proposed that coreleased epinephrine may activate presynaptic β-receptors, thereby initiating a positive feedback loop by which the release of norepinephrine is enhanced. *In vitro* experiments have indeed provided some evidence to support this proposal (6).

High plasma levels of epinephrine have been documented in many forms of stress, including surgery, severe trauma, and myocardial infarction. It is in these conditions that transient hypokalemia can occur independently of gastrointestinal or renal potassium losses (31–34). Epinephrine may therefore participate in stress-related hypokalemia.

Conversely, an abnormal rise in plasma potassium has been documented during physical exercise in healthy subjects who had been pretreated with propranolol (35,36). Along the same lines, a rise in plasma potassium has been observed during surgery with cardiopulmonary bypass in patients who were receiving propranolol (37), whereas potassium usually falls in patients who are not receiving β-blockers (31).

Not only epinephrine but also norepinephrine is high in the abovementioned forms of stress. This reflects the increased sympathetic activity that is a characteristic feature of these conditions. That presynaptic facilitation of norepinephrine release by epinephrine could play a role in such conditions is supported by the observation that propranolol reduces the increase in plasma norepinephrine following myocardial infarction (10).

Increased sympathetic drive to the heart together with hypokalemia may predispose to cardiac arrhythmias, particularly in patients with myocardial infarction. Both of these arrhythmogenic changes can be favorably influenced by β-blockers, which may well contribute to the so-called cardioprotective effect of these drugs. This cardioprotective property appears to be shared by nonselective β-blockers, as well as by β_1-selective blockers (for review, see 38). Although at therapeutic doses the selectivity of these drugs may be lost, the evidence

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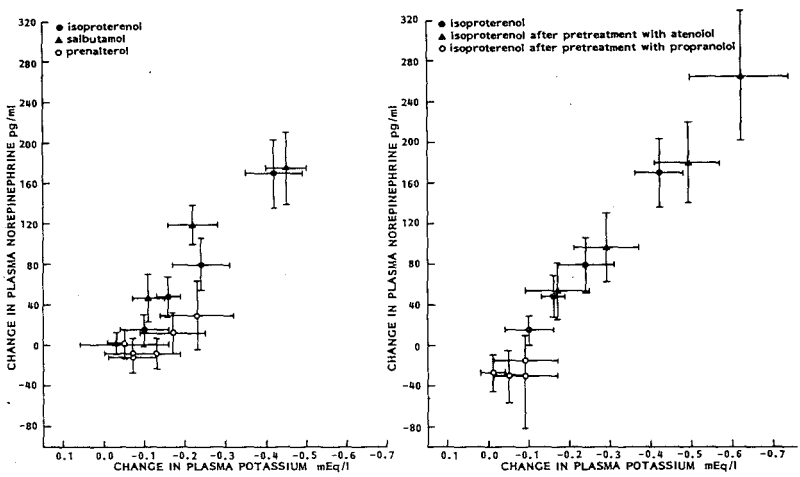

FIG. 6. Correlations between the effects of isoproterenol, salbutamol, and prenalterol on plasma potassium and norepinephrine **(left)** and correlations between the effects of isoproterenol on plasma potassium and norepinephrine when the patients were untreated, after treatment with atenolol and after treatment with propranolol **(right).** The agonists were administered as indicated for Figs. 1–3 and 5.

that β_2- rather than β_1-receptors are involved in stress-induced hypokalemia and presynaptic facilitation of sympathetic transmitter release warrants further consideration of the clinical significance of β-blocker selectivity.

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Stress Levels of Adrenaline Amplify the Blood Pressure Response to Sympathetic Stimulation*

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The possibility that sympathetic pressor responses are modulated by adrenaline-mediated facilitation of neuronal noradrenaline release was explored in 17 subjects with borderline hypertension. Infusion of adrenaline, which raised plasma adrenaline by a factor of 8 to 9, augmented the rise in systolic and diastolic arterial pressure induced by standardized cold pressor and isometric exercise tests. The heart rate response to these tests was not affected. When a low dose of propranolol was given on top of the adrenaline infusion before the cold pressor test, the blood pressure response to cold exposure was not different from the response observed when the test was performed during saline infusion. Plasma noradrenaline was higher during adrenaline infusion then during saline infusion, both before and after the cold pressor and isometric exercise tests, and the effect of adrenaline on plasma noradrenaline was antagonized by propranolol. These observations are consistent with the hypothesis that stress levels of circulating adrenaline may amplify sympathetic pressor responses by facilitation of the release of transmitter noradrenaline.

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Keywords: Adrenaline, noradrenaline, pre-synaptic β-adrenoceptors, stress, hypertension.

Introduction

In vitro studies have shown that the release of noradrenaline from sympathetic nerves is enhanced by β-adrenoceptor agonists [1,2]. The receptors involved appear to be of the β_2-subtype [3-5], and are probably sensitive to the naturally occurring β-adrenoceptor agonist, adrenaline [2,5-8]. These findings have led to the hypothesis of modulation of neuronal noradrenaline release by adrenaline through activation of pre-synaptic β-adrenoceptors located on sympathetic nerve endings. In this way adrenaline may indirectly increase sympathetic vasoconstrictor tone and thereby cause a rise in blood pressure [9].

In the present experiments we studied the effect of adrenaline infusion on the blood pressure response to a reflex increase in sympathetic nervous activity. We also measured the effect of adrenaline on the plasma level of noradrenaline.

Subjects

Seventeen untreated subjects with borderline hypertension, aged 18-46 years, were studied. Their blood pressure in the outpatient clinic ranged from 140 to 160 mmHg systolic and 90 to 100 mmHg diastolic. Routine investigations, including urine analysis, blood chemistry, electrocardiogram (ECG) and chest X-ray, revealed no signs of cardiac or renal disease. All subjects gave their consent after a thorough explanation of the procedures and the purpose of the study.

Methods

The studies were performed while the subjects were supine and resting. Heart rate was derived from the ECG. Blood pressure was measured directly through a cannula in the radial artery. Drug infusions were given via a

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forearm vein.

All subjects performed a series of cold pressor tests by immersion of one hand up to the wrist in ice-water for 60 s. Isometric exercise tests were carried out in eight subjects by handgrip with one hand at 50% of maximal voluntary contraction for 60 s. Heart rate, systolic, diastolic and integrated mean arterial pressure were continuously recorded. An arterial blood sample was drawn within 30 s before and 15 s after completion of the test.

The blood samples were collected in chilled 10-ml tubes containing 12 mg of glutathione and 19 mg of ethylene-bis(oxyethylenenitrile)-tetraacetic acid (EGTA). The tubes were immediately centrifuged at 4°C. Part of the plasma (2.25 ml) was deproteinized with 3 mol/l trichloroacetic acid (0.25 ml), before storage at −20°C. Assays of adrenaline and noradrenaline were carried out in the deproteinized samples, within 2 weeks after blood collection. All samples of one subject were run in the same assay batch, in duplicate, both with and without internal standard. Adrenaline was determined by the radioenzymatic catechol-O-methyltransferase (COMT) method as described by Peuler and Johnson [10]. This method can also be used for determination of noradrenaline. However, in experiments with plasma enriched in adrenaline we have found that high levels of adrenaline occasionally interfere with the noradrenaline determination, resulting in erroneously elevated values for noradrenaline. To avoid this problem in the present investigations, where high levels of adrenaline occur, we determined noradrenaline separately by the selective radioenzymatic phenylethanolamine-N-methyltransferase (PNMT) method described by Henry *et al.* [11]. With this method the addition of adrenaline up to 2000 pg/ml (11 nmol/l) *in vitro* did not interfere with the noradrenaline determination.

Study protocol

Saline was infused at a rate of 5.4 ml/h for 30-45 min before baseline values were recorded. Two cold pressor tests were then performed 60 min apart, with three isometric exercise tests at 15-min intervals in between. Subsequently, the infusion was switched to adrenaline, 30 ng/min (0.16 μmol/min) per kg body weight in an equal volume of saline. This was not noticed by the subjects. After 30 min the tests were repeated in the same sequence as before. In seven subjects propranolol, 0.5 mg i.v, was injected while the infusion of adrenaline was continued. After 20 min two more cold pressor tests were performed, now 20 min apart. To examine the possibility of an order effect the following two experiments were performed. First, the adrenaline infusion was continued in four subjects for a total of 120 min without adding propranolol, and an additional cold pressor test was performed at the end of this period. Second, a cold pressor test was performed in five subjects 30 min after changing back from adrenaline to saline infusion.

Statistical analysis

Results of the two cold pressor tests during adrenaline infusion were averaged and compared with the average of the two tests during saline infusion and with the average of the two tests during adrenaline plus propranolol. Results of the isometric exercise tests were analysed in a similar way. Data are presented as means ± s.e.m.

Differences between responses during infusion of saline, adrenaline and adrenaline plus propranolol were compared by three-way analysis of variance, with type of treatment, subjects and time after beginning of the test considered as sources of variation. For this analysis the variance due to treatment was compared with that due to the interaction between treatment and subjects. This analysis showed significant effects of treatment, but there was also a significant interaction between treatment and time ($P < 0.001$ for the blood pressure response to cold exposure). We therefore subsequently evaluated the effects of treatment at each time point by Student's t-test for paired observations. The latter test was also used to compare the differences in plasma catecholamine levels. A P value < 0.05 was considered to be significant.

Results

Effects of adrenaline infusion

Adrenaline, 30 ng/min (0.16 μmol/min) per kg body weight, caused an eight- to ninefold rise in plasma adrenaline. This was sufficient to exert a detectable cardiovascular effect. There was also a significant rise in plasma noradrenaline (Table 1).

Table 1. Effects of adrenaline during supine rest (n = 17)

	Saline 30 min	Adrenaline 30 min	P
Arterial pressure (mmHg)			
Systolic	148 ± 2	148 ± 3	NS
Mean	101 ± 2	91 ± 1	<0.001
Diastolic	80 ± 1	69 ± 1	<0.001
Heart rate (beats/min)	73 ± 2	82 ± 2	<0.001
Adrenaline (nmol/l)	0.47 ± 0.07	4.17 ± 0.55	<0.001
Noradrenaline (nmol/l)	1.29 ± 0.17	1.56 ± 0.21	<0.05

NS, not significant.

With the cold pressor test during control saline infusion there was a gradual rise in both systolic and diastolic arterial pressure over the 60-s period. Heart rate rose more abruptly within 10 s. The pressor effect of cold exposure was increased by adrenaline infusion. The increments of systolic and diastolic arterial pressure in the 20-60-s period were significantly greater than during saline infusion. The rise in heart rate was not altered by adrenaline (Fig. 1). Thirty minutes after the adrenaline infusion was switched back to saline the pressor response to cold exposure did not differ from the response during the first saline period. Thus, it seems unlikely that the increased pressor response observed during adrenaline was due to an order effect.

Isometric exercise also caused a gradual rise in both systolic and diastolic arterial pressure, and a more abrupt rise in heart rate. Adrenaline infusion had an effect on the pressor response to isometric exercise similar to the effect observed with the cold pressor test, and again the

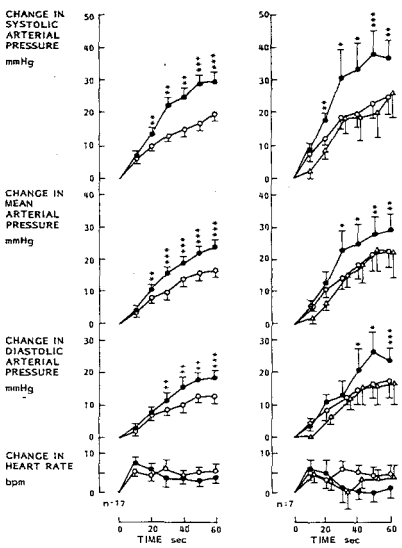

Fig. 1. Cold pressor test, during infusion of saline (○), adrenaline (●) and adrenaline after administration of propranolol (△).*P < 0.05,**P < 0.01,***P < 0.001.

heart rate response was not altered (Fig. 2).

In both tests plasma noradrenaline rose significantly during adrenaline infusion but not during control saline infusion (Fig. 3). The levels of noradrenaline that were reached after the tests were higher during adrenaline infusion than during saline infusion. The tests had no significant effect on plasma adrenaline.

Effects of propranolol injection

The effects of adrenaline on arterial pressure and heart rate were fully antagonized by propranolol, 0.5 mg i.v, and blood pressure and heart rate after adrenaline plus propranolol and with the control saline infusion were not different. The blood pressure and heart rate responses to cold exposure after adrenaline plus propranolol were also not different from the responses during saline infusion (Fig. 1). After infusion of adrenaline for 120 min, without adding propranolol, the pressor response to cold exposure was enhanced to the same extent as it was after 30 and 90 min of infusion. It is therefore unlikely that the diminished pressor response to cold exposure during adrenaline plus propranolol, compared with adrenaline alone, was due to an order effect.

With adrenaline plus propranolol, plasma adrenaline was higher then with adrenaline alone (Table 2). In contrast, plasma noradrenaline was lower with

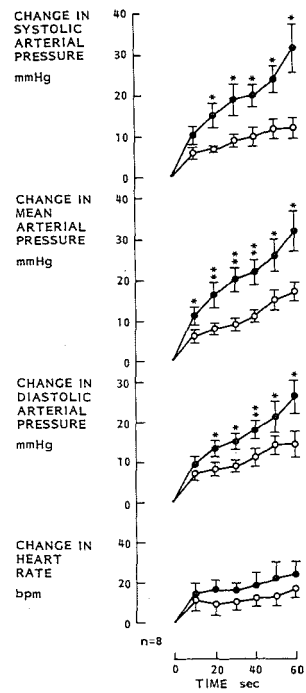

Fig. 2. Isometric handgrip exercise, during infusion of saline (○) and adrenaline (●). *P < 0.05, **P < 0.01.

adrenaline plus propranolol than with adrenaline alone, both before and after the cold pressor test (Fig. 4).

Discussion

Pressor responses

The present study was aimed at the possible role of adrenaline-mediated modulation of noradrenaline release in cardiovascular homeostasis. We therefore infused adrenaline at such a rate that its plasma level remained within the physiological range. This range is from less than 0.25 nmol/l at rest to about 5-7.5 nmol/l during heavy exercise [12]. During infusion of adrenaline a plasma level ranging from 1.5 to 6.5 nmol/l was reached in our subjects.

The cold pressor test evokes a sympathetic reflex, which leads to vasoconstriction and a rise in blood pressure, and this is prevented by α-adrenoceptor blockade [13,14]. Beta-adrenoceptor blockade does not

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Table 2. Effects of adrenaline alone and adrenaline plus propranolol during supine rest (n = 7).

	Saline 30 min	Adrenaline 30 min	Adrenaline plus propranolol 20 min	P*
Arterial pressure (mmHg)				
Systolic	152 ± 3	157 ± 3	154 ± 4	NS
Mean	102 ± 2	94 ± 2	102 ± 2	<0.001
Diastolic	81 ± 2	72 ± 1	82 ± 2	<0.001
Heart rate (beats/min)	70 ± 4	84 ± 4	70 ± 4	<0.001
Adrenaline (nmol/l)	0.56 ± 0.08	4.63 ± 0.68	6.44 ± 0.90	<0.01
Noradrenaline (nmol/l)	1.95 ± 0.24	2.27 ± 0.36	1.48 ± 0.25	<0.05

*Difference between adrenaline and adrenaline + propranolol.

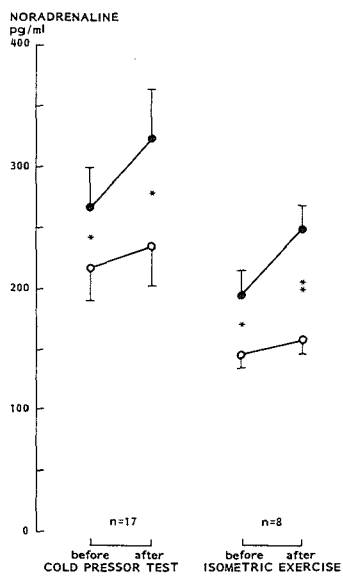

Fig. 3. Plasma noradrenaline concentration before and after cold pressor and isometric exercise tests, during infusion of saline (○) and adrenaline (●). $^{*}P < 0.05$, $^{**}P < 0.01$.

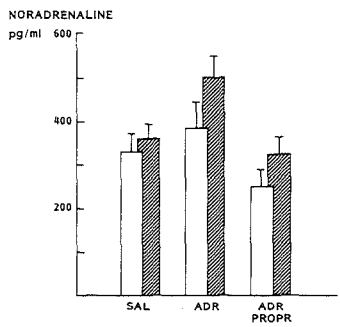

Fig. 4. Plasma noradrenaline before (open bars) and after (hatched bars) cold pressor test (n = 7), during infusion of saline (SAL), adrenaline (ADR) and adrenaline after administration of propranolol (ADR PROPR). Levels after propranolol were lower than with adrenaline alone, both before ($P < 0.05$) and after the test ($P < 0.01$).

change the blood pressure response, although it does attenuate the rise in heart rate [13,15,16]. In the present study adrenaline significantly augmented the pressor response to cold exposure, and this effect was antagonized by propranolol. The data, therefore, indicate that activation of β-adrenoceptors by adrenaline is capable of enhancing a physiological response mediated by α-adrenoceptors. This would fit with evidence from *in vitro* experiments that the release of transmitter noradrenaline is facilitated by adrenaline via activation of pre-synaptic β-adrenoceptors [2,5-8].

Isometric exercise has been used as another stimulus of sympathetic activity. This stimulus is known to increase heart rate and cardiac output, but the rise in blood pressure is mainly due to vasoconstriction, since it is prevented by blockade of α-adrenoceptors but not by β-receptor blockade [15,17-19]. With this stimulus, too, the α-adrenoceptor-mediated rise in blood pressure was augmented in our subjects by adrenaline infusion.

Theoretically the augmented pressor response to these tests might have been due to increased post-synaptic sensitivity, although we do not know of data in the literature to support such a post-synaptic mechanism. Moreover, this would not explain the increase in plasma noradrenaline observed.

Adrenaline, in similar doses as we have given, has been found to increase plasma renin activity [20]. We cannot therefore exclude a facilitatory effect of adrenaline on noradrenaline release in our experiments via the activation of pre-synaptic angiotensin II receptors [21]. However, in previous experiments we have shown that the β-agonist-induced increase in plasma noradrenaline is mediated by β_2- rather than β_1-receptors [22], whereas

stimulation of renin release is a β_1-effect [23-25].

Adrenaline infusion did not alter the heart rate response in the cold pressor and isometric exercise tests. This is not unexpected, since the rise in heart rate is probably caused by vagal withdrawal rather than by sympathetic stimulation [19].

Plasma noradrenaline

Adrenaline infusion caused a significant rise in plasma noradrenaline in our subjects, and this effect was especially evident at the end of the cold pressor and isometric exercise tests. This is further support for the concept of adrenaline-mediated facilitation of neuronal noradrenaline release.

That adrenaline increased plasma noradrenaline by a tyramine-like effect seems unlikely. Adrenaline has little, if any, indirect sympathomimetic activity [26], and there was no indication of tachyphylaxis in our experiments. Moreover, the effect of adrenaline was abolished by propranolol, and this can hardly be ascribed to its ability to block the neuronal uptake of adrenaline. Propranolol is very weak in this respect [27] and we used the drug in a low dose.

One could argue that the fall in diastolic pressure after adrenaline might have led to the observed increase in plasma noradrenaline, through baroreflex stimulation of sympathetic activity. However, the increase in heart rate and pulse pressure after adrenaline would be expected to cause reflex suppression of sympathetic activity [28]. Moreover, baroreflex-induced increased sympathetic activity is an insufficient explanation for the observed enhancement of pressor responses to cold exposure and isometric exercise during adrenaline infusion.

The possibility of an alteration in the clearance rate of plasma catecholamines has also to be considered. However, adrenaline infusion has been reported to increase the clearance rate of plasma noradrenaline [29], and this would tend to cause a drop in plasma noradrenaline and not the rise observed here. Propranolol causes a decrease of the clearance rate of plasma catecholamines [29]. This could explain why, in the present study, the increased plasma level of adrenaline during adrenaline infusion was further increased by giving propranolol on top of adrenaline. In contrast, noradrenaline fell after propranolol. This suggests that, with high levels of circulating adrenaline, propranolol is capable of suppressing noradrenaline release.

An increase in plasma noradrenaline after adrenaline infusion has been reported by others using doses ranging from about 10 to 30 ng/min (0.05-0.16 μmol/min) per kg body weight [30-32]. This effect was not seen with higher doses of adrenaline [21,33]. By activating pre-synaptic α-adrenoceptors these high doses may have counteracted the facilitatory effect that is mediated by pre-synaptic β-adrenoceptors.

Adrenaline that is infused or secreted into the circulation may reach the pre-synaptic receptor sites on sympathetic nerve endings either directly via diffusion or indirectly after neuronal uptake and subsequent co-release together with noradrenaline [7].

So far, data on the effect of adrenaline infusion on both plasma noradrenaline and on cardiovascular responses to sympathetic stimulation appear to suggest that pre-synaptic β-adrenoceptors have a physiological role in blood pressure control. It has been proposed that these receptors are involved in the pathogenesis of hypertension [9] and in some therapeutic effects of β-adrenoceptor blockade [34]. The results of the present study are in keeping with these hypotheses.

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Is β_1-Antagonism Essential for the Antihypertensive Action of β-Blockers?

HIERONYMUS H. VINCENT, ARIE J. MAN IN 'T VELD, FRANS BOOMSMA, FRANS H. M. DERKX, AND MAARTEN A. D. H. SCHALEKAMP

SUMMARY **Both nonselective β-blockers and β_1-selective blockers are effective antihypertensive agents. β_1-Blockade generally is considered to be responsible for their antihypertensive action, whereas β_2-blockade is regarded as undesirable. These common assumptions notwithstanding, the mechanism by which β-blockers lower blood pressure remains unknown. To examine the possibility that β_2-blockade may contribute to the antihypertensive action of β-blocker therapy, we studied the cardiovascular effects of compound ICI 118551, a β_2-selective blocker. First, we showed that 50 mg t.i.d. orally is a β_2-selective dose. In contrast to propranolol, 80 mg t.i.d., or atenolol, 100 mg once a day, 50 mg of ICI 118551 t.i.d. failed to block β_1-mediated inotropic stimulation and stimulation of renin by isoproterenol. We then performed a double-blind, placebo-controlled trial in patients with mild essential hypertension to compare this compound with propranolol, 80 mg t.i.d., and showed that ICI 118551 significantly decreased systolic and diastolic blood pressure. This antihypertensive effect was demonstrated by direct as well as by indirect blood pressure measurements. Thus, contrary to prevailing thought, β_2-blockade has an antihypertensive effect independent of, and distinct from, β_1-blockade. (Hypertension 9: 198–203, 1987)**

KEY WORDS • **β_1-adrenergic receptors • β_2-adrenergic receptors • presynaptic β-adrenergic receptors • β-adrenergic receptor antagonists • hypertension • isoproterenol • norepinephrine • renin**

NONSELECTIVE β-blockers and β_1-selective blockers are both effective in lowering blood pressure. β_1-Adrenergic receptor blockade generally is thought to be essential for this effect, and β_2-blockade is often regarded as an undesirable property that increases the chance of unfavorable side effects.[1] On the other hand, the mechanism by which β-blockers lower blood pressure is not known, and β_1-selective blockers also have their drawbacks. A β-blocker that is highly selective for β_2-adrenergic receptors, ICI 118551 (DL-erythro-3-isopropylamine-1-[7-methyl-4-indanyloxy]-2-butanol hydrochloride), is now available for clinical use.[2] In the present study we found that this compound has an antihypertensive action in a dose that blocks the β_2-adrenergic receptor–mediated responses to isoproterenol but has no detectable effect on β_1-adrenergic receptor–mediated responses.

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Patients and Methods

Nineteen men (mean age, 36 years; age range, 21–50 years) were studied. Their blood pressure in the outpatient clinic was 140 to 160 mm Hg systolic and 90 to 105 mm Hg diastolic. Routine clinical investigations revealed no cause for their hypertension. There were no signs of cardiac or renal disease. After the purpose and the procedures of the study had been explained, the patients gave their consent to participate. The study was approved by the hospital ethical review committee. Drug treatment, if any, was withdrawn 3 weeks before the study. Nine patients (mean age, 35 years) were enrolled in a double-blind, placebo-controlled crossover trial comparing the β_2-selective antagonist ICI 118551 with propranolol. Four of them had never been treated before. Patients were randomized to receive placebo, followed by ICI 118551, placebo, and propranolol or placebo, followed by propranolol, placebo, and ICI 118551. Each treatment period lasted 1 week. ICI 118551 (50 mg t.i.d.) was given as a syrup, and propranolol (80 mg t.i.d.) was given as tablets. Either placebo tablets or placebo syrup was added to active treatment, and both were given in the placebo periods.

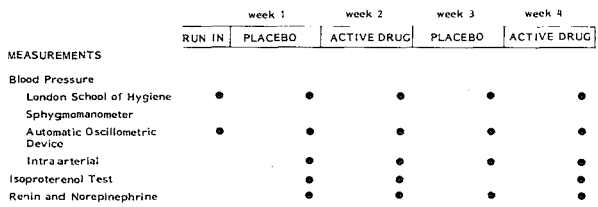

FIGURE 1. *Study protocol.*

The times at which the various measurements were made are shown in Figure 1. The patients rested in the supine position for 15 minutes, and then three blood pressure measurements were made with a blind sphygmomanometer (London School of Hygiene).[3] Then a series of blood pressure measurements was made every 5 minutes for 1 hour using an automatic oscillometric device (Accutorr, Datascope, Paramus, NJ, USA).[4] At the end of each week of placebo or active treatment, blood pressure was also measured intra-arterially. All blood pressure measurements were made 2 to 4 hours after the last dose of placebo or active drug. Blood samples were drawn from the arterial line, collected in chilled, heparinized tubes, and immediately centrifuged. Plasma was stored at −20°C before assay of active renin.[5] Plasma was deproteinized by 10% (vol/vol) of 3 M trichloroacetic acid and stored at −20°C for a maximum of 2 weeks before assay of norepinephrine.[6]

A continuous infusion of isoproterenol was given at the end of Week 1 on placebo and at the end of Weeks 2 and 4 on active drug. The dose of isoproterenol was increased every 15 minutes. Before infusion and at the end of each dose step, intra-arterial pressure, heart rate, and duration of electromechanical systole[7] were measured and blood samples were drawn for determination of plasma renin, norepinephrine, and potassium concentrations.

The effects of the first 2 hours of active drug on blood pressure, heart rate, renin, and norepinephrine were assessed at the beginning of Weeks 2 and 4. The first dose of active drug was given 30 to 45 minutes after the isoproterenol infusion had been stopped. In 10 patients isoproterenol was infused while they were untreated, after 1 week of propranolol, 80 mg t.i.d., and after 1 week of atenolol, 100 mg once daily. These data are presented in order to compare the shifts of the isoproterenol dose-response curves caused by β_2-adrenergic receptor blockade with those caused by β_1-adrenergic receptor blockade.

Data are presented as means ± SEM. Student's paired *t* test was used for comparison. A *p* value of less than 0.05 was considered significant.

Results

Short-term Effects of ICI 118551 and Propranolol

Intra-arterial pressure after the first dose of either ICI 118551 or propranolol did not change in the 2 hours that it was measured (Table 1). In contrast, heart rate was lowered by the two drugs. Plasma renin was significantly lowered by propranolol but not by ICI 118551. Plasma norepinephrine rose after propranolol and was unchanged after ICI 118551 (Figure 2).

Effects of a 1-Week Treatment with ICI 118551 or Propranolol

After 1 week of treatment systolic and diastolic pressures, measured both directly and indirectly, were significantly reduced by ICI 118551 as well as by propranolol (Table 1, Figures 3 and 4). Heart rate and plasma renin were also reduced by both drugs after 1

TABLE 1. *Short-Term and Long-Term Effects of ICI 118551 and Propranolol*

	ICI 118551 (n = 9)			Propranolol (n = 9)		
	Before	2 hr	1 wk	Before	2 hr	1 wk
Intra-arterial blood pressure (mm Hg)						
Systolic	157±2	160±5	150±3*	154±5	154±6	139±6†
Diastolic	86±3	90±2	80±2*	84±3	86±9	72±3†
Heart rate (beats/min)	68±3	61±3*	61±3*	69±3	59±3†	55±3†
Norepinephrine (nmol/L)	1.78±0.20	1.65±0.20	1.55±0.17	1.37±0.20	1.95±0.24*	1.90±0.30*
Active renin (μU/ml)	21±7	13±4	10±3*	21±7	7±6†	6±5†

Values are means ± SEM. Blood pressure, norepinephrine, and renin measured before and 2 hours after the first dose and after 1 week of treatment. Data after 1 week were compared with corresponding data after placebo.
*$p < 0.05$, †$p < 0.01$, compared with pretreatment values.

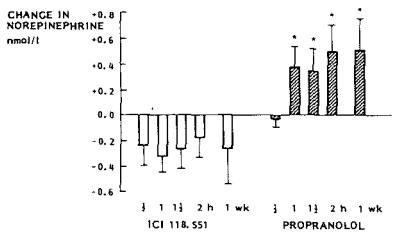

FIGURE 2. *Change in norepinephrine levels after short-term administration and after 1 week of treatment with ICI 118551 or propranolol. Asterisk indicates significant difference ($p<0.05$) compared with placebo values.*

week of treatment. Plasma norepinephrine rose after propranolol but did not change after ICI 118551 (see Figure 2).

Isoproterenol Infusion Tests

As described in Methods, the effects of β_2-selective blockade on the responses to isoproterenol infusion were compared with the effects of β_1-selective blockade by studying two groups of patients. One group received ICI 118551 and propranolol, the other received atenolol and propranolol. In the first group intra-arterial pressure before isoproterenol infusion was 160 ± 4/87 ± 2 mm Hg without β-blocker treatment, 150 ± 3/80 ± 2 mm Hg after 1 week of ICI 118551, and 139 ± 6/72 ± 3 mm Hg after 1 week of propranolol. In the second group the pressure was 152 ± 4/77 ± 3 mm Hg without treatment, 130 ± 5/62 ± 2 mm Hg after 1 week of atenolol, and 130 ± 5/66 ± 2 mm Hg after 1 week of propranolol. Thus, the blood pressure values before isoproterenol infusion were comparable in the two groups.

ICI 118551 did not alter the inotropic effects of isoproterenol (i.e., shortening of electromechanical systole [QS_2I] and rise in systolic pressure; Figure 5). Neither did it alter the effect of isoproterenol on plasma renin (Figure 6). In contrast, these responses were shifted by a dose factor of eight or more by both atenolol and propranolol. The heart rate response was shifted by a dose factor of about two after ICI 118551, by a factor of four after atenolol, and by a factor of 16 after propranolol. The effects of isoproterenol on diastolic pressure, plasma norepinephrine, and plasma potassium were abolished by ICI 118551 and propranolol. After atenolol treatment these effects were shifted by a dose factor of two.

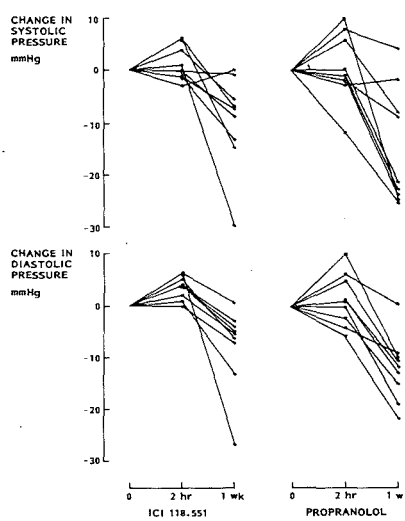

FIGURE 3. *Intra-arterial pressure response 2 hours and 1 week after ICI 118551 and after propranolol. Each line represents one patient.*

Discussion

β_2-Selectivity of ICI 118551

ICI 118551 is a highly selective and specific β_2-adrenergic receptor antagonist in vitro.[2] The present study shows that ICI 118551, given in a dosage of 50 mg t.i.d., is also β_2-selective in vivo. The drug antagonized the effects of isoproterenol on diastolic pressure, plasma norepinephrine, and plasma potassium, which are β_2-adrenergic responses.[8-11] ICI 118551 did not antagonize the rise in systolic pressure or the shortening of electromechanical systole (QS_2I) caused by isoproterenol, which are inotropic responses mediated by β_1-adrenergic receptors. Neither did it antagonize the effect of isoproterenol on plasma renin. This effect of isoproterenol also is regarded as a β_1-adrenergic response.[12] The chronotropic effect of isoproterenol, which is a mixed β_1/β_2-adrenergic response,[13] was antagonized by a factor of only two by ICI 118551, as compared with a factor of 16 by propranolol. When the shifts of the β_1-adrenergic responses (systolic pressure, QS_2I, renin) and of the β_2-adrenergic responses (diastolic pressure, norepinephrine, potassium) caused by ICI 118551 were compared with the shifts caused by atenolol, the β_2-selectivity of ICI 118551 was even greater than the β_1-selectivity of atenolol, at least in the doses we have used.

Antihypertensive Effect of ICI 118551

The main finding of this study is that ICI 118551, in a β_2-selective dose, lowered blood pressure. This observation is at variance with earlier reports by Robb et al.[14] and by Dahlof et al.[15] who could not demonstrate

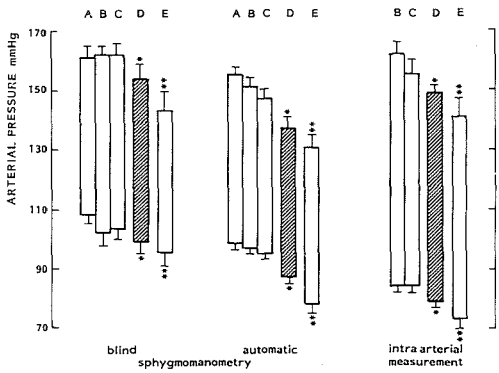

FIGURE 4. *Average blood pressure before treatment (run in; A), after placebo (B), after placebo (wash out; C), after ICI 118551 (D), after propranolol (E). Single* ($p<0.05$) *and double* ($p<0.01$) *asterisks indicate significant difference compared with placebo.*

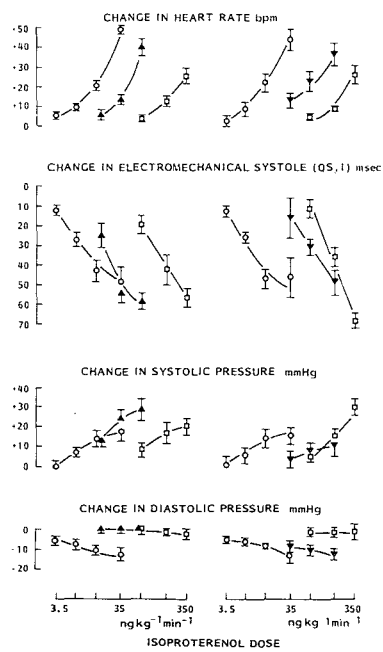

FIGURE 5. *Isoproterenol dose-response curves before treatment (○) and after treatment with ICI 118551 (▲), atenolol (▼), or propranolol (□).*

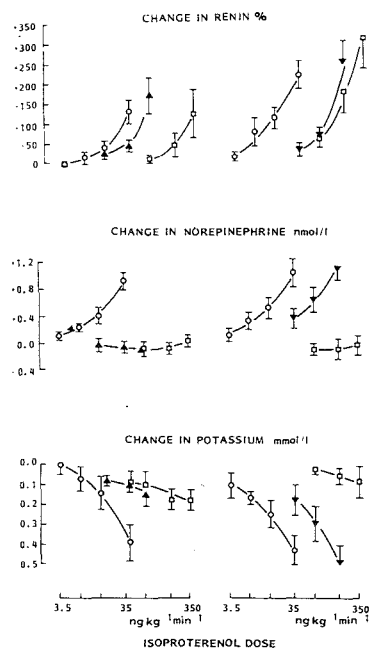

FIGURE 6. *Isoproterenol dose-response curves before treatment (○) and after treatment with ICI 118551 (▲), atenolol (▼), or propranolol (□).*

an antihypertensive effect of ICI 118551. The mean age of the patients (48 and 46 years, respectively) in those two studies was higher than that in our study (35 years). Moreover, the patients in the two negative studies had all been treated previously, whereas four of our patients had never been treated before. This difference suggests that their patients had hypertension of longer duration. It could be that β_2-blockade is more effective in an earlier stage of the disease. It may also be important that Dahlof et al.[15] did not use placebo controls after the run-in phase. This omission might obscure the antihypertensive effect of ICI 118551 by a gradual return of hypertension, not only during the run-in phase but also during ICI 118551 treatment.

Mechanism of Action of ICI 118551

The mechanism by which β-blockers lower blood pressure remains a matter of debate.[16–19] Cardiac, vascular, or hormonal changes and effects on body fluid volumes have been suggested to play a role.[20, 21] β-Blockers with different ancillary properties, such as partial agonist activity, β_1-adrenergic receptor selectivity, or hydrophilicity, all lower blood pressure. β_1-Adrenergic receptor blockade generally is considered the common denominator of this effect. The current study, however, shows that selective blockade of β_2-adrenergic receptors also lowers blood pressure. Firm conclusions on the anatomical site of this β_2-adrenergic receptor, centrally, presynaptically, or postsynaptically in the heart, kidneys, or blood vessels, cannot be drawn from our data.

Currently, there is little evidence that β_2-adrenergic receptors in the central nervous system are involved in blood pressure regulation. We have no information on changes in cardiac output, vascular resistance, or body fluid volumes after ICI 118551 treatment. The drug did lower the basal levels of plasma renin, which might have contributed to its antihypertensive effect. Plasma norepinephrine rose with propranolol treatment but did not change with ICI 118551 treatment. This difference might be explained by different effects of the two drugs on the clearance of plasma norepinephrine. Heart rate (and therefore probably cardiac output) fell more during propranolol than during ICI 118551 treatment. Indeed, changes in plasma norepinephrine level during β-blockade seem to reflect changes in the clearance of this neurotransmitter rather than changes in its release.[17, 22–24]

One of the theories explaining the antihypertensive effect of β-blockers implicates a role for presynaptic β_2-adrenergic receptors, which serve to facilitate norepinephrine release from sympathetic nerves. Activation of these receptors by epinephrine might cause hypertension.[25, 26] Blockade of these receptors would decrease norepinephrine release and thereby lead to diminished activation of cardiac β-adrenergic receptors and vascular α-adrenergic receptors. This mechanism might account for the antihypertensive effect of β_2-blockade, although it does not provide an explanation for the antihypertensive action of β_1-blockade. The rise in renin in response to isoproterenol was not antagonized by ICI 118551, which confirms its β_2-selectivity. Yet the drug lowered the basal level of renin. Again, this response may be explained by presynaptic inhibition of norepinephrine release, leading to diminished activation of postsynaptic β_1-adrenergic receptors in the kidney.

Whatever the precise mechanism of the fall in blood pressure after β_2-blockade, our data demonstrate that β_1-blockade is no always essential for the antihypertensive effect of β-blocker therapy.

Acknowledgments

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Chapter 6

BETA-2 AGONISTS INCREASE PLASMA RENIN THROUGH BETA-1 ADRENOCEPTOR ACTIVATION BY TRANSMITTER NORADRENALINE. EVIDENCE FOR FUNCTIONAL PRESYNAPTIC BETA-RECEPTORS IN THE KIDNEY.

Short title:
Beta-2 adrenoceptor mediated stimulation of renin release

Authors: Hieronymus H. Vincent M.D., Arie J. Man in't Veld M.D., Frans H.M. Derkx M.D., Frans Boomsma Ph.D and Maarten A.D.H. Schalekamp M.D.

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SUMMARY

Adrenergic stimulation of renin release is known to be mediated by activation of beta-1 adrenoceptors on juxtaglomerular cells. However, when systemically infused, both non-selective and beta-2 selective agonists increase renin release. In order to investigate the mechanism whereby beta-2 stimulation may increase renin, we compared the effects of infusion of the beta agonists prenalterol (beta-1), salbutamol (beta-2) and isoprenaline (beta-1 plus beta-2) on plasma renin in subjects with mild essential hypertension. Isoprenaline was infused before and during treatment with the betablockers atenolol (beta-1), ICI 118551 (beta-2) and propranolol (beta-1 plus beta-2). The effect on plasma renin was compared with the effects on electromechanical systole (QS2-index) and diastolic blood pressure, as a measure of activation of beta-1 and beta-2 adrenoceptors respectively. The concomitant changes in plasma noradrenaline were also measured.

For a given shortening of the QS2-index, the rise of renin in response to prenalterol was less than after salbutamol and isoprenaline. Prenalterol had no effect on diastolic pressure. For a given decrease in diastolic pressure, the rise of renin in response to salbutamol was similar to that after isoprenaline. The dose related rise in renin after salbutamol and isoprenaline was closely and linearly correlated with the rise in noradrenaline. This was associated with a drop in diastolic pressure at low agonist doses but there was no further change in diastolic pressure at higher doses. These data indicate that beta-2 receptor activation is capable of stimulating renin and that this beta-2 effect is not solely caused by baroreflex mediated sympathetic stimulation in response to vasodilatation. The antagonistic effect of both atenolol, in a beta-1 selective dose, and propranolol on the isoprenaline induced rise in renin was equal to their antagonistic effect on the isoprenaline induced shortening of the QS2-index. In contrast, selective beta-2 blockade with ICI 118551 had little antagonistic effect on these responses to isoprenaline. Thus, the beta-2 mediated rise in renin depends on beta-1 receptor activation. Baseline plasma levels of renin were decreased by all three betablockers. The data, taken together, are compatible with the view that beta-2 agonists, through activation of renal presynaptic beta receptors, increase the release of transmitter noradrenaline, which acts on postsynaptic beta-1 receptors to stimulate the release of renin.

Key words: renin, noradrenaline, presynaptic beta adrenoceptors, beta adrenoceptor agonists, betablockers

Chapter 6

INTRODUCTION

From in-vitro experiments it appears that the release of renin from juxtaglomerular cells induced by beta adrenoceptor agonists is mediated by beta-1 adrenoceptors [1-3]. Renin release elicited by low frequency stimulation of the renal nerves or by infusion of beta agonists in intact animals also depends on beta-1 receptor activation [4-6]. In man, exercise induced renin release is also a beta-1 effect [7-9] and, according to most investigators, the same is true for the increase in plasma renin in response to beta agonist infusion [for review of the literature see ref. 10]. Some observations, however, indicate that plasma renin is increased by beta-2 agonists and not by beta-1 selective agonists [11-14]. The present study was undertaken to clarify the mechanism whereby beta-2 agonists may stimulate renin in man. We have previously found that plasma noradrenaline is increased by systemic infusion of beta agonists, which is an indication of stimulated sympathetic activity. This effect appears to be mediated by beta-2 receptors [15]. It seems therefore possible that the increase in renin by infusion of beta-2 agonists is an indirect effect, via increased sympathetic nerve stimulation of beta-1 receptors on juxtaglomerular cells.

SUBJECTS AND METHODS

Forty subjects with mild essential hypertension were studied. Their blood pressure in the outpatient clinic ranged from 140 tot 160 mmHg systolic and from 90 tot 105 mmHg diastolic. They showed no signs of cardiac or renal disease and were not on any medical treatment. All subjects had given their informed consent and the study protocol was approved by the hospital ethical review committee.

Isoprenaline was given by continuous intravenous infusion in increasing doses to 21 male subjects. In 10 subjects (aged 20-45 yr) each dose step lasted 30 min (group I) and in 11 subjects the dose was increased every 15 min (group II). In group I the isoprenaline infusion was given before and after 7 days treatment with the non-selective beta antagonist propranolol (80 mg t.i.d.) and also after 7 days treatment with the beta-1 selective antagonist atenolol (100 mg once daily). In group II isoprenaline was infused before and after 7 days treatment with propranolol (80 mg t.i.d.) and also after 7 days treatment with the beta-2 selective antagonist ICI 118551 [16] (50 mg t.i.d.), in random order. The beta-1 selective agonist prenalterol was given intravenously in increasing doses to 9 subjects (aged 19-45 yr, 5 males) (group III). Each dose was infused over 5 min at 15 min intervals. The beta-2 selective agonist salbutamol was given by continuous intravenous infusion in increasing doses to 12 subjects (aged 16-50 yr, 8 males), each dose step lasting 15 min.

A 20-gauge cannula was inserted into the radial artery for blood sampling and blood pressure measurements. Infusions were given via a forearm vein with the patient resting supine. Prior to each beta-agonist infusion, saline was infused for 30-45 min at a rate of 22 ml/hr until heart rate and blood pressure were

stable. Blood samples for renin and noradrenaline were taken just before the beta-agonist infusion was started and at the end of each dose step.

Heart rate and intra-arterial pressure were continuously recorded. The duration of electromechanical systole corrected for heart rate (QS2-index) was measured according to Lewis et al [17]. Inotropic stimulation, as reflected by a shortening of the QS2-index was taken as a measure of beta-1 receptor stimulation. Vasodilatation, as reflected by a decrease in diastolic arterial pressure, was taken as a measure of beta-2 receptor stimulation. Blood was collected into chilled heparinized tubes and immediately centrifuged. For the measurement of noradrenaline the plasma was then deproteinized with 10% trichloroacetic acid. The deproteinized samples were stored at -20°C for no longer than 2 weeks before assay. Renin (the enzymatically active form) was measured by RIA as described previously [18] and noradrenaline was measured radioenzymatically, using phenylethanol-N-methyltransferase (PNMT) and (3H)-S-adenosyl-L-methionine [19]. Data are presented as mean and SEM. Changes from baseline were analysed by Student's t-test for paired observations. A p-value of less than 0.05 was considered to be significant.

RESULTS

Baseline plasma renin and noradrenaline levels are shown in Fig. 1. They were not different in the 4 groups of subjects before betablocker treatment. Renin was decreased by all three betablockers i.e. propranolol, atenolol and ICI 118551. Noradrenaline was increased by propranolol and was not altered by atenolol and ICI 118551.

Fig. 2 compares the effects of isoprenaline, prenalterol and salbutamol. At the highest dose, heart rate (not shown) was increased by 44 (SEM 5) beats/min with isoprenaline, by 24 (2) beats/min with prenalterol and by 22 (3) beats/min with salbutamol. Renin rose by 128 (34) % ($p<0.001$) at the highest dose of isoprenaline, by only 68 (25) % ($p<0.05$) at the highest dose of prenalterol and by 100 (39) % ($p<0.01$) at the highest dose of salbutamol. For a given shortening of the QS2-index the rise in renin in response to prenalterol was less than after isoprenaline and salbutamol. Prenalterol had no effect on diastolic pressure. For a given decrease in diastolic pressure the rise in renin in response to salbutamol was similar to that after isoprenaline. Thus, not only beta-1 receptor activation but also beta-2 receptor activation caused an increase in renin.

The responses of renin and the QS2-index to isoprenaline were antagonized by both the beta-2 selective antagonist ICI 118551 and by the non-selective beta antagonist propranolol but much less so by ICI 118551 than by propranolol (Fig. 3). The effect of isoprenaline on diastolic pressure was abolished by both ICI 118551 and propranolol. Thus, the rise in renin in response to isoprenaline depended on beta-1 receptor stimulation. This is confirmed by the data shown in Fig. 4. The responses of renin and the QS2-index to isoprenaline were antagonized to a similar extent by the beta-1 selective antagonist atenolol and the non-selective beta antagonist propranolol, whereas the effect

on diastolic pressure was much less antagonized by atenolol than by propranolol.

As can be seen in Fig. 5, the effect of isoprenaline and salbutamol on renin was closely and linearly correlated with the effect on noradrenaline. Moreover, for a given increase in noradrenaline, the increase in renin was similar with both drugs. This, together with the results obtained with isoprenaline after treatment with the beta-1 selective blocker atenolol, suggests that beta-2-mediated stimulation of noradrenaline release with subsequent activation of beta-1 receptors is the mechanism that underlies the effect of both salbutamol and isoprenaline on renin. The beta-1 selective agonist prenalterol increased renin but had little effect on noradrenaline, which suggests that in this case the effect on renin is caused by stimulation of beta-1 receptors by prenalterol itself.

It is further of interest that the diastolic blood pressure was decreased by the lowest doses of isoprenaline and salbutamol and that this was accompanied by little increase in noradrenaline. In contrast, diastolic pressure was not further decreased at higher doses of isoprenaline and salbutamol, whereas the largest increments in noradrenaline occurred at these doses (Fig. 5).

DISCUSSION

By measuring the effects of various beta agonists and antagonists on plasma renin and by comparing these effects with haemodynamic responses that are known to be selectively mediated by either beta-1 or beta-2 receptors, we could demonstrate that renin is increased by beta-2 stimulation with salbutamol and decreased by beta-2 blockade with ICI 118551. Beta-1 stimulation in addition to beta-2 stimulation, as occurs with isoprenaline, had little extra effect on renin. Also, isolated beta-1 stimulation with prenalterol had only a modest effect on renin as compared with salbutamol and isoprenaline. Yet, the results obtained with isoprenaline after pretreatment with either the selective beta-1 blocker atenolol, the selective beta-2 blocker ICI 118551 or the non-selective betablocker propranolol indicate that beta-2 agonist infusion in the intact individual causes an increase in renin via stimulation of beta-1 receptors and that this beta-1 effect is not a consequence of insufficient selectivity of the beta-2 agonist being used.

We observed a modest increase of renin in response to prenalterol but others did not see any effect [11,13,14]. Prenalterol is a partial beta-1 agonist. Because of this, the effect on renin may depend on the prevailing sympathetic nervous outflow to the kidney. Prenalterol may have little effect on renin when the prevailing sympathetic activity is high.

Theoretically, there are several ways in which beta-2 receptor stimulation may cause an increase in renin. Firstly, one has to consider that juxtaglomerular cells may be endowed with beta-2 receptors, and that stimulation of these receptors may lead to increased release of renin. However, the antagonistic effect of the beta-1 selective blocker atenolol and the non-selective betablocker propranolol on the response of renin to

isoprenaline was correlated with the degree of beta-1 blockade (response of the QS2-index) and not with the degree of beta-2 blockade (response of diastolic blood pressure). This argues against a role of beta-2 receptors located on juxtaglomerular cells.

Secondly, plasma potassium is known to be lowered in a dose dependant way by infusion of beta-2 agonists. At the highest doses we have used both isoprenaline and salbutamol lowered plasma potassium by approximately 0.5 mmol/l [15]. Dietary potassium deprivation may be associated with in increase in renin [20]. However, experiments with isolated perfused rat kidneys have demonstrated that although renin secretion is increased after potassium depletion, changes in the potassium concentration of the perfusate were without effect [21]. Experiments with rat kidney slices have also shown that variations of extracellular potassium concentration from 2.5 to 4.0 mmol/l had no effect on renin secretion [22]. More importantly, studies in dogs have shown that renin in plasma is increased by adrenaline infusion when the plasma level of potassium is kept constant and that this effect on renin is not greater when the potassium level is not maintained [23]. Isoprenaline infusion in dogs has also been reported to increase renin in the absence of changes in plasma potassium [24].

A third possibility is that renin release is stimulated as a consequence of activation of vascular postsynaptic beta-2 receptors. Activation of these vascular receptors causes vasodilatation and a lowering of blood pressure. This in turn may stimulate renin release through the renal baroreceptor mechanism or through the arterial baroreflex induced increase in sympathetic activity. However, stimulation of the renal baroreceptor mechanism is in itself insufficient to explain the rise of renin in response to beta-2 agonist infusion, since this response depended on stimulation of beta-1 receptors. Furthermore, with infusion of salbutamol and isoprenaline the plasma levels of renin and noradrenaline rose in a dose dependent way, whereas diastolic pressure decreased only at the lowest dose but showed no further decrease at higher doses. Also, both renin and diastolic pressure were decreased by the beta-2 selective blocker ICI 118551. Thus, baroreflex mediated stimulation of renin release is unlikely to be the sole cause of the rise of renin in plasma.

Finally the rise in renin after beta-2 agonist infusion may be explained by presynaptic facilitation of sympathetic transmitter release within the kidney. In-vitro experiments have shown that activation of presynaptic beta-2 receptors located on sympathetic nerve endings has a facilitatory effect on noradrenaline release in various tissues, including the kidney [25,26]. In our subjects plasma renin was increased in parallel with noradrenaline after beta-2 agonist infusion. It seems therefore likely that at least part of the rise in renin is caused by increased release of transmitter noradrenaline. Indeed, in dogs it has been shown that the increase in renin after systemic infusion of adrenaline in a low concentration depends on intact renal innervation and that the effect on renin is antagonized by beta-1 as well as by beta-2 antagonists. From these observations it was concluded that beta-2 receptor

stimulation by adrenaline leads to increased renin through presynaptic facilitation of noradrenaline release in the kidney [27]. The observation that in our subjects plasma renin was lowered by the beta-2 selective blocker ICI 118551 as well as by the beta-1 selective blocker atenolol is in accordance with the above study in dogs. That plasma noradrenaline was not lowered by ICI 118551 is not in contradiction with an effect on presynaptic beta receptors, since the clearance of noradrenaline is decreased after betablocker treatment [28].

We conclude that both beta-1 and beta-2 adrenoceptor activation by systemic infusion of beta agonists can increase renin release. The effect of beta-2 receptor activation on renin is most likely an indirect one; it depends on activation of postsynaptic renal beta-1 receptors by transmitter noradrenaline. Although a reflex increase of sympathetic tone in response to vasodilatation may contribute to this indirect effect, it is likely that activation of presynaptic renal beta-2 receptors with subsequent facilitation of sympathetic transmitter release is the more important underlying mechanism.

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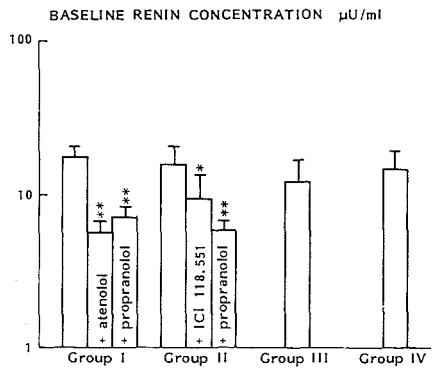

Fig. 1A. Preinfusion values of plasma renin concentration in the 4 patient groups. In group I and II values after betablocker treatment were compaired with those without treatment.
* p<0.05 ** p<0.01

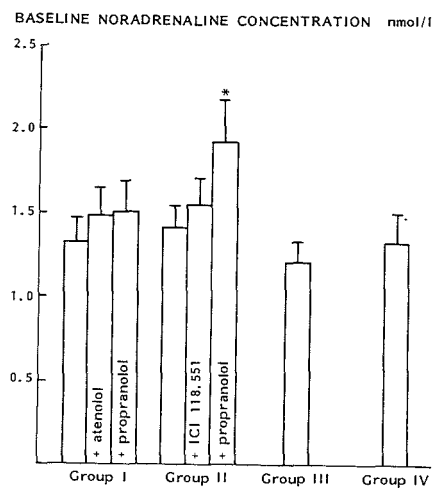

Fig. 1B. Preinfusion values of plasma noradrenaline concentration in the 4 patient groups. In group I and II values after betablocker treatment were compaired with those without treatment.
* p<0.05 ** p<0.01

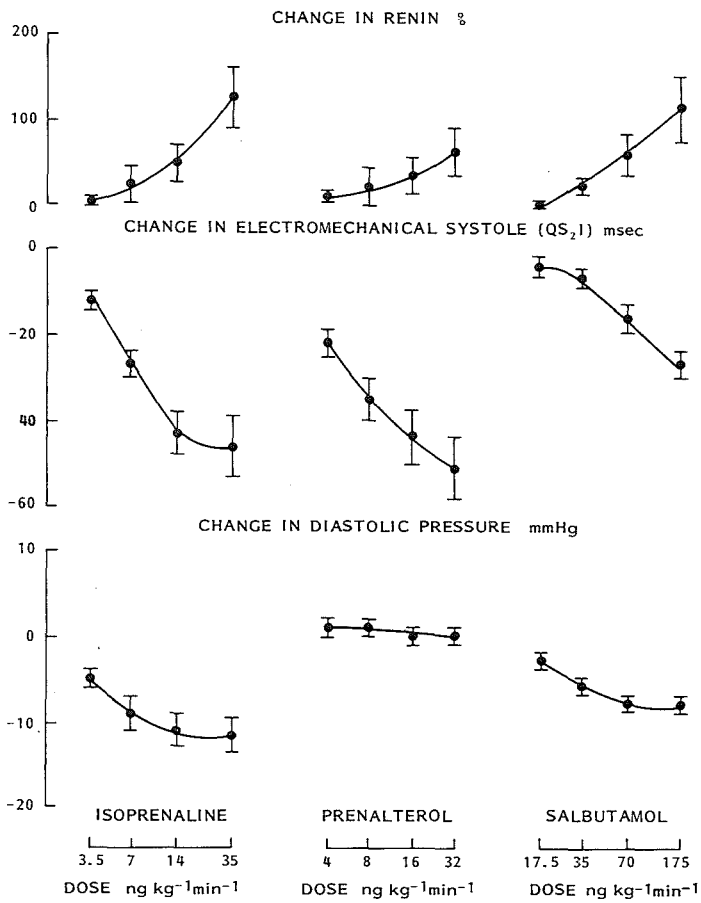

Fig. 2. Effects of the beta agonists isoprenaline (non-selective), prenalterol (beta-1 selective) and salbutamol (beta-2 selective) on renin and on duration of electromechanical systole and diastolic blood pressure.

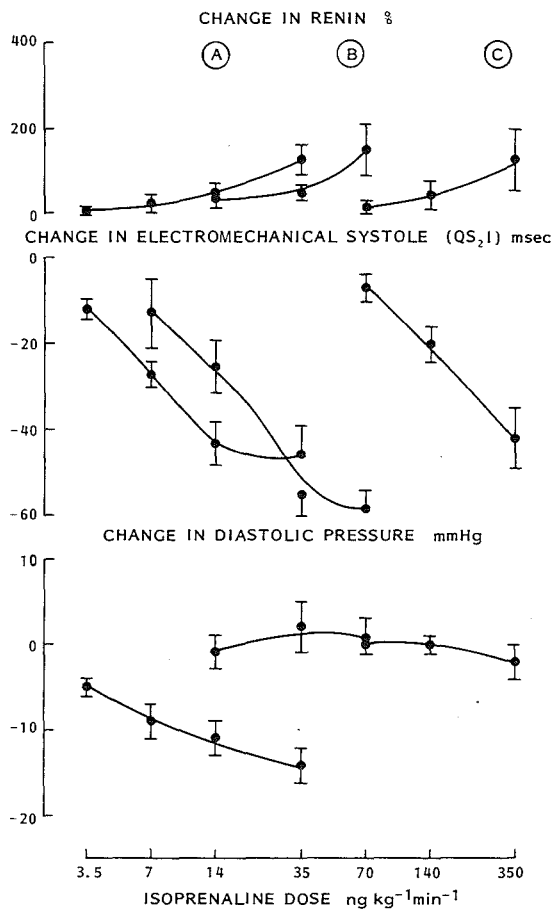

Fig. 3. Effects of the beta agonist isoprenaline on renin and on duration of electromechanical systole and diastolic pressure.
A: untreated
B: after ICI 118551 (beta-2 selective blocker)
C: after propranolol (non-selective betablocker)

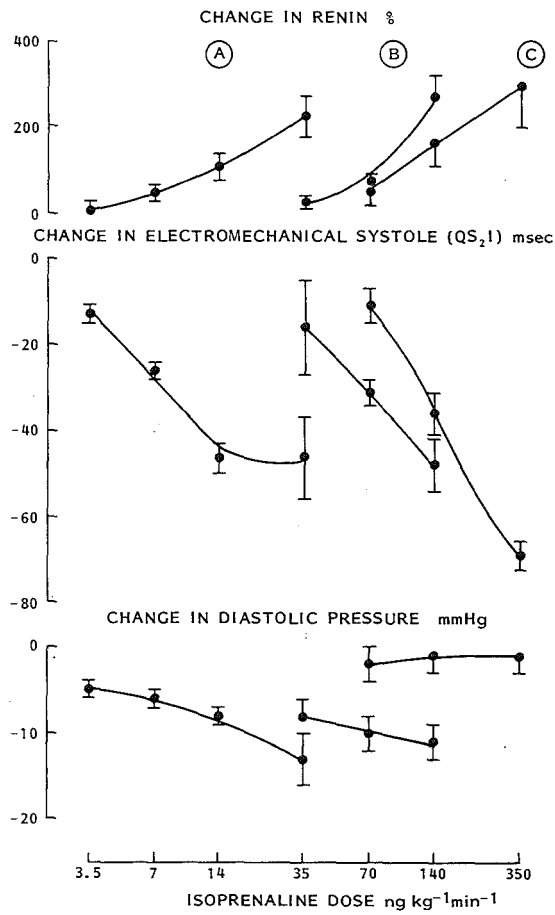

Fig. 4. Effects of the beta agonist isoprenaline on renin and on duration of electromechanical systole and diastolic pressure.
A: untreated
B: after atenolol (beta-1 selective blocker)
C: after propranolol (non-selective betablocker)

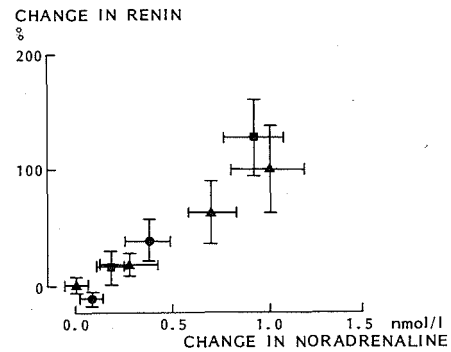

Fig. 5 a. Changes in plasma noradrenaline and renin during beta agonist infusion.
● : isoprenaline (non-selective) (15 min dose steps)
▲ : salbutamol (beta-2 selective)

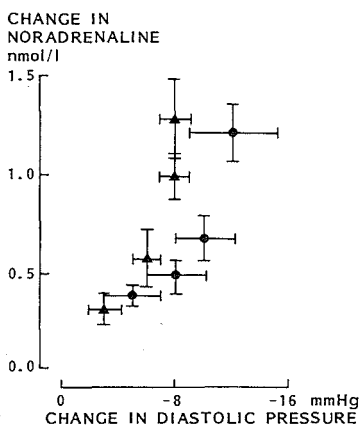

Fig. 5 b. Changes in diastolic blood pressure and plasma noradrenaline during beta agonist infusion.
● : isoprenaline (non-selective) (15 min dose steps)
▲ : salbutamol (beta-2 selective)

Chapter 7

GENERAL DISCUSSION

As discussed in the introduction (Chapter 1), several in-vitro studies and a number of animal studies have provided evidence for the existence of so-called presynaptic beta adrenoceptors. These are beta-2 type receptors located on sympathetic nerve endings, which regulate the amount of transmitter released in respons to nerve stimulation. Activation of these presynaptic receptors by the hormone adrenaline facilitates the release of the neurotransmitter, noradrenaline. In this way presynaptic beta receptors could be involved in blood pressure regulation, and it has been speculated that presynaptic beta blockade could account for the antihypertensive effect of betablocker therapy.

The questions addressed in this thesis are the following: 1) Can presynaptic beta receptors be shown to be functionally active in intact man. 2) What, if any, is their physiological role. 3) Are these receptors involved in the antihypertensive effect of betablocker therapy.

Effect of beta agonists and antagonists on plasma noradrenaline

Our first approach was to study the effects of beta agonists and antagonists with different beta-1 / beta-2 selectivity (Chapters 2 and 3). Beta-2 receptor activation with isoprenaline or salbutamol caused a 75% rise in plasma noradrenaline concentration. Beta-1 receptor activation had no such effect. Our finding that isoprenaline increases plasma noradrenaline has been confirmed by Goldstein et al [1]. These authors, using the same doses as we did, found an 81% rise of plasma noradrenaline. They further found that plasma adrenaline was unchanged or decreased, which may be taken as evidence that isoprenaline led to noradrenaline release from sympathetic nerves rather than from the adrenals. These observations are taken as evidence for presynaptic facilitation of neuronal noradrenaline release. With regard to this interpretation three possible points of criticism must be considered. First, beta-2 receptor activation causes vasodilatation and may thereby lower blood pressure. This in turn may cause stimulation of sympathetic nervous activity via the arterial baroreflex mechanism. In our opinion this is unlikely for several reasons. It is questionable whether baroreflex mediated changes in sympathetic activity are ever reflected by changes in noradrenaline as large as in our study [2]. Furthermore, the increase in blood pressure was small (sometimes blood pressure actually increased) and heart rate and pulse pressure rose. The latter would rather have the opposite effect on the baroreflex. A further argument that the isoprenaline induced rise in plasma noradrenaline is not mediated by the baroreflex comes from a recent study by Arnold et al [3]. These authors administered the anticholinergic drug atropine before infusion of isoprenaline and found that the heart rate response curve was shifted to the left, implying the presence of a reflex increase in vagal tone during isoprenaline infusion. Thus a

reflex withdrawal of sympathetic tone may be inferred, and this would cause a fall rather than a rise in plasma noradrenaline concentration.

Second, a rise in plasma noradrenaline could result from a decrease of its metabolic clearance rate. However, beta adrenoceptor stimulation has been found to increase, not decrease, noradrenaline clearance [4]. The finding by Goldstein et al that, during isoprenaline infusion, plasma adrenaline concentration tended to fall [1] is indeed an indication that catecholamine clearance was increased.

Third, beta adrenoceptor activation raises plasma renin and will thereby lead to higher angiotensin-II concentrations. Angiotensin-II, like adrenaline, has been shown to exert a presynaptic facilitatory effect on noradrenaline release [5]. The rise in noradrenaline after beta agonist infusion might therefore be secondary to its effect on renin. This is supported by the observation by Majewski et al [6] that, in a pithed rabbit, inhibition of noradrenaline release by propranolol no longer occurred after pretreatment with the angiotensin converting enzyme inhibitor captopril. He suggested that the inhibition of noradrenaline release after propranolol was in fact due its effect on renin rather than to blockade of presynaptic beta receptors. In our studies, however, the effects of isoprenaline on renin and on noradrenaline were clearly dissociated. Atenolol and propranolol lowered plasma renin concentration to the same extent (see Chapter 6), yet atenolol had little effect on the noradrenaline response to isoprenaline, whereas propranolol abolished this response.

The data presented in Chapters 2 and 3 and the reports from the literature discussed above provide evidence that beta-2 adrenoceptor activation can stimulate or facilitate noradrenaline release from sympathetic nerves in a direct way.

Effects of adrenaline on plasma noradrenaline and on cardiovascular responses

The question regarding the physiological significance of presynaptic beta-2 receptors was addressed in a number of experiments where we studied the effects of the natural beta-2 agonist, adrenaline, on the response to sympathetic nervous stimulation. Cold exposure (one hand in ice water) and isometric forearm exercise were used to evoke a sympathetic nervous response, which was reflected by a rise in blood pressure. Adrenaline was infused in a dose that caused plasma adrenaline concentration to rise to 800 pg/ml, which is within the physiological range. Similar concentrations are reached during moderate levels of stress [7]. During adrenaline infusion base line heart rate rose by 8 beats/min and diastolic pressure fell by 10 mmHg. Plasma noradrenaline rose by 30%. The blood pressure response to the cold pressor and isometric exercise tests was significantly increased. The rise in heart rate, which is attributed to a decrease of parasympathetic activity, was not affected. After intravenous injection of propranolol, 0.5 mg, baseline heart rate and blood pressure returned to control levels. The observation that heart rate was not lowered below control levels is important, since this indicates that

propranolol in this dose blocked beta-2 receptors selectively. After propranolol the amplification by adrenaline of the blood pressure response to sympathetic stimulation was abolished. Thus, adrenaline, in physiological concentrations, increases noradrenaline and amplifies the blood pressure response to sympathetic nerve activity. This effect is mediated by beta-2 type receptors.

A recent study by Floras et al [8] corroborates this finding. These authors reported that infusion of either isoprenaline or adrenaline into the forearm, in doses that caused no systemic effects, augmented the vasoconstrictor response to another sympathetic stimulus, lower body negative pressure. The effect of adrenaline outlasted the duration of the infusion by at least 30 min, which argues for the possibility that an acute elevation of plasma adrenaline concentration causes a longlasting increase in vascular resistance. This longlasting effect is likely to be due to adrenaline uptake into the sympathetic neuron and subsequent 'co-release' together with noradrenaline. Interestingly, adrenaline attenuated the vasoconstrictor response to infused noradrenaline. This may be taken as evidence that the augmented blood pressure response to sympathetic stimulation during adrenaline infusion was indeed due to increased noradrenaline release rather than to an increased alpha receptor sensitivity. Further confirmation comes from a study from our own laboratory [9]. In normal volunteers intravenous infusion of adrenaline, 15 ng/min per kg body weight, raised plasma levels of adrenaline to 250 pg/ml, corresponding to mild forms of stress. In this study the dose of adrenaline was lower than in our previous experiments, but again the blood pressure response to cold exposure and isometric exercise was augmented. Moreover, resting blood pressure was significantly increased for up to 18 h after the infusion had been stopped. All these data are in accordance with a physiological role for adrenaline to facilitate the release of noradrenaline from sympathetic nerves. This effect may last much longer than the elevation of plasma adrenaline.

Effect of a beta-2 selective antagonist on blood pressure

If it is true that adrenaline raises blood pressure through presynaptic beta-2 type receptors, beta-2 receptor blockade is expected to have a blood pressure lowering effect. The question wether blockade of presynaptic beta-2 receptors lowers blood pressure was addressed in a clinical trial, described in Chapter 5. In 9 subjects with essential hypertension, the effects of a beta-2 selective antagonist ICI 118551 and of the non-selective antagonist propranolol were compared with placebo according to a randomized crossover design. ICI 118551 did not block the beta-1 receptor mediated inotropic stimulation of the heart and stimulation of renin by isoprenaline, but it abolished a number of beta-2 receptor mediated responses, i.e. the fall in diastolic pressure, the rise in noradrenaline and the fall in plasma potassium. Two hours after ingestion of either ICI 118551 or propranolol, heart rate had decreased and blood pressure was unchanged. After one week, however, both systolic and diastolic pressure, whether measured indirectly or intra-arterially, were significantly decreased by beta-2 selective blockade as well as

by propranolol. Furthermore, both drugs lowered heart rate and renin. Thus, a comparison of beta-2 selective blockade with non-selective betablockade showed that the time course and pattern of the responses of heart rate, blood pressure and renin were in fact very similar. The data are compatible with presynaptic beta-2 blockade leading to inhibition of noradrenaline release and a fall in blood pressure.

At first sight the observation that, generally, beta-1 selective blockers and non-selective betablockers lower blood pressure equally well seems to argue against a role of presynaptic beta-2 blockade. However, it is likely that, in the doses clinically used, beta-1 selective blockers also block presynaptic beta receptors to a significant extent. On the other hand, in our study the magnitude of the fall in blood pressure tended to be greater after propranolol than after ICI 118551. This would suggest that beta-1 blockade does contribute to the antihypertensive action of betablocker therapy.

It has further been argued that presynaptic inhibition of noradrenaline release ought to be reflected in a fall in plasma noradrenaline concentration. In our study we found a tendency for plasma noradrenaline to fall after ICI 1181551 but this was not significant. After propranolol noradrenaline was actually increased. It must be pointed out, however, that sometimes plasma noradrenaline may not accurately reflect noradrenaline release [10,11]. Specifically, it has been reported that after beta blockade noradrenaline clearance is decreased. In some studies betablocker therapy did lower the noradrenaline release rate and urinary catecholamine excretion, especially in those subjects who responded to betablocker therapy with a fall in blood pressure [12-15]. Furthermore, an increase in noradrenaline after propranolol may not only be explained by an effect on noradrenaline clearance but also by a temporary baroreflex mediated increase in sympathetic nervous tone to counteract the consequences of cardiac beta-1 receptor blockade. Such an event is not incompatible with concomitant presynaptic inhibition of noradrenaline release.

Another criticism against a role of presynaptic beta blockade in the antihypertensive action of betablockers concerns the change in pheripheral vascular resistance. Whereas presynaptic blockade of noradrenaline release would be expected to lead to a fall in vascular resistance, most studies have in fact reported an increase. However, as has been pointed out by Man in't Veld and Schalekamp [16], the antihypertensive effect of betablocker therapy may be delayed by an initial increase in vascular resistance, but at the time blood pressure is falling this fall is actually parallelled by a fall in vascular resistance.

Effects of beta agonists and antagonists on plasma renin

From the study described in Chapter 5 we concluded that beta-2 blockade leads to a fall in renin. Data in the literature, regarding the beta receptor subtype involved in renin release, are somewhat contradictory. This prompted us to analyse in detail the renin response to beta receptor agonists and antagonists with different selectivity. The results are presented in Chapter 6. Effects of isoprenaline before and after treatment with the

antagonist atenolol (beta-1), ICI 118551 (beta-2) or propranolol (non-selective beta antagonist) were examined. The response of renin was compared to the shortening of cardiac electromechanical systole and to the fall in diastolic arterial pressure as parameters of beta-1 and beta-2 receptor activation respectively. This comparison of different betablockers indicated that the isoprenaline induced rise in renin was mediated by beta-1 receptors. When, however, isoprenaline was compared to the beta-2 agonist salbutamol and to the beta-1 agonist prenalterol, it was found that beta-2 receptor stimulation was mainly responsible for the rise in renin. The data are best explained by presynaptic beta-2 receptor activation facilitating the release of noradrenaline in the kidney. Noradrenaline subsequently acts on postsynaptic beta-1 receptors to cause a rise in renin release. Indeed, after isoprenaline and after salbutamol there was a close linear correlation between the rise in noradrenaline and the rise in renin.

That beta-1 receptor stimulation per se had little effect on renin needs to be explained. It may be of importance that prenalterol is not a full but rather a partial beta-1 receptor agonist. With a high prevailing level of sympathetic nervous activity in the kidney, prenalterol may cause little further beta-1 receptor stimulation. In contrast, facilitation of transmitter release may have its greatest effect under these circumstances.

The rise in renin after beta-2 receptor stimulation is not likely to be due to a reflex increase in sympathetic tone. It has previously been argued that isoprenaline and salbutamol are likely to cause a reflex fall in sympathetic tone. Furthermore, while there was a close linear correlation between the rise in renin and the rise in noradrenaline, there was no such correlation with the fall in diastolic pressure. The data are therefore interpreted as evidence that presynaptic beta-2 receptor stimulation increases noradrenaline release and thereby leads to a postsynaptic beta-1 receptor mediated response, i.e. a rise in renin.

Our conclusions may be summarized as follows:

1) Beta-2 agonists increase plasma noradrenaline, most likely reflecting a direct influence on neuronal release of noradrenaline.
2) The beta-2 agonist-induced increase in noradrenaline is associated with a postsynaptic beta-1 response, namely a rise in renin.
3) Adrenaline, in physiological concentrations, increases plasma noradrenaline. This effect is also mediated by beta receptors.
4) Adrenaline, in physiological concentrations, amplifies the postsynaptic alpha receptor mediated blood pressure response to standardized sympathetic stimulation. This effect of adrenaline is also mediated by beta receptors.
5) Treatment of essential hypertension with a selective beta-2 receptor antagonist causes a fall in blood pressure.

In view of the above conclusions there is now good evidence that presynaptic beta-2 receptors exist in humans and that these

receptors play a role in blood pressure regulation. Activation of these receptors by the hormone adrenaline enhances the cardiovascular effect of sympathetic nervous activity. Blockade of these receptors leads to a fall in blood pressure in patients with essential hypertension.

Thus the hormone adrenaline serves a hitherto unrecognized function. It amplifies the signal transmission in the sympathetic nervous system. One may envisage the usefulness of such a function. Physiological studies have shown that noradrenaline, after its release, may act on presynaptic alpha receptors to serve a negative feed-back function on further noradrenaline release. In this way noradrenaline will increase the 'signal-to-noise ratio', especially at low stimulation frequencies. The facilitating effect of adrenaline through presynaptic beta receptors is likely to be most effective at high stimulation frequencies. Both functions are in fact complementary and serve to increase the 'gain' of the sympathetic nervous system.

As a clue to the physiological implications of the presynaptic effect of adrenaline one may further consider the circumstances when adrenaline levels are highest. These circumstances can generally be referred to as 'stress', either with or without physical activity. With physical activity, metabolic waste products from exercising muscles cause vasodilattion and a fall in vascular resistance. Blood pressure is maintained by a rise in cardiac output. After stopping muscular exercise, cardiac output falls abruptly but vascular resistance remains decreased for a while, leading to a temporary drop in blood pressure below pre-exercise level. At this stage plasma adrenaline levels have come down but high levels of adrenaline will still be present in sympathetic nerves. Adrenaline may be released from these nerves and act on presynaptic receptors to augment the vasoconstrictor response to sympathetic activity. In this way a precipitous drop in blood pressure is prevented.

If stress is not associated with muscular exercise, there seems to be no need for such a mechanism. Now high levels of adrenaline will perhaps lead to hypertension. In this context it is of interest that in patients with essential hypertension plasma levels of both adrenaline and noradrenaline are somewhat higher than in normotensive controls [17-20]. A betablocker is therefore a logical choice for the treatment of essential hypertension.

The mechanism whereby betablockers lower blood pressure is unknown. A fall in cardiac output, resetting of the baroreflex, postsynaptic blockade of renin release, a central effect and presynaptic inhibiton of noradrenaline release have all been put forward as possible explanations. As discussed above, our finding that beta-2 receptor blockade contributes to the antihypertensive effect of betablocker therapy provides evidence for the importance of presynaptic beta receptors. It is conceivable that, in some patients, a low dose of a non-selective betablocker will be as effective as a high dose of a beta-1 selective blocker. If this is the case, one might prefer a non-selective betablocker for those patients who experience side effects related to the fall in cardiac output due beta-1 receptor blockade.

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SAMENVATTING

Diverse experimenten in vitro en enkele onderzoekingen bij dieren hebben aanwijzingen opgeleverd dat sympathische zenuwvezels beschikken over zogenaamde presynaptische beta receptoren. Het zou gaan om receptoren van het beta-2 type, die gelegen zijn op het uiteinde van de zenuwvezels. Wanneer de sympathische zenuw electrisch wordt gestimuleerd, wordt noradrenaline, dat in de uiteinden van de zenuw ligt opgeslagen, aan de omgeving afgegeven. Deze afgifte wordt versterkt (gefaciliteerd) door activatie van de presynaptische beta receptoren. Het doel van dit proefschrift was te onderzoeken: 1) of presynaptische beta receptoren bij de mens functioneren, 2) in hoeverre deze receptoren een rol spelen bij cardiovasculaire reacties op bepaalde vormen van fysieke stress, en 3) of het bloeddruk verlagende effect van betablockers mede door presynaptische beta blokkade tot stand komt.

Effecten van beta agonisten en antagonisten op plasma noradrenaline

Eerst onderzochten we het effect van diverse beta agonisten en antagonisten op plasma noradrenaline. Daarbij werden de veranderingen in noradrenaline vergeleken enerzijds met de stijging van de hartfrequentie, de verkorting van de electromechanische systole (QS2I) en de stijging van plasma renine (beta-1 effecten) en anderzijds met de daling van de diastolische bloeddruk en van plasma kalium (beta-2 effecten).

Bij patienten met essentiele hypertensie bleek infusie van isoprenaline de hartfrequentei te doen stijgen met 40 slagen/min, terwijl het plasma noradrenaline steeg met 75%. Na voorbehandeling met de beta-1 antagonist atenolol was de dosis-werkingscurve voor de stijging van noradrenaline in geringe mate naar rechts verschoven. Na de niet selectieve beta antagonist propranolol werd echter, ook bij een zelfde mate van beta-1 stimulatie, geen noradrenaline stijging gezien. Vervolgens vergeleken we de beta-1 agonist prenalterol met de beta-2 agonist salbutamol in doseringen waarbij elk de hartfrequentie deed stijgen met 20 slagen/min. Na salbutamol steeg het plasma noradrenaline met 75%, maar na prenalterol veranderde het noradrenaline niet. Hieruit blijkt dat activatie van beta receptoren, behorende tot het beta-2 type, leidt tot een toename van de plasma noradrenaline concentratie.

Dat infusie van isoprenaline het plasma noradrenaline verhoogt is later bevestigd door Goldstein e.a. [1]. Zij vonden bovendien dat hierbij het plasma adrenaline gelijk bleef of iets daalde. Een en ander past bij beta-2 receptor gemedieerde facilitatie van de noradrenaline afgifte uit de sympathische

zenuwvezels.

Op deze conclusie zijn drie punten van kritiek mogelijk. Beta-2 receptor activatie kan door vaatverwijding de bloeddruk verlagen, zodat wellicht via de baroreflex de sympathische zenuwactiviteit toeneemt. Wij menen dat dit niet de verklaring is voor de waargenomen stijging van noradrenaline omdat naast een geringe bloeddruk daling, en soms zelfs een stijging, steeds een sterke stijging van polsdruk en hartfrequentie optrad, waarbij eerder een reflex afname van de sympathische activiteit zou worden verwacht. Recent werd door Arnold e.a. [2] aangetoond dat infusie van isoprenaline bij de mens leidt tot een reflex toename van de parasympathische activiteit. Algemeen wordt aangenomen dat een baroreflex gemedieerde toename van parasympathische activiteit gepaard gaat met een afname van de sympathische activiteit.

Beta receptor activatie verhoogt de concentratie van renine en angiotensine-II in plasma. Naast presynaptische beta-2 receptoren zijn er ook presynaptische angiotensine-II receptoren beschreven, die eveneens de afgifte van transmitter noradrenaline bevorderen [3].De waargenomen stijging van noradrenaline zou wellicht via de presynaptische faciliterende angiotensine-II receptoren op de sympathische zenuwvezel kunnen verlopen. In onze experimenten was het van isoprenaline op renine echter duidelijk gedissocieerd van het effect op noradrenaline. Atenolol en propranolol antagoneerden in gelijke mate de stijging van renine. Atenolol had echter weinig invloed op de stijging van noradrenaline terwijl propranolol deze stijging volledig blokkeerde.

Tenslotte zou een stijging van noradrenaline ook nog door een afname van de klaring kunnen worden veroorzaakt. Van beta stimulatie is echter bekend dat dit de klaring van catecholamines juist verhoogt [4]. Op grond van onze eigen bevindingen en genoemde gegevens uit de literatuur mag daarom worden geconcludeerd dat beta-2 receptor activatie bij onze patienten rechtstreeks heeft geleid tot een toename van de noradrenaline afgifte door sympathische zenuwvezels, hetgeen een sterke aanwijzing is voor het functioneren van presynaptische beta-2 receptoren.

Effecten van adrenaline op plasma noradrenaline en op de cardiovasculaire respons

De hierboven besproken farmacologische studies werden aangevuld met meer fysiologische studies, waarbij de invloed werd bestudeerd van adrenaline op het effect dat sympathische zenuw stimulatie in het eindorgaan teweegbrengt. Bij patienten met essentiele hypertensie en bij normotensieve vrijwilligers werden de zgn. 'cold pressor-test' en een isometrische inspanningstest uitgevoerd, eerst tijdens infusie van fysiologisch zout, daarna tijdens adrenaline 30 ng/min per kg lichaamsgewicht. Door adrenaline infusie steeeg het plasma adrenaline tot hoge fysiologische waarden, gemiddeld tot 800 pg/ml. Het plasma noradrenaline steeg met 30%. De hartfrequentie steeg 8 slagen/min

en de diastolische bloeddruk daalde 10 mmHg. De bloeddrukstijging op beide stimulatietesten, die door alpha receptoren wordt gemedieerd, was tijdens adrenaline infusie significant toegenomen. De verandering in de hartfrequentie, die aan afname van parasympathische activiteit wordt toegeschreven, werd niet door adrenaline beinvloed. Tijdens de adrenaline infusie werd aan een aantal patienten propranolol, 0,5 mg intraveneus, toegediend. Propranolol deed de hartfrequentie en bloeddruk juist tot de uitgangswaarden terugkeren. (Dat de hartfrequentie niet verder daalde suggereert dat propranolol in deze dosering selectief de beta-2 receptoren blokkeerde.) Na propranolol daalde het plasma noradrenaline tot beneden de controlewaarde. Tevens bleek de versterkende invloed van adrenaline op de bloeddruk-respons volledig geblokkeerd. Uit deze experimenten blijkt dat adrenaline, in een fysiologische concentratie, via beta receptor activatie leidt tot een stijging van het plasma noradrenaline en tot een versterking van de alpha receptor gemedieerde response op sympathische stimulatie.

Recent werd een vergelijkbare bevinding gedaan door Floras e.a. [5]. Als stimulus werd door deze onderzoekers gebruik gemaakt van een negatieve druk aangelegd om de benen. Dit leidde via een sympathische respons tot vaatvernauwing, die aan de onderarm d.m.v. plethysmografie werd gemeten. Wanneer isoprenaline of adrenaline in de arteria brachialis werd toegediend, in zo lage doseringen dat hiervan geen systemisch effect werd gezien, bleek deze respons significant toegenomen. Een verhoogde receptor gevoeligheid kon worden uitgesloten, omdat de vaatvernauwing na infunderen van noradrenaline niet was toegenomen. Het effect van adrenaline hield aan tot minstens 30 min na beeindiging van de infusie. Binnen onze eigen groep onderzochten Blankenstijn e.a. [6] het effect van een 6 uur durende intraveneuze infusie van adrenaline 15 ng/min per kg lichaamsgewicht, waarbij plasma spiegels werden bereikt van 250 pg/ml. Het effect van adrenaline werd vergeleken met dat van noradrenaline en met dat van fysiologisch zout. Ook in deze relatief lage dosering bleek adrenaline de bloeddruk respons op de cold pressor test en op isometrische inspanning te versterken. Na het beeindigen van de infusie bleef de bloeddruk gedurende minstens 18 uur verhoogd ten opzichte van de controle waarden. Een en ander maakt waarschijnlijk dat presynaptische facilitatie van de noradrenaline afgifte onder invloed van adrenaline een fysiologische rol kan spelen bij het handhaven of verhogen van de bloeddruk, en dat deze rol niet beperkt blijft tot de tijd waarin de plasma adrenaline spiegel verhoogd is. Dit laatste pleit voor de mogelijkheid dat adrenaline na neuronale opname weer wordt afgegeven om dan opnieuw presynaptische receptoren te activeren [7].

Effect van een beta-2 selectieve antagonist op de bloeddruk

Als het zo is dat adrenaline via beta-2 receptoren de bloeddruk kan verhogen, is ook te verwachten dat beta-2 blokkade de bloeddruk kan verlagen. Daarom werd het effect op de bloeddruk

bestudeerd van een beta-2 selectieve blocker, ICI 118551. In een dubbelblinde placebo-gecontroleerde crossover trial werd het effect van een week behandeling met ICI 118551, 3 dd 50 mg, vergeleken met placebo en met een week propranolol, 3 dd 80 mg. Door metingen van bloeddruk, hartfrequentie, systolische tijdsintervallen, plasma kalium, noradrenaline en renine tijdens infusie van isoprenaline werd vastgesteld dat de gebruikte dosis van ICI 118551 inderdaad beta-2 selectief was. Voor zowel ICI 118551 als voor propranolol gold dat, 2 uur na inname van de eerste tablet, de hartfrequentie was gedaald terwijl de bloeddruk niet was veranderd. Na een week voortgezette behandeling, echter, bleken systolische en diastolische bloeddruk, indirect en intra-arterieel gemeten, zowel na beta-2 selectieve blokkade als na propranolol te zijn verlaagd. Verder was na beide betablockers de hartfrequentie en de plasma renine concentratie verlaagd. Met andere woorden, bij vergelijking van de effecten van beta-2 selectieve blokkade met niet-selectieve beta blokkade bleken de patronen van de verandering van hartfrequentie, renine en bloeddruk, ook qua beloop in de tijd, sterk overeen te komen. De gegevens zijn dan ook goed verenigbaar met de hypothese dat presynaptische beta-2 blokkade, door inhibitie van de noradrenaline afgifte, de bloeddruk verlaagt. Wel moet worden opgemerkt dat na beta-2 selectieve blokkade de bloeddrukdaling minder leek te zijn dan na propranolol. Het is daarom aannemelijk dat beta-1 blokkade bijdraagt aan de bloeddruk verlagende werking van betablockers.

Als argument tegen de bijdrage van presynaptische beta blokkade aan de bloeddruk verlagende werking van betablockers is aangevoerd dat inhibitie van de noradrenaline afgifte tot uiting had moeten komen in een daling van de plasma spiegel van noradrenaline. Wij vonden na beta-2 blokkade een niet significante daling van de noradrenaline spiegel en na propranolol een stijging. Hierbij moet worden opgemerkt dat na beta blokkade de noradrenaline klaring afneemt [4,8,9]. Verder zijn er aanwijzingen dat de daling van het hartminuutvolume na beta blokkade een reflex toename van de sympathische activiteit kan veroorzaken [10]. Beide mechanismen zullen een stijging van het plasma noradrenalne tot gevolg hebben. In een aantal studies is gevonden dat de noradrenaline afgifte of uitscheiding in de urine na beta blokkade daalt, vooral bij diegenen bij wie ook de bloeddruk daalt [11-14]. Ook blijkt de bloeddrukdaling na beta blokkade door een daling van de vaatweerstand verklaard te moeten worden [10]. Deze observaties passen juist goed bij het mechanisme van presynaptische beta blokkade.

Effect van beta agonisten en antagonisten op plasma renine

Voor wat betreft het type betareceptor dat betrokken is bij de stimulatie van renine afgifte zijn de gegevens in de literatuur niet eensluidend. Dit was de aanleiding om een nadere analyse te maken van de renine response op de verschillende door ons gebruikte betareceptor agonisten en antagonisten. De verandering in het renine werd vergeleken met de gelijktijdige verkorting van

de electromechanische systole (QS2I) en daling van de diastolische bloeddruk, parameters van respectievelijk beta-1 en beta-2 stimulatie. Bij eenzelfde diastolische drukdaling veroorzaakten isoprenaline en de beta-2 agonist salbutamol een gelijke stijging van het plasma renine. Bij eenzelfde verkorting van de QS2I veroorzaakte de beta-1 agonist prenalterol een geringere stijging van renine dan isoprenaline. Uit bestudering van de effecten van isoprenaline infusie zowel voor en na propranolol als voor en na atenolol bleek dat beide betablockers de renine respons curve in dezelfde mate naar rechts verschoven als de curve van de QS2I-verandering. Uit bestudering van de effecten van isoprenaline voor en na de beta-2 antagonist ICI 118551 en voor en na propranolol bleek dat beide betablockers de diastolische drukdaling geheel teniet deden en dat propranolol een aanzienlijke verschuiving van de renine respons curve bewerkstelligde, terwijl ICI 118551 hierop weinig invloed had. Met andere woorden, zowel beta-1 als beta-2 stimulatie kunnen het plasma renine doen stijgen. Tevens kon worden aangetoond dat de renine response moest verlopen via beta-1 receptor stimulatie. Na toediening van isoprenaline of van salbutamol was er een positieve lineaire correlatie tussen de stijging van renine en die van noradrenaline, maar niet tussen de stijging van noradrenaline en de diastolische drukdaling. In het licht van wat hiervoor werd besproken kunnen de gegevens het best worden geinterpreteerd als een sterke aanwijzing dat presynaptische beta-2 receptor stimulatie de noradrenaline afgifte verhoogt en daardoor een postsynaptische beta-1 receptor gemedieerde response, in casu een stijging van het renine, veroorzaakt.

CONCLUSIES

Onze conclusies kunnen als volgt worden samengevat.

1) Beta-2 agonisten verhogen het plasma noradrenaline, waarschijnlijk door een rechtstreekse invloed op de neuronale afgifte van noradrenaline.
2) De door beta-2 agonisten geinduceerde stijging van noradrenaline gaat gepaard met een postsynaptische beta-1 respons, namelijk een stijging van het plasma renine.
3) Adrenaline, in fysiologische concentraties, verhoogt het plasma noradrenaline. Dit effect komt ook tot stand door betareceptor stimulatie.
4) Adrenaline, in fysiologische concentraties, leidt tot een versterking van een postsynaptische alpha response, namelijk de bloeddrukstijging tijdens bepaalde gestandaardiseerde vormen van fysieke stress. Dit effect van adrenaline komt eveneens tot stand door beta receptor stimulatie.
5) Behandeling van hypertensie met een beta-2 selectieve antagonist veroorzaakt een daling van de bloeddruk.

Deze conclusies zijn sterke argumenten dat de mens beschikt over presynaptische beta-2 receptoren, die een rol spelen bij de

regulatie van de bloeddruk. Activatie van deze beta-2 receptoren door het hormoon adrenaline versterkt het effect van sympathische zenuwstimulatie door toegenoemen afgifte van de neurotransmitter noradrenaline. Blokkade van beta-2 receptoren leidt tot een daling van de bloeddruk bij patienten met essentiele hypertensie.

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CURRICULUM VITAE

The author was born on februari 11th 1954 in Schiedam, the Netherlands. In 1972 he obtained the diploma Gymnasium β at Scholengemeenschap Spieringshoek in Schiedam. The same year he commenced the medical study at the medical faculty, now Erasmus University, in Rotterdam. He graduated from medical school in januari 1979. In march 1979 he started his training in Internal Medicine in the University Hospital Dijkzigt, Rotterdam, department of Internal Medicine I, headed by Prof. Dr. J.J. Gerbrandy and later by Prof. Dr. M.A.D.H. Schalekamp. The studies described in this thesis were started during this training period. From march 1985 the author works in the same department as an internist/nephrologist.

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