Studies with β-adrenoceptor antagonists in essential hypertension

Studies met β-receptorblokkerende middelen bij essentiële hypertensie

Proefschrift

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Contents

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2. van den Meiracker A. H., Man in 't Veld A. J., Ritsema van Eck, Schalekamp M. A. D. H.: Systemic and renal vasodilation after β-adrenoceptor blockade with pindolol: A hemodynamic study on the onset and maintenance of its antihypertensive effect. *Am Heart J.* 1986; 112: 368-374.

3. van den Meiracker A. H., Man in 't Veld A. J., Fischberg D. J., Molinoff P. B., Ritsema van Eck H. J., Boomsma F., Derkx F. H. M., Schalekamp M. A. D. H.: Acute and long-term effects of acebutolol on systemic and renal hemodynamics, body fluid volumes, catecholamines, active renin, aldosterone, and lymphocyte β-adrenoceptor density. *J. Cardiovasc Pharmacol* 1988; 11: 413-423.

4. van den Meiracker A. H., Man in 't Veld A. J., Ritsema van Eck H. J., Boomsma F., Derkx F. H. M., Schalekamp M. A. D. H.: The clinical pharmacology of bopindolol, a new long-acting β-adrenoceptor antagonist in hypertension. *Clin Pharmacol Ther* 1987; 42: 411-419.

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6. van den Meiracker A. H., Man in 't Veld A. J., Boomsma F., Molinoff P. B., Schalekamp M. A. D. H.: Hemodynamic and β-adrenergic receptor adaptations during long-term β-adrenoceptor blockade. Studies with acebutolol, atenolol, pindolol and propranolol in hypertensive patients. Submitted for publication.

7. van den Meiracker A. H., Man in 't Veld A. J., Boomsma F., Blankestijn P. J., Schalekamp M. A. D. H.: Epinephrine-induced enhancement of sympathetic activity in man; inhibition by non-selective as well as β_1-selective β-adrenoceptor blockade. Submitted for publication.

1. General introduction

1.1 History

The concept of β-adrenoceptor antagonism represents a clear example of logical scientific development in therapeutic research. In 1948 R. P. Ahlquist (1) published his classical paper "A study of the adrenotropic receptors", in which he suggested that there were two distinct types of adrenotropic receptors, which he destignated as α and β. The α-receptors mediating mainly exitatory, and the β-receptors inhibitory functions, with the important exception that in the heart the exitatory receptors were of the β type.

Ten years later in 1958 Powell and Slater (2) showed that dichloroisoprenaline inhibited the effects of isoprenaline on tracheal and vascular smooth muscle, and in the same year Moran and Perkins (3) showed that it also inhibited the effects of adrenergic agents on the heart. However, the high level of partial agonist acitivity of dichloroisoprenaline restricted its potential use in clinical practice.

A crucial contribution to the development of β-adrenoceptor blocking agents was made by J.W. Black. He hypothesized that blockade of the action of the sympathetic nervous system on the heart might be a new approach in the treatment of patients with angina pectoris. A major break-through came in 1960 with the synthesis of pronethalol, a compound in which the two chloride ions of dichloroisoprenaline were replaced by another phenyl ring. The properties of pronethalol, a non-selective β-adrenoceptor antagonist with only a small degree of partial agonist activity, as well its usefulness in the treatment of patients with angina pectoris were described in 1962 (4,5).

In 1967 A. M. Lands and colleaques, by studying the effects of different sympathetic amines on a number of organ systems, classified β-adrenoceptors into two subtypes: β_1 and β_2 (6). β_1-Receptors mediating responses in the heart, and β_2-receptors

mediating responses in vascular and bronchial smooth muscle. Independently of the study of Lands et al it was reported in 1968 that the β-adrenoceptor antagonist practolol reduced exercise-induced tachycardia, but not isoprenaline-induced vasodilatation in man (7). This drug, therefore, appeared to be the first β_1-selective adrenoceptor antagonist that was used in man.

1.2 Antihypertensive Effect of β-Adrenoceptor Antagonists

According to the idea of J.W. Black β-adrenoceptor antagonists were originally developed for diminishing myocardial oxygen requirements, thereby benefiting patients with ischaemic heart disease. From animal studies there was no indication that β-adrenoceptor antagonists possessed antihypertensive properties. The fact that this catagory of drugs lowered blood pressure in man was therefore completely unexpected. In 1964 Prichard and Gillam described the antihypertensive effect of pronethalol (8). Later in that year the antihypertensive properties of propranolol were reported by the same authors (9).

For several reasons there was initially considerable resistance to the idea of the use of β-adrenoceptor antagonists in hypertension.

1. The antihypertensive effect was unexpected.
2. The mechanism underlying the antihypertensive effect was unknown.
3. The drugs were cardiodepressive, i.e. they lowered cardiac output, whereas hypertension in general is caused by a high vascular resistance.
4. These drugs did not reduce blood pressure acutely since the fall in cardiac output was compensated for by an increase in vascular resistance.

However, due to their efficacy and overall good toleralability the popularity of using β-adrenoceptor antagonists in the treatment of hypertension gradually increased. In the early 1980's β-adrenoceptor antagonists were the most commonly prescribed antihypertensive drugs in many West-European countries.

1.3 Classification of β-Adrenoceptor Antagonists

All β-adrenoceptor antagonists have in common to inhibit competitively the effect of catecholamines on the β-adrenergic receptor. Apart from this a number of the compounds possess so called ancillary pharmacological properties such as β_1-selectivity, partial agonist activity and membrane stabilizing activity.

Selective β_1-antagonism was first demonstrated in 1968 with practolol (7), and subsequently other compounds with this property have been described (Table). The term cardioselectivity has been introduced to indicate that the drugs selectively block cardiac β_1-adrenoceptors, and that bronchial β_2-adrenoceptors would remain unblocked. This implied safety factor in patients with asthma is, however, relative as selectivity diappears with higher doses (10). Furthermore, radioligand-binding studies have shown, that the human heart and the larger airways contain both β_1 and β_2-adrenoceptors mediating the same response (11,12). β_1-Adrenoceptor selectivity seems therefore a more accurate term than cardioselectivity apart from the fact that other β_1-adrenoceptors like for instance those on juxtaglomerular cells are also blocked by β_1-selective drugs.

Some β-adrenoceptor antagonists, when they occupy the receptors, besides inhibiting the effects of catecholamines, also possess a certain degree of stimulatory activity of their own. This stimulatory effect is called partial agonist activity (PAA), often referred to as intrinsic sympathomimetic activity (ISA) (13). The expression of PAA is most distinct when background sympathetic tone is low. For example, at night, pindolol, a β-adrenoceptor antagonist with a relatively high degree of PAA, shows in man its stimulant action leading to an increase in heart rate, whereas during the day heart rate with this drug is reduced (14,15). Furthermore, in patients with chronic autonomic failure in whom residual sympathetic tone is very low, pindolol increases heart rate and cardiac output (16). Although data are limited there is evidence that the ISA of non-selective as well as β_1-selective antagonists is restricted predominantly to the β_2-adrenoreceptor (17).

Membrane stabilizing activity (MSA) is a non-β-adrenoceptor-

mediated property, which is shared by some of the β-adrenoceptor antagonists (Table). Local anaesthetic activity, a quinidine-like effect, serotonin antagonism, inhibition of phospholipase A are among of the different mechanisms which may underlie the MSA of β-adrenoceptor antagonists (18). Initially it was suggested that the MSA of propranolol was responsible for its beneficial effect in cardiac arrhytmias (19). All the available evidence nowadays indicates that MSA does not contribute to the therapeutic effects of β-adrenoceptor antagonists in arrhytmias, hypertension or angina pectoris.

The solubility of β-adrenoceptor blocking agents in lipid or water is conventionally expressed as the n-octanol-water partition coefficient (Table). Although no ancillary property in a strict sense this partition coefficient is an important determinant of the pharmocokinetic profile of drugs, including β-adrenoceptor antagonists. As a general rule lipid-soluble drugs as opposed to water-soluble drugs are more rapidly and more completely absorbed from the gastrointestinal tract, they are extensively metabolised by the liver (first pass effect), they have a higher binding to plasma proteins, and they are more widely distributed to all body tissues. Lipid-soluble drugs have the ability to cross easily the blood-brain barrier (20,21). The clinical implication of this phenomenon is that central nervous system side-effects more frequently occur with lipophilic than with hydrophilic β-adrenoceptor antagonists.

1.4 Mechanism of Antihypertensive Action

Despite extensive research the mechanism of the antihypertensive action of β-adrenoceptor antagonists remains uncertain. It is generally agreed that antagonism of the β-adrenoceptors *per se* underlies the antihypertensive effect of these drugs. This is concluded from the fact that the dextro-isomer of propranolol, which lacks β-adrenoceptor blocking properties, does not produce any fall in blood pressure in man (22).

Besides their unknown mechanism of action, another puzzling feature of β-adrenoceptor blocking agents is their delayed antihypertensive response. After acute administration heart rate and

Table: *Pharmacological characteristics of β-adrenoceptor antagonists*

Agent	β_2-selectivity	Partial agonist activity	Membrane stabilising activity	Partition* coefficient
Acebutolol	+	+	+	0,68
Alprenolol	—	+	+	12,4
Atenolol	++	—	—	0,015
Betaxolol	++	—	±	3,89
Bisoprolol	+++	—	—	0,82
Metropolol	++	—	±	0,98
Oxprenolol	—	++	+	2,28
Penbutolol	—	+	+	179
Pindolol	—	+++	±	0,82
Propranolol	—	—	++	20,2
Sotalol	—	—	—	0,039
Timolol	—	—	—	1,16

* octanol/water at pH 7.4 and 37° C.

cardiac output are almost immediately reduced, but, owing to a compensatory increase in total peripheral vascular resistance, blood pressure remains initially unchanged. During continued treatment the cardiodepressant effect is usually maintained, but blood pressure is then reduced. As a consequence, the fall in blood pressure is caused by a decrease of the initially elevated vascular resistance. Originally it was believed that the full antihypertensive effect of β-adrenoceptor agents was not seen for several weeks after starting of treatment (23), but more recent studies suggest that this delay is in the range of hours to days (24). The precise interval, however, is not known, since detailed haemodynamic studies during onset of the antihypertensive action of β-adrenoceptor agents have not been performed.

β-Adrenergic receptors are located in virtually every organ, but with regard to blood pressure control, only those β-adrenergic receptors which are located in the heart, the central nervous system, the peripheral sympathetic nervous system and the kidney require consideration. Theoretically, blockade of β-adrenergic receptors in these organs may result in a fall in blood pressure through a decrease in cardiac output, a reduction in central sympathetic outflow and/or a resetting of the arterial baroreflex, a decrease

of the peripheral release of noradrenaline, or inhibition of renin release. The pros and cons of these several possibilities will be discussed extensively in the subsequent chapters of this thesis. Although an increased peripheral vascular resistance is a uniform finding in established essential hypertension, this does not mean that the pathogenetic moments ultimately resulting in this increased resistance are similar in each patient. The possibility has therefore to be considered that in different patients different mechanisms may contribute to the blood pressure lowering effect of β-adrenoceptor blocking agents.

1.5 Aim of the Thesis

The studies described in this thesis were aimed to extend our understanding of the mode of action of β-adrenoceptor blocking agents in essential hypertension. For this purpose the literature, concerning the acute and chronic effects of 10 different β-adrenoceptor blocking agents, on haemodynamics, plasma catecholamines, and active renin, was extensively reviewed. (Chapter 2). In addition a number of orginal studies were performed. In these studies the acute and chronic effects of β-adrenoceptor antagonists with different ancillary properties on haemodynamic and hormonal parameters were investigated. The observation periods in the acute studies lasted for 24 hours. These observation periods gave us the opportunity to study in detail the sequence of haemodynamic and hormonal events that take place during the onset of the blood pressure lowering effect of β-adrenoceptor antagonists after starting treatment.
In chapters 3, 4 and 5 the haemodynamic effects of those β-adrenoceptor antagonists with varying degrees of partial agonist activity, respectively the non-selective antagonist pindolol, the β_1-selective antagonist acebutolol and the non-selective antagonist bopindolol, are described. In chapter 6, the time course of changes in systemic and central haemodynamics, plasma catecholamines, and active renin during the onset of the antihypertensive effect of propranolol, atenolol, acebutolol and pindolol is described. Chapter 7 presents the long-term effects of pindolol, propranolol,

acebutolol and atenolol on systemic and renal haemodynamics. Also presented in this study are the effects of the drugs on the density of beta-adrenergic receptors on lymphocyte membranes. Disease or drug-induced alterations in the number of lymphocyte β-adrenergic receptors may reflect changes in the number of β-adrenoceptors in less accessible organs as the heart and lungs (25,26). Depending on whether β-adrenoceptor antagonists possess partial agonist activity or not the number of lymphocyte β-adrenergic receptors may decrease or increase. An increased density of β-adrenergic receptors may underlie the so-called hyperadrenergic state, which sometimes occurs after abrupt withdrawal of β-adrenoceptor antagonists (27).
An impairment of the release of noradrenaline from sympathetic nerves through inhibition of postganglionic presynaptic β-adrenoceptors is one of the proposed antihypertensive mechanisms of β-adrenoceptor antagonists. Adrenaline may be the natural agonist of the presynaptic β-adrenoceptor, which is of the β_2-subtype, and in this way adrenaline may play a role in the pathogenesis of certain forms of hypertension (28-30). Chapter 8 shows that resting and activated sympathetic activity is enhanced by a low intravenous dose of adrenaline as was described previously by us (31). In addition this chapter describes the effects of non-selective as well as β_1-selective β-adrenoceptor antagonism on this adrenaline-induced enhancement of sympathetic activity. Finally, in chapter 9 some general conclusions that can be drawn from our studies are discussed.

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2. The effect of β blockers on total peripheral resistance

2.1 Summary

A review of the relevant literature indicates that the effects of β blockers on cardiac output and plasma renin or penetration of these drugs into the brain and cerebrospinal fluid are not essential for their antihypertensive effect. Reduction in blood pressure during long-term β-blocker therapy is always associated with reduction of total peripheral resistance. Evidence is presented suggesting that blockade of presynaptic β receptors underlies this vasodilatator effect of β blockers.

2.2 Introduction

After β blockers had been introduced for the treatment of angina pectoris (1), it was soon discovered that this type of drug is also effective in lowering blood pressure (2-4). A great variety of β blockers are now available for clinical use (5). They differ in their so-called ancillary properties, such as intrinsic sympathomimetic activity (ISA) or partial agonist activity, β_1-receptor selectivity, and hydrophilicity, but they all lower blood pressure. The underlying mechanism for this effect is still an enigma (5-7). Undoubtedly, this mechanism is related to blockade of β receptors, because the dextroisomers of β blockers do not lower blood pressure (8). Reduction of adrenergic nerve activity, cardiac output, and renin release by blockade of central, cardiac, and juxtaglomerular β receptors, respectively, has been proposed and refuted as being important. Recently, attention has been focused on so-called presynaptic β receptors located on postganglionic sympathetic neurons as a site at which β blockers may act to lower blood pressure. Blockade of these receptors causes inhibition of norepinephrine release (9-21). This would cause a reduction

in cardiac, renal, and vaso-constrictor nerve transmission, which may add to the effects of postsynaptic β blockade (10,12-14,16,20-23).

Our knowledge of the pharmacological actions of β blockers with different ancillary properties has enormously increased during the past two decades. At first sight, the recognition that these different compounds are about equally effective in lowering blood pressure seems to make it difficult to identify a single underlying mechanism (5,24-31). However, a more thorough comparative analysis of the different actions of β blockers in terms of changes in cardiac output, vascular resistance, plasma renin, and norepinephrine may contribute to our understanding of the underlying mechanism of their antihypertensive action. This led us to search the medical literature for these actions of β blockers. Lipophilicity and plasma protein binding of these drugs were taken into account as possible determinants of a central action.

2.3 Review of the literature

Selection of papers

A computer-assisted search of the literature on the effects of 10 different β blockers on systemic hemodynamics, plasma renin, and plasma norepinephrine was carried out. The MEDLAR system of the National Library of Medicine and DATA BASE of Excerpta Medica were used for this purpose. The selected papers are shown in Table 1 (37-151). For reasons of standardization, only studies performed in the supine resting state have been included. A separate analysis was made for acute and long-term studies. "Acute" refers to those studies in which β blockers were administered intravenously, and "long-term" refers to studies on hypertensive patients who were treated orally for more than 1 week. Studies on the effect on plasma renin were included only when performed in patients who had no dietary sodium restriction. Renin in these studies was measured by radioimmunoassay of angiotensin I that was generated in vitro at neutral or slightly acidic pH with endogenous renin substrate. In most studies on the effect on plasma norepinephrine, specific radioenzymatic techniques were used, with

the exception of two studies in which a fluorometric method was employed. In those cases where more than one study on a given β blocker was available, the mean values were calculated. When four or more studies on one β blocker were available, the standard error of the mean was also calculated. The relevant ancillary properties and physicochemical characteristics are given in Table 1(32-36, 152-154).

Association between antihypertensive effect and reduction in vascular resistance, a phenomenon common to all β blockers

Intravenous injection of a β blocker devoid of intrinsic sympathomimetic activity (ISA) is followed by a drop in heart rate and cardiac output (Fig. 1). Arterial pressure, however, changes very little, and total peripheral resistance must therefore be raised. This pattern is observed with all β blockers devoid of ISA irrespective of the property of β_1-receptor selectivity. The cardiodepression by β blockers with ISA appears to be inversely proportional to their pharmacologically defined degree of partial agonist activity or ISA. The acute blood pressure lowering effect of β blockers with and without ISA is similar. Therefore, the increment in total peripheral resistance in response to a given degree of cardiodepression is also inversely proportional to the degree of ISA. Similar to what has been observed with β blockers without ISA, the resistance response to cardiodepression after β blockers with ISA is also not modified by absence or presence of relative selectivity for β_1 receptors.

During long-term therapy the different β blockers appear to be roughly equally effective in lowering blood pressure. Because there is little difference between the acute and long-term effects of a given β blocker on cardiac output (Fig. 1), the decrease in arterial pressure that is observed with long-term treatment is an indication of a decrease in total peripheral resistance. Thus, in the end, all β blockers, no matter their effect on cardiac output, lower blood pressure by shifting vascular resistance to a lower level. In other words, the decrease in arterial pressure during long-term β-blocker therapy is always associated with a decrease in vascular resistance (Fig. 2). However, because on their different effects on cardiac

Table 1. *Literature survey on the effects of 10 different β blockers on hemodynamics, plasma renin activity, and plasma norepinephrine concentrations*

	Pindolol	Practolol	Alprenolol	Oxprenolol	Acebutolol	Penbutolol	Metoprolol	Atenolol	Propranolol	Timolol
Characteristics										
Intrinsic sympathomimetic activity	+++	++	+	+	±	±	—	—	—	—
β-Adrenoceptor selectivity	$\beta_1+\beta_2$	β_1	$\beta_1+\beta_2$	$\beta_1+\beta_2$	β_1	$\beta_1+\beta_2$	β_1	β_1	$\beta_1+\beta_2$	$\beta_1+\beta_2$
Log P octanol water	0.82	0.79	2.61	2.28	0.68	1.70	0.98	0.02	20.20	1.26
Plasma protein binding (%)	40	0	85	80	25	95	10	5	95	10
Acute hemodynamic studies										
References (n = 44)	37-41	39,40,42-46	43,47-51	52-56	44,57-59	60	43,51,56,61-64	39,40,43	39,44,49,65	66,67
Number of patients (n = 430)	34	54	47	61	42	25	54	15	62	36
Dosage (mg i.v.)	1.3 (1.0-1.8)	40 (20-45)	8 (5-10)	10 (5-14)	55 (30-70)	4	13 (10-20)	4 (2-5)	9 (2-15)	0.6 (0.3-1.0)
Observation period (min)	40 (15-90)	30 (15-90)	20 (15-35)	18 (15-30)	30 (30)	30	15 (10-20)	50 (15-90)	35 (15-90)	30 (30)
Long-term hemodynamic studies										
References (n = 41)	68-78	79	80,81	53-55,72,82	72-83	72,84	72,80,85	72,86-90	65,72,91-95	66,80,93,96-99
Number of patients (n = 482)	92	8	17	29	25	19	39	99	67	87
Dosage (mg/day)	18 (5-30)	2,400	500 (400-600)	230 (200-280)	800 (400-1,200)	45 (40-50)	175 (125-200)	230 (100-600)	320 (240-360)	40 (20-60)
Observation period (weeks)	12 (1-64)	1	27 (6-48)	8 (3-12)	5 (4-5)	29 (5-48)	34 (5-48)	66 (3-250)	20 (4-80)	27 (4-64)
Studies on plasma renin activity										
References (n = 54)	100-110	110-113	114,115	110,116-118	83,119,120	121	106,122-125	90,126-130	101,105,106 123,131-138	139,140
Number of patients (n = 784)	143	49	68	27	73	14	42	105	245	18
Dosage (mg/day)	28 (5-45)	400 (200-800)	500 (300-900)	115 (40-240)	1300 (800-2,000)	100	280 (150-450)	160 (50-300)	185 (80-240)	30
Observation period (weeks)	4 (1-12)	8 (1-24)	7 (4-12)	2 (1-3)	4 (1-6)	24	6 (4-12)	7 (3-16)	5 (1-16)	8
Studies on plasma norepinephrine										
References (n = 17)	104,108,141-146	—	—	—	—	121	122,125,144	147	95,148-151	—
Number of patients (n = 217)	104	—	—	—	—	5	25	16	67	—
Dosage (mg/day)	22 (10-30)	—	—	—	—	50	270 (200-300)	200	260 (240-320)	—
Observation period (weeks)	8 (1-16)	—	—	—	—	24	4 (4)	5	3 (1-8)	—

The data on dosages and observation periods of the various β-blockers are presented as means (and ranges). The value of log P octanol/water is an estimate of liposolubility: The higher this value, the more lipophilic the β-blocker in question is.

output, resistance is lower on β blockers with ISA than on β blockers lacking this property. On a β blocker with ISA, the initial rise in resistance in response to cardiodepression is relatively small or even absent, depending on the degree of ISA.

Suppression of plasma renin, an epiphenomenon
The effects on plasma renin of β blockers with and without ISA are different. There is a close correlation between the degree of cardiodepression and the degree of renin suppression, indicating that the effect of the different β blockers on renin is determined by their degree of ISA (Fig. 3). Contrary to what one may expect, the change in total peripheral resistance with longterm β-blocker therapy appears to be inversely correlated with the effect on plasma renin. This makes it unlikely that suppression of renin is the cause of the decrease in vascular resistance and, for that matter, the decrease in blood pressure during long-term β blockade.

Changes in plasma norepinephrine, an unsettled issue
Plasma norepinephrine is raised during long-term treatment with β blockers devoid of ISA. In contrast, pindolol, which exerts the most pronounced degree of ISA, lowers plasma norepinephrine (Fig. 4). The clearance of plasma norepinephrine is reduced by β-blockers without ISA, and this could explain why plasma norepinephrine is increased (151, 155). Thus, unchanged or even raised plasma levels of norepinephrine might still be compatible with a reduced sympathetic activity and diminished spillover of the sympathetic transmitter from the synaptic cleft. The reduced clearance rate of norepinephrine from plasma during treatment with β blockers devoid of ISA might be related to their cardiodepressant effect — an effect that is associated with reduced blood flow through liver and lungs, where norepinephrine is known to be removed from the circulation (156). Pindolol does not cause a reduction of blood flow through these organs (157), and the fall in plasma norepinephrine with this drug might therefore reflect reduced transmitter release.

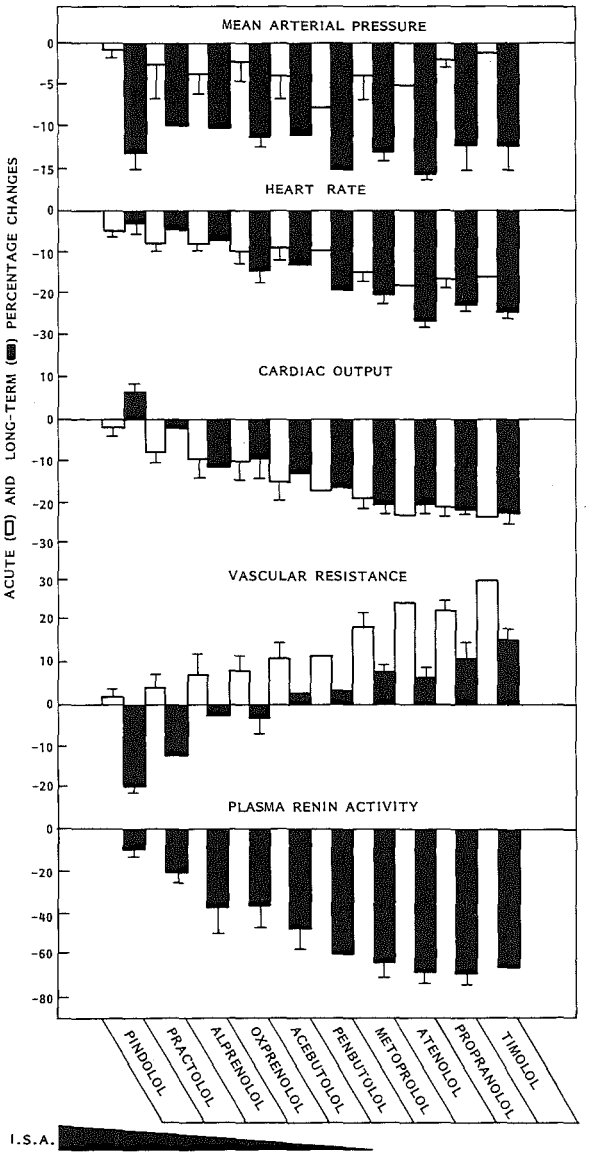

Fig. 1. Effects of 10 β blockers on hemodynamics and plasma renin activity under basal conditions. Data are presented as mean percentage changes (±SEM). The drugs are depicted according to a decreasing degree of intrinsic sympathomimetic activity (ISA) from left to right. Metoprolol, atenolol, propranolol, and timolol lack the property of ISA.

2.4 Interpretation of literature review

Different β blockers have similar antihypertensive efficacy despite dissimilar effects on heart rate, cardiac output, vascular resistance, plasma renin, and plasma norepinephrine. This is further illustrated in Figure 5, in which the interrelations between heart rate, plasma renin activity, and vascular resistance are shown, during long-term treatment with the 10 different β blockers.

Differences in liposolubility between β blockers, as estimated by their value of log P octanol/water or their plasma protein binding (Table 1), have no influence on the onset of their antihypertensive action or on the magnitude of this effect (7,101,108,158-160). The equal antihypertensive effect of the different types of β blockers is difficult to reconcile with theories that imply blockade of either central β receptors or post-synaptic cardiac or juxtaglomerular β receptors as the common underlying key mechanism.

An alternative explanation would be the reduction of vasoconstrictor nerve transmission by blockade of presynaptic β receptors. This, in our view, provides an unconstrained explanation for the decrease in vascular resistance as a characteristic hemodynamic feature of the response to long-term β-blocker therapy in hypertension. The effects on the plasma level of norepinephrine that have been reported are compatible with this hypothesis when allowance is made for changes in the clearance of plasma norepinephrine.

Presynaptic β receptors are perhaps also important in the pathogenesis of hypertension. Epinephrine has been implicated as the natural stimulant of these receptors (161). In low concentrations, epinephrine enhances the release of norepinephrine in the rabbit (162), and this catecholamine can induce hypertension in the rat, an effect that is prevented by β blockade (163). β-Receptor agonists and low doses of epinephrine cause a rise in plasma norepinephrine in man (164,165). Some patients with essential hypertension have a raised plasma level of epinephrine (166-170). These patients show increased α-receptor-mediated vasoconstriction, which is reversed by propranolol (171,172).

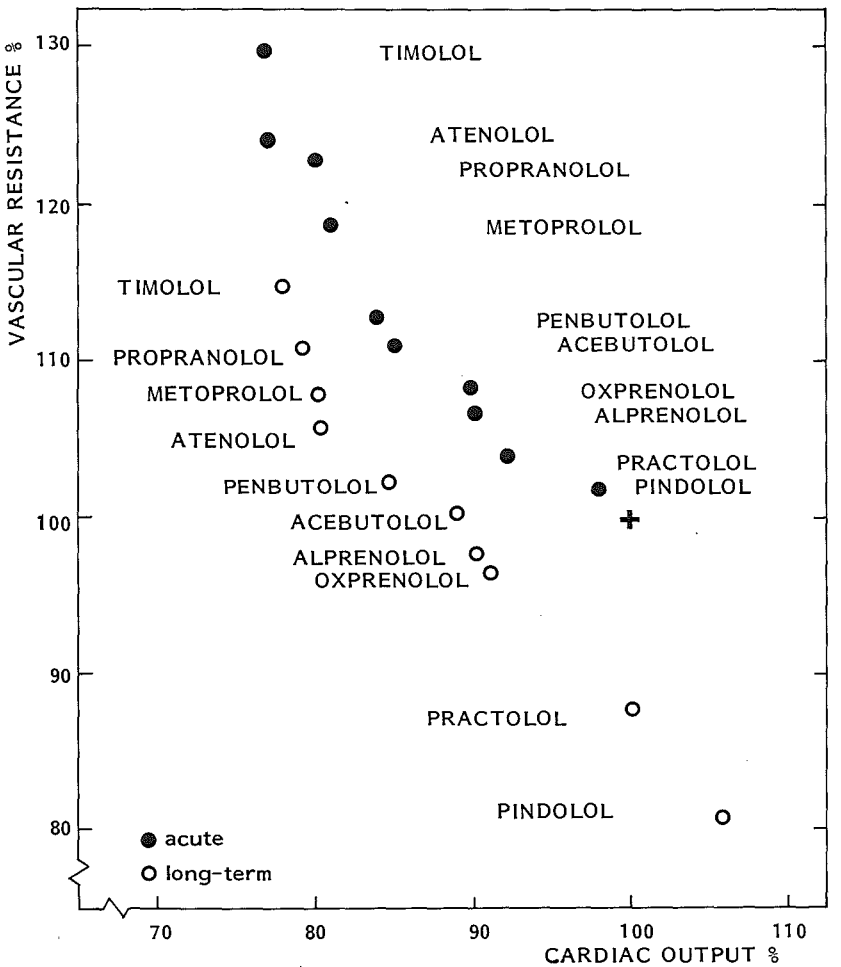

Fig. 2. Relationship between cardiac output and total peripheral resistance after acute intravenous β blockade (•) and after long-term oral β blockade (○) in hypertension with 10 β-blockers. Data are taken from Figure 1. The cross indicates the pretreatment value.

The importance of epinephrine-stimulated norepinephrine release via presynaptic β-receptor activation is further illustrated by the amplification of the pressor effect of reflex sympathetic stimulation by cold exposure and isometric exercise (165). This amplification

is abolished by propranolol. Epinephrine may therefore act on presynaptic β receptors to increase sympathetic vasoconstrictor nerve transmission. Competitive antagonism by β blockers of epinephrine's action at presynaptic β-receptor sites provides an elegant explanation for their vasodilator and antihypertensive effect, an effect that is common to all β blockers despite their different ancillary properties.

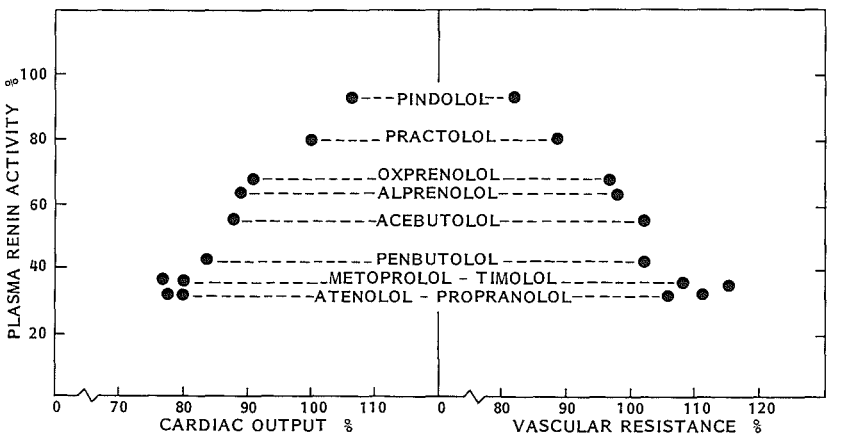

Fig. 3. Plots of cardiac output and total peripheral resistance vs. plasma renin activity during long-term therapy of hypertension with 10 different β blockers.

2.5 Observations on the time course of hemodynamic changes after the onset of β blockade

The present analysis summarizes the acute and longterm hemodynamic effects reported in the literature for 10 different β blockers, as well as their effects on plasma renin activity and plasma norepinephrine in supine resting hypertensive patients. One point of criticism of any conclusions based on the results of a review of this kind could be aimed at the validity of combining results from many different trials with different subjects, investigators, protocols, methods of measurement, and dosage range for the β blockers under study. However, with the wide range of studies used, it is difficult to imagine that consistent bias would have arisen. Moreover, since changes in heart rate, cardiac output,

vascular resistance, plasma renin activity, and plasma norepinephrine concentration show a graded response according to one of the well-defined properties of these agents — their degree of ISA — it is difficult to deny the general impression as outlined in Figures 1-5. One other criticism is less easy to overcome, although it seems somewhat less relevant. For reasons of standardization, only studies in supine resting subjects who had no restriction of their dietary sodium intake were included in this review. It is quite clear that the effects of the different β blockers during exercise, or at least during normal ambulation, may show smaller differences among the various agents. A review of this kind has not been attempted because of the relative paucity of these data and the lack of standardization of activities in different studies.

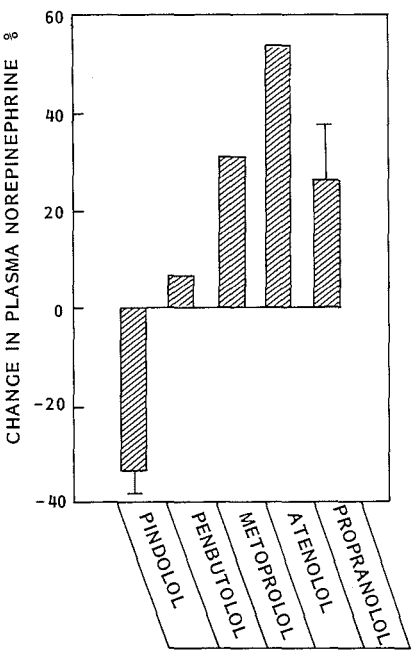

Fig. 4. Effects of five β blockers on basal plasma norepinephrine values. Mean percentage changes (±SEM) are shown.

One very important criticism is that some conclusions are based on interpolation between acute and long-term studies. Detailed hemodynamic studies during the onset of the antihypertensive action of β blockers, however, are lacking. Therefore we studied

the hemodynamic changes after starting β-blocker therapy with pindolol for 24 h in 10 patients with uncomplicated essential hypertension (173). Heart rate and intraarterial pressure were measured for 24 h on placebo and after starting pindolol 10 mg b.i.d. During the second study cardiac output was also measured every hour by the thermodilution technique. During both 24-h studies the patients had complete bed rest. Before and 2, 6, 12 and 24 h after treatment with pindolol was started, venous blood was sampled for determination of plasma norepinephrine and active plasma renin.

One hour after pindolol, arterial pressure was already reduced significantly compared with baseline and placebo values ($p < 0.05$) (Fig. 6). The maximum anti-hypertensive effect was seen 3-4 h after dosing (—15 ± 3% compared with placebo; $p < 0.001$). The maximum drop in systemic pressure was caused by vasodilatation; systemic vascular resistance had fallen by 25 ± 6% ($p < 0.01$) at that time, without significant effects on heart rate and cardiac output, as compared with baseline values. In comparison with placebo values, however, heart rate was significantly reduced from 80 ± 3 to 67 ± 2 beats per minute ($p < 0.01$). After 24 h, arterial pressure and systemic vascular resistance were reduced by 14 ± 2% ($p < 0.01$) and 25 ± 4% ($p < 0.001$), respectively. By that time cardiac output was increased by 16 ± 5% ($p < 0.05$). The decreases in arterial pressure and systemic vascular resistance were not associated with a rise in plasma norepinephrine.

Active plasma renin was normal or low in all patients (8.6 ± 1.5 μU/ml; normal range 5-35 μU/ml). It fell significantly after pindolol by 38 ± 3% ($p < 0.05$). The fall in arterial pressure, however, was inversely correlated with suppression of plasma renin ($r = -0.58$, $p < 0.01$).

This study shows that the nonselective β-blocker pindolol, with considerable ISA, after oral dosing, exerts its full antihypertensive effect within the absorption phase of the drug. Apparently there is no time delay between the blockade of β receptors per se and the antihypertensive effect. This contrasts with β blockers devoid of ISA (91,174,175). The onset and maintenance of the

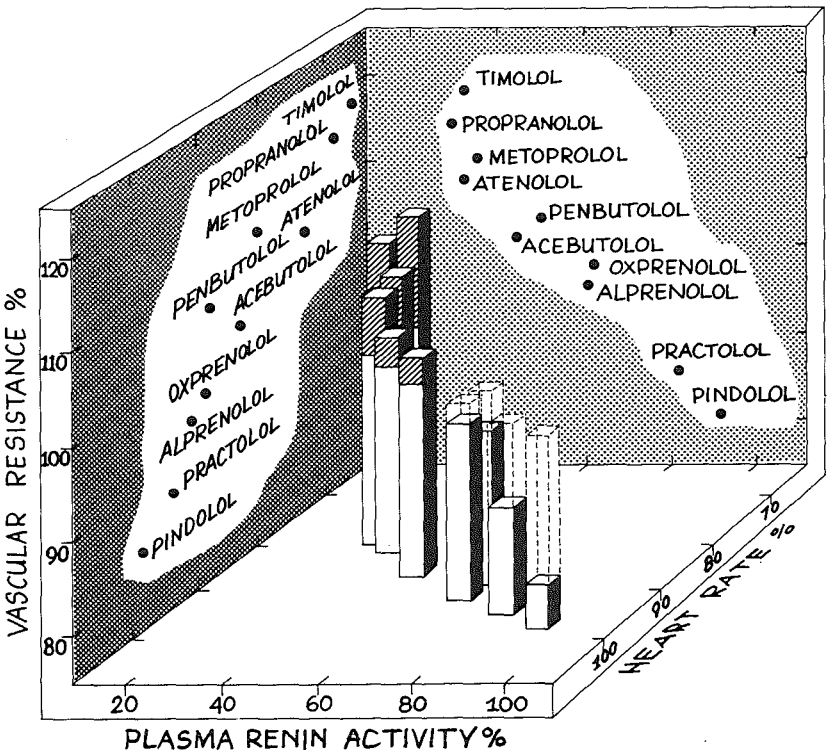

Fig. 5. Correlations between plasma renin activity, heart rate, and vascular resistance during long-term treatment of hypertension with various β blockers. Heart rate and plasma renin activity are directly correlated during treatment with the various β blockers. Both variables are determined by the quantity of ISA of a given β blocker. Despite the variable effects of the β blockers on heart rate and plasma renin activity, their hypotensive effect is of approximately equal magnitude. Plasma renin activity and vascular resistance are inversely correlated. This strongly suggests that the fall in vascular resistance that is always associated with a reduction of mean arterial pressure cannot be caused directly by reduction of plasma renin activity. Thus the lower the heart rate and plasma renin activity during treatment with the different β blockers, the higher the absolute value of vascular resistance, as indicated by the columns. The hatched parts of the columns indicate increments of vascular resistance above 100% — i.e., the pretreatment value (timolol, propranolol, atenolol, metoprolol, acebutolol, and penbutolol). The transparent parts of the columns indicate the supplement of vascular resistance to 100% (pindolol, practolol, oxprenolol, and alprenolol).

antihypertensive effect of pindolol is associated with a reduction of the elevated vascular resistance in essential hypertension. This also contrasts with the hemodynamic profile of β blockers lacking ISA, whether or not they are cardioselective (Figs. 1-5).
The vasodilator activity of pindolol cannot easily be explained by suppression of plasma renin activity. Although pindolol reduced plasma renin, blood pressure reduction and renin suppression were inversely correlated. Despite its vasodilatator and antihypertensive effects, pindolol did not increase plasma norepinephrine. This suggests that sympathetic activity was reduced by pindolol. Such an effect through blockade of central and/or peripheral presynaptic β receptors would also elegantly explain the vasodilatator properties of pindolol.

2.6 Future prospects in vasodilatating β-blockers

Long-standing arterial hypertension is characterized hemodynamically by a normal or subnormal cardiac output and an elevated vascular resistance. It appears that β blockers devoid of ISA are worsening the underlying hemodynamic abnormality of long-standing arterial hypertension; i.e., they increase vascular resistance further. Depending on the degree of ISA, β blockers possessing this property leave the elevated vascular resistance relatively untouched or decrease it somewhat below pretreatment levels. Just as propranolol has been the prototype of all β blockers for over 20 years, pindolol is the prototype for the vasodilatating β blockers. This drug is unique in that its effect of ISA on the heart more or less equals the loss of basal sympathetic tone on this organ as caused by the concomitant blockade of cardiac β receptors. Therefore a baroreflex-mediated increment in vasoconstrictor nerve activity in response to cardiodepression after β blockade is prevented. Acutely and in the long run, the fall in arterial pressure during treatment with pindolol is associated with a normal cardiac output at rest and return of vascular resistance to normal. More recent developments in β-blocker research have lead to a number of alternative ways to manipulate the characteristically elevated vascular resistance in hypertension (Ta-

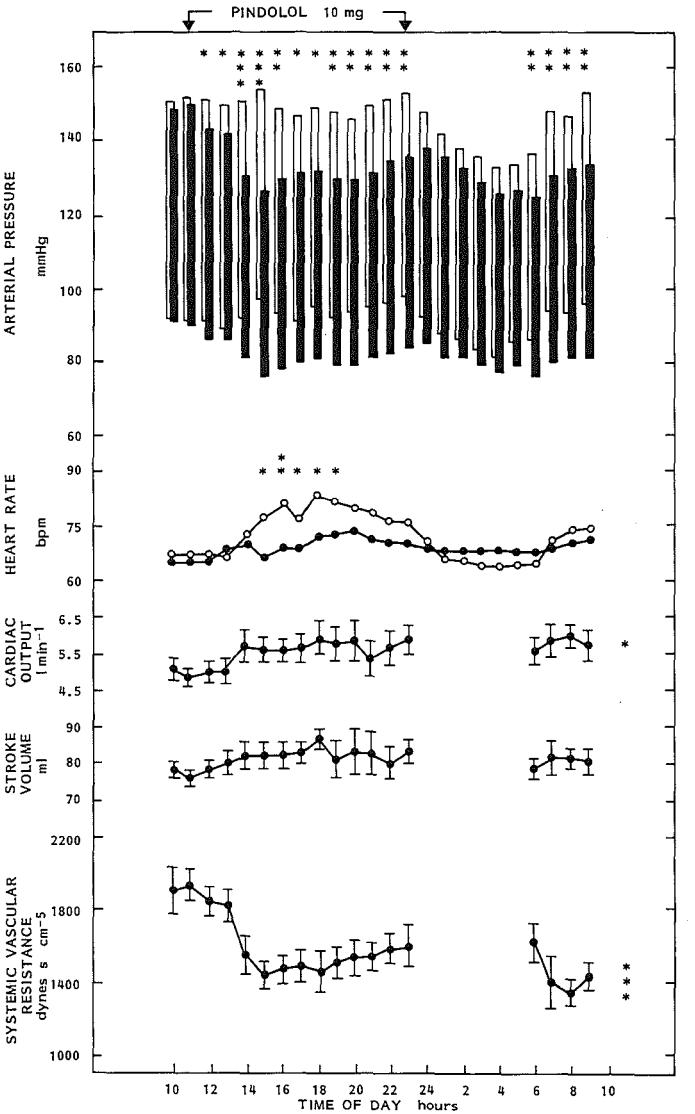

Fig. 6. Acute hemodynamic changes after pindolol, 10 mg b.i.d., open symbols, placebo; closed symbols, pindolol. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are presented as mean values ± SEM. For statistical analysis, Student's *t* test for paired observations was used. P values smaller than 0.05 were considered to indicate statistically significant differences from either placebo (arterial pressure and heart rate) or baseline (all other parameters) values.

ble 2). Examples of these efforts are the combination of ISA, α_1- or α_2-receptor blockade and/or direct vasodilatating properties in the enantiomers of a single β-blocker molecule. The practical significance of these potentially interesting developments in β-blocker research remains to be established.

Table 2. *The vasodilating β-blockers*

	Receptor blockade				ISA	Direct
	α_1	α_2	β_1	β_2	β	vasodilatation
Pindolol			+	+	+++	
Labetalol	±		+	+	±	
Celiprolol		±	+		+	
Prizidilol			+	+		+
Carvedilol			+	+		+
Medroxalol	±		+	+	+	+
Bucindolol	±	±	+	+	+	+

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3. Systemic and renal vasodilation after beta-adrenoceptor blockade with pindolol: a hemodynamic study on the onset and maintenance of its antihypertensive effect

3.1 Summary

Pindolol, a beta blocker with considerable partial agonist activity (PAA), was studied in 10 hypertensive subjects. The maximal fall in mean arterial pressure (MAP) was seen 3 to 4 hours after oral dosing with 10 mg of pindolol (—15 ± 3%, mean ± SEM). This was caused by a reduction in total peripheral resistance (TPR), which amounted to 25 ± 4% after 24 hours. Cardiac output increased by 16 ± 5%. Cardiac filling pressures and pulmonary artery pressure did not change. Increasing the dose of pindolol, from 5 mg twice a day to 15 mg twice a day over a 3-week period, caused no further change in MAP. After 3 weeks, the fall in MAP (—11 ± 2%) was maintained by reduced TPR (—26 ± 6%), whereas cardiac output and stroke volume were increased by 16 ± 6% and 26 ± 6%. Renal blood flow and glomerular filtration rate did not change. Beta blockers devoid of PAA lower cardiac output, whereas the elevated TPR in hypertension is unchanged. The hemodynamic profile of pindolol essentially differs from that of beta blockers devoid of PAA.

3.2 Introduction

Increased vascular resistance in the hallmark of practically all forms of clinical hypertension. Therefore, it seems logical to treat this disease with drugs that act primarily on the resistance vessels. A major challenge to this view has been the introduction of the beta-adrenoceptor antagonists, of which propranolol was the first

representative drug to be used on a large scale. Depression of cardiac output and an initial increase in vascular resistance are prominent features of these drugs. However, after these initial changes, arterial pressure is ultimately lowered by the return of vascular resistance toward its pretreatment level (1). Thus, in the long run, the antihypertensive effect of propranolol is associated with reduced cardiac output and unchanged or even increased vascular resistance.

It has been suggested that the initial rise in vascular resistance after propranolol is caused by unopposed alpha-adrenoceptor-mediated vasoconstriction after blockade of vasodilator beta-2 adrenoceptors. A strong argument against this assumption is provided by the observation that the hemodynamic profiles of beta-1-selective and non-selective beta-adrenoceptor antagonists are not different (2). Apparently, vascular beta-2 adrenoceptors do not contribute substantially to the control of vascular resistance, at least under resting conditions.

It has been reported that during long-term treatment with the nonselective beta-adrenoceptor antagonist pindolol, cardiac output is unchanged (3). Consequently, the long-term antihypertensive effect of pindolol should be characterized by reduced vascular resistance. Such a hemodynamic profile of a beta blocker could be related to a relatively high degree of partial agonist activity (PAA). Pindolol's PAA on cardiac beta adrenoceptors is sufficient to maintain a normal cardiac output, at least acutely and under resting conditions (4). However, detailed studies on the time course of the short-term vs long-term hemodynamic effects of pindolol are lacking. In the present study we compared the hemodynamic responses to the first dose of pindolol for 24 hours with its effects after 3 weeks of chronic treatment.

3.3 Methods

Ten male patients with mild-to-moderate essential hypertension participated in the study. They were selected for the study, in the outpatient clinic, if they had sitting blood pressures over 160/95 mm Hg, when untreated, on at least three different occasions.

The mean age of the patients was 45 years (range 27 tot 53 years). Routine clinical and laboratory investigations did not reveal any cause for their hypertension. A history or clinical signs of coronary or valvular heart disease, congestive heart failure, cerebrovascular disease, renal disease, or chronic obstructive lung disease were all negative. After the aim of the study and the procedures to be used had been explained, all patients gave their consent to participate in this study. The study protocol was approved by the local hospital ethical review committee.

Study design.
The study was designed as a single-blind, placebo-controlled trial which lasted for 6 weeks. Antihypertensive and other medication, if any, was discontinued at least 3 weeks prior to the study. Placebo was then given for 3 weeks. At the end of the first and second weeks the subjects were taken into the clinical pharmacology unit for 2 days. During their hospital stay patients had complete bed rest. During the first admission, intraarterial pressure was continuously monitored for 24 hour during placebo. Cardiac output, renal plasma flow, and glomerular filtration rate were measured by noninvasive methods. After these measurements had been completed, an intravenous isoproterenol infusion was given. During the second stay in the hospital, which also lasted for 2 days, pindolol, 10 mg twice a day, was given for the first time, and the acute effects on blood pressure and cardiac output were studied by invasive methods for 24 hours after 2 hours of baseline readings. Thus, the 24-hour blood pressure profile during placebo could be compared with the profile during the 24 hours when beta blocker therapy was started. At the end of the 24-hour observation period, blockade of cardiac and vascular beta adrenoceptors was assessed by isoproterenol infusion.
After the third week, at which time the patients were back on placebo, a dose titration with pindolol was carried out during weeks 4, 5, and 6. The patients were seen at weekly intervals in the outpatient clinic. Pindolol was given, 5 mg twice a day and 10 mg twice a day during weeks 4 and 5, respectively. In five patients, 20 mg twice a day was given during week 6 because

blood pressure was higher than 140/90 mm Hg. The average dose of pindolol at the end of the sixth week was 15 ± 2 mg (mean ± SEM) twice a day. Noninvasive measurements of cardiac output, renal plasma flow, and glomerular filtration rate were repeated at the end of the sixth week. All hemodynamic measurements were performed during the morning hours after patients had been recumbent for at least 1 hour.

Invasive measurements.
Arterial pressure was measured twice over 24 hours with the Oxford System (5). The brachial artery of the nondominant arm was cannulated after local anesthesia with a 2% lidocaine solution. A Teflon catheter, 1.0 mm in diameter (Plastimed, Saint-Leu-La Foret, France), was introduced by the Seldinger technique. The catheter was connected to a miniature transducer-perfusion device (Northwick Park Hospital, London, England), and the pressure signal was continuously recorded on magnetic tape (Medilog Recorder II, Oxford Medical Instruments, Oxford, England). The analogue signal was digitized during replay of the tape at 60 times real-time with a sampling frequency of $33^1/_3$ samples/second real-time. The quantitation level of this procedure is 0.3 to 0.5 mm Hg. Traces were analyzed beat by beat by means of Hewlett-Packard 2113 E computer system. They were scrutinized for beat loss, damping, and movement artifacts. These events accumulated to 1% to 2% of all data and were excluded from analysis. The mean values for systolic, diastolic, and integrated mean arterial pressure and for heart rate were computed over hourly periods. The values of these parameters in the 24-hour period in which active drug treatment was started were compared with the corresponding values obtained during 24 hours on placebo. Cardiac output was measured by means of a 7F triple-lumen, flow-directed, Swan-Ganz thermodilution catheter (Edwards Laboratories, Irvine, Calif.). The catheter was inserted percutaneously into an antecubital vein by means of the Seldinger technique. Cardiac output was measured in triplicate by bolus injections of 10 ml of ice-cold dextrose 5%, every hour, but not between 11 pm and 7 am, because in a pilot study measurements appeared

to awaken the patients. Systemic arterial pressure and pulmonary artery pressure were monitored by means of Gould Statham P23 ID transducers with zero reference at midaxillary level. The pressure signals were continuously recorded on a Hewlett-Packard 7754A recorder. Right atrial pressure and pulmonary capillary wedge pressure were measured immediately before and after the cardiac output determinations. Mean values for systemic and pulmonary artery pressures were obtained by electronic integration of the respective analogue signals. Heart rate was derived from the continuously monitored ECG. Cardiac output and pressures were always measured with patients in a strictly horizontal position for at least 15 minutes. After baseline readings for 2 hours, the first oral dose of pindolol, 10 mg, was given, and the second dose of 10 mg was given 12 hours later. Values relevant to body size were converted to 1.73 m^2 body surface area. The following hemodynamic variables were derived: SVR = (MAP — RAP) X 80/CO and PVR = (MPAP — PCWP) X 80/CO, where MAP = mean systemic arterial pressure (mm Hg), RAP = mean right atrial pressure (mm Hg), MPAP = mean pulmonary artery pressure (mm Hg), PCWP = mean pulmonary capillary wedge pressure (mm Hg), CO = cardiac output (L/min), SVR = systemic vascular resistance (dynes · sec · cm^{-5}), and PVR = pulmonary vascular resistance (dynes · sec · cm^{-5}).

During the isoproterenol infusions arterial pressure was also measured directly and heart rate was derived from the ECG. Isoproterenol was infused through an indwelling cannula (Venflon, 18G, Viggo AB, Helsingborg, Sweden) in a forearm vein. Baseline values were obtained after continuous infusion of saline solution at a flow rate of 22 ml/hr for 20 minutes. The infusion was then switched to isoproterenol, 3.5, 7, 14, and 35 ng kg^{-1} min^{-1} during placebo and 70, 140, 350, and 700 ng kg^{-1} min^{-1} after pindolol. The dosage of isoproterenol was increased every 10 minutes until a rise in heart rate of at least 25 bpm was obtained. The average values for heart rate and diastolic pressure during the last 2 minutes of each dose step were used to construct log dose-response curves. For each patient the dosage of isoproterenol required for increasing heart rate by 25 bpm (CD_{25}) was calculated.

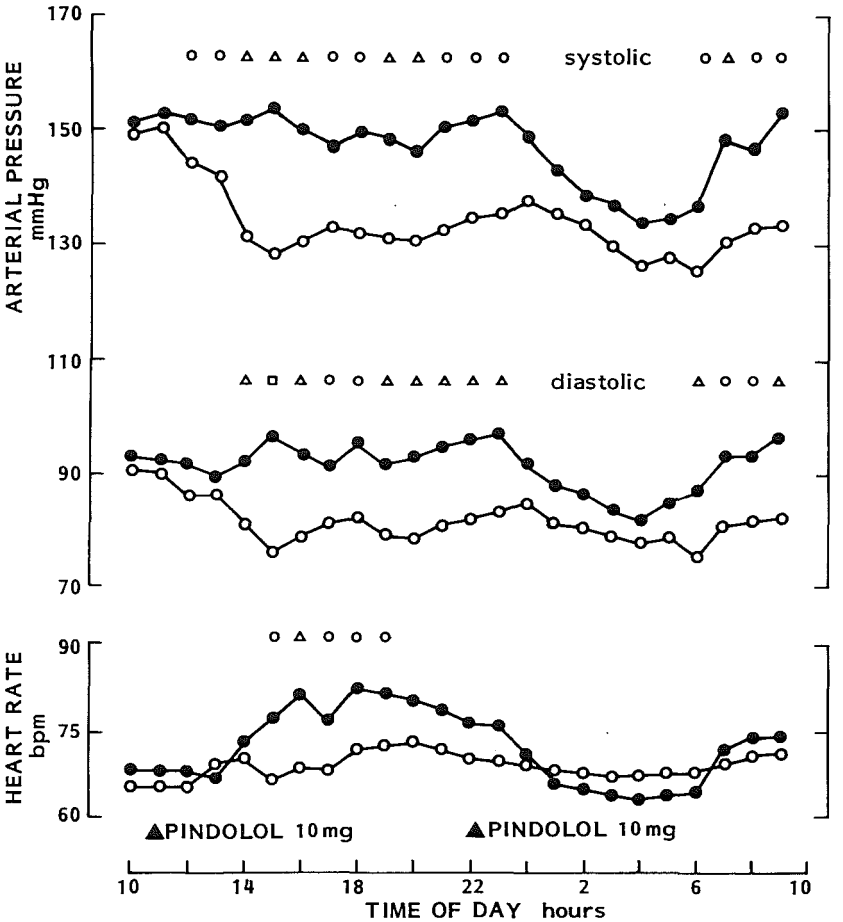

Fig. 1. Comparison of diurnal pattern of hourly mean values of systolic and diastolic arterial pressure and heart rate during placebo (●—●) and after pindolol was begun (○—○). Pindolol, 10 mg orally, was given for the first time at 11 am, 2 hours after measurements were started. ○ = $p < 0.05$; △ = $p < 0.01$; □ = $p < 0.001$ for differences between pindolol and placebo.

Noninvasive measurements.

Supine blood pressure and heart rate were measured weekly. Measurements were made every 5 minutes for 1 hour by a semiautomatic oscillometric device (Datascope, Accutorr I, Datascope Corp.,

Paramus, N.J.) between 8 and 10 am. The values for systolic and diastolic blood pressure, MAP, and heart rate were averaged. At the end of the blood pressure measurements supine cardiac output was measured by an isotope dilution technique (6,7) at the end of the first and sixth weeks. ^{99m}Technetium-labeled human serum albumin (Technescan HSA, Mallinckrodt Diagnostica BV, Petten, The Netherlands), 100 to 200 μCi, was used as the indicator. After a rapid intravenous injection of the isotope, time-concentration curves were recorded for 2 minutes by precordial counting of radioactivity with the use of a single probe. Additional recordings were made after 5 and 10 minutes when blood was sampled for measurement of radioactivity. Cardiac output was calculated by means of the Stewart-Hamilton formula. The method yields a good correlation with the dye-dilution technique ($n = 57$, $r = 0.92$), and the coefficient of variation of duplicate measurements with the isotope dilution technique is 6% ($n = 38$) (6). The heart rate was measured from a simultaneously recorded ECG. Cardiac output and stroke volume were expressed per 1.73 m^2 body surface area. Systemic vascular resistance was calculated as follows: SVR = MAP X 80/CO.

A constant-infusion technique was used for renal function studies (8,9). Effective renal plasma flow and glomerular filtration rate were estimated by means of the clearance of ^{131}I-hippuran and ^{125}I-thalamate (Amersham, UK). The priming dose for hippuran was 0.3 to 0.4 μCi/kg body weight and for thalamate, 0.08 to 0.1 μCi/kg body weight. The sustaining infusion rates were 0.2 and 0.05 μCi/min, respectively. The clearance of the isotopes was determined at steady state after 90 and 105 minutes. Renal blood flow was calculated by means of central venous packed cell volume and assuming 75% renal extraction of hippuran (9). Glomerular filtration rate and renal blood flow were corrected for 1.73 m^2 body surface area. Renal vascular resistance (RVR) was derived as: RVR = MAP X 80/RBF, where RBF = renal blood flow (L/min).

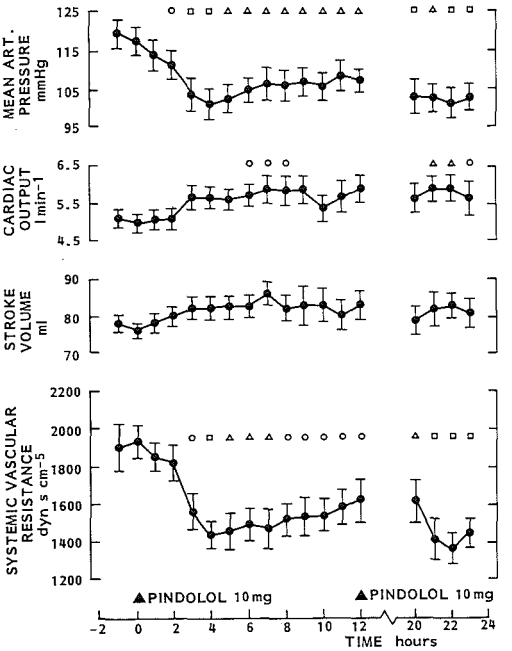

Fig. 2. Time course of acute effects of pindolol on systemic hemodynamics. $\circ = p < 0.05$; $\Delta = p < 0.01$; $\square = p < 0.001$ vs baseline values.

Statistics.
Data are presented as mean values ± SEM. Student's two-tailed t test for paired observations was used for comparison. p values < 0.05 were considered to indicate a statistically significant difference.

3.4 Results

Acute hemodynamic effects.
Systemic arterial pressure and heart rate during placebo between 9 and 11 am did not differ from the corresponding values shortly before pindolol was given (Fig. 1). One hour after pindolol, systolic pressure was already reduced ($p < 0.05$). The maximum antihypertensive effect was observed 4 hours after dosing. Arterial pressure at that time was decreased from 153 ± 6 to 128 ±

6 mm Hg systolic ($p < 0.01$) and from 97 ± 4 to 76 ± 4 mm Hg diastolic ($p < 0.001$). Heart rate had not changed at that time as compared to the baseline values between 9 and 11 am. However, as compared to the corresponding placebo values obtained 1 week previously, heart rate was reduced from 77 ± 3 to 66 ± 2 bpm ($p < 0.05$). During the night arterial pressure and heart rate on pindolol were not different from placebo values. After 24 hours arterial pressure was reduced from 153 ± 5 to 134 ± 4 mm Hg systolic ($p < 0.05$) and from 96 ± 3 to 82 ± 2 mm Hg diastolic ($p < 0.01$). Heart rate was not changed at that time. The reduction of arterial pressure was caused by a decrease in systemic vascular resistance (Fig. 2). After 4 hours, when the antihypertensive effect of pindolol was maximal, mean arterial pressure was decreased by 15 ± 3% ($p < 0.001$). Systemic vascular resistance had dropped by 25 ± 6% ($p < 0.001$) at that time, without significant changes in cardiac output and stroke volume. After 24 hours, mean arterial pressure and systemic vascular resistance were reduced by 14 ± 2% ($p < 0.01$) and 25 ± 4% ($p < 0.001$), respectively, while cardiac output was increased by 16 ± 5% ($p < 0.05$). Cardiac filling pressures, pulmonary artery pressure, and pulmonary vascular resistance did not change throughout the 24-hour observation period (Fig. 3).

Long-term hemodynamic effects.

Systolic and diastolic arterial pressure were reduced after 1 week of treatment with pindolol, 5 mg twice a day, as compared to placebo values at the end of weeks 1 and 3 (Fig. 4). Increasing the dose of pindolol in the following 2 weeks did not cause any further change in arterial pressure. After 3 weeks of treatment arterial pressure was reduced from 152 ± 3 to 137 ± 3 mm Hg systolic ($p < 0.01$) and from 98 ± 3 to 89 ± 2 mm Hg diastolic ($p < 0.01$). Heart rate was unchanged. The long-term antihypertensive effect of pindolol (decrease in mean arterial pressure 11 ± 2%, $p < 0.01$) was maintained by a reduced systemic vascular resistance (26 ± 6%, $p < 0.01$, comparison between weeks 1 and 6). Cardiac output and stroke volume after 3 weeks of treatment were increased by 16 ± 6% ($p < 0.01$) and 26

± 6% ($p < 0.001$), respectively.

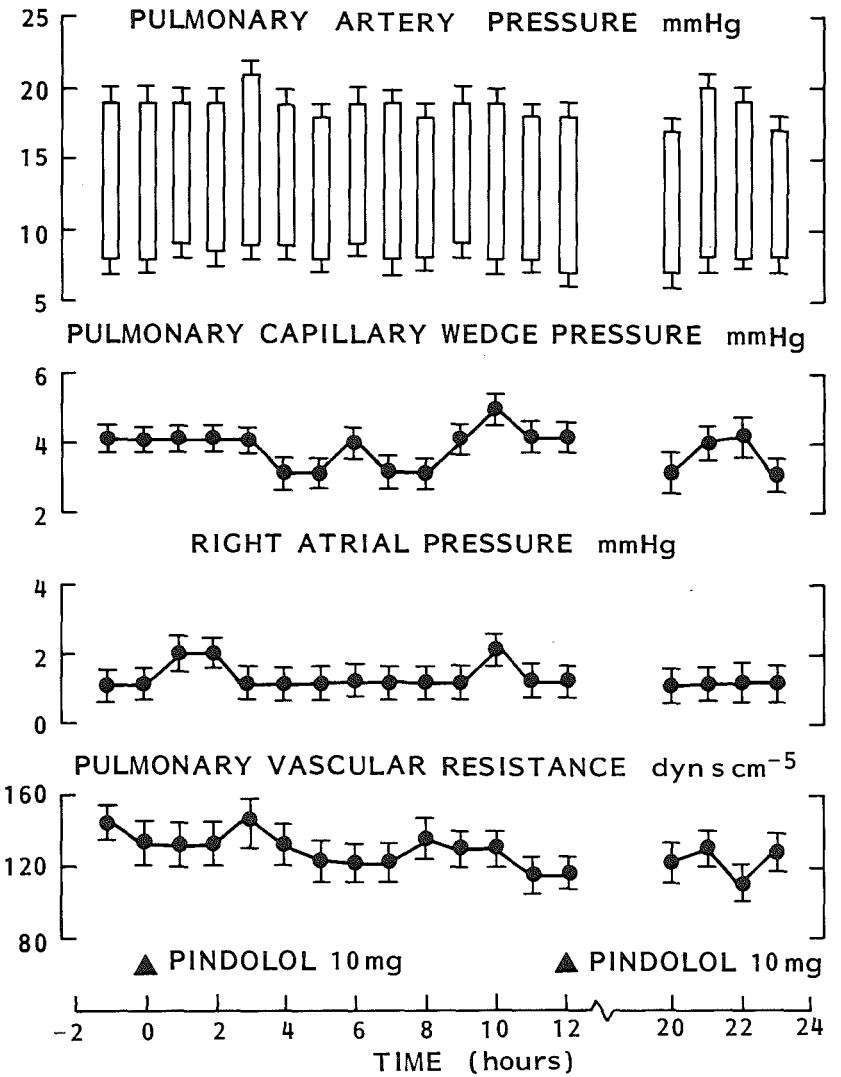

Fig. 3. Time course of acute effects of pindolol on central hemodynamics.

Effects on renal hemodynamics.
The vasodilator effect of pindolol was also observed in the renal

vascular bed. Renal vascular resistance after 3 weeks of treatment was decreased by 14 ± 4% ($p < 0.01$) (Table I). This reduction in renal vascular resistance was not associated with significant changes in renal blood flow or glomerular filtration rate.

Isoproterenol infusion.
The mean CD_{25} during placebo was 30 ng kg^{-1} min^{-1} (range 25 to 35). Twelve hours after the second dose of pindolol, 10 mg, it was increased to 520 ng kg^{-1} min^{-1} (range 190 to 700), indicating significant cardiac beta-adrenoceptor blockade (Fig. 5). Complete abolition of the fall in diastolic pressure during isoproterenol infusion after pindolol indicated that vascular beta-2 adrenoceptors were also blocked.

3.5 Discussion

Hemodynamics of pindolol.
The present study confirms and extends an earlier observation, that the nonselective beta-adrenoceptor antagonist pindolol lowers arterial pressure in the long run through vasodilation (3). Our study shows that the maximum of this vasodilator effect is already seen within 4 hours after oral administration of 10 mg. The vasodilator effect of pindolol appears to be fully responsible for its acute as well as its long-term antihypertensive effects. Small but signifcant increments in cardiac output and stroke volume were observed, both acutely and chronically. The vasodilator effect of pindolol is also observed in the renal vascular bed, so that renal perfusion is not compromised despite the fall in arterial pressure. Thus, the hemodynamic profile of pindolol is essentially different from the profile of beta-adrenoceptor antagonists lacking PAA. These drugs lower arterial pressure in the presence of reduced cardiac output and unchanged or even increased vascular resistance (see Man in 't Veld and Schalekamp (2) for review of 41 studies including 482 patients).

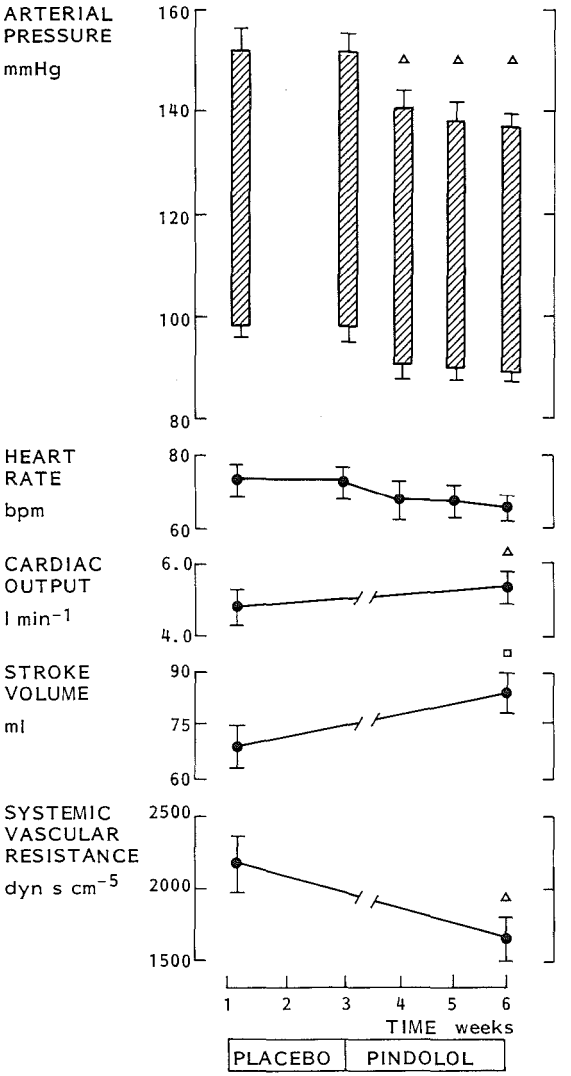

Fig. 4. Long-term effects of pindolol on systemic hemo-dynamics. Dose of pindolol during week 4:5 mg twice a day; during week 5:10 mg twice a day; during week 6:15 ± 2 mg twice a day. ○ = $p < 0.05$; △ = $p < 0.01$; □ = $p < 0.001$ vs placebo values.

Table 1. *Effects of long-term treatment with pindolol on renal hemodynamics*

	Placebo	Pindolol	p Value
Glomerular filtration rate (ml/min)	97 ± 4	99 ± 4	NS
Renal blood flow (ml/min)	996 ± 39	1031 ± 52	NS
Renal vascular resistance (dynes · sec · cm^{-5})	10,084 ± 706	8479 ± 534	< 0.01

Time course of hemodynamic changes.
Detailed hemodynamic studies at the time of onset of the antihypertensive effect of beta-adrenoceptor antagonists are scarce. Recently, two of these studies, one with the beta-1-selective antagonist atenolol (10) and one with the nonselective antagonist timolol (11), have been reported. After atenolol and timolol any reduction in arterial pressure was not observed within 3 to 4 hours after dosing. The present study shows that after pindolol arterial pressure was already reduced after 1 hour, whereas the maximum antihypertensive effect was observed within 4 hours. This time course confirms an early study with pindolol by Anavekar et al (12). The reason for the observed difference in the time of onset of the blood pressure reduction between beta-adrenoceptor antagonists with different ancillary properties could be that with drugs without PAA, the antihypertensive effect is initially offset by reflex vasoconstriction in response to the fall in cardiac output. Pindolol's PAA is apparently sufficient to compensate for the loss of basal sympathetic tone on the heart, so that cardio-depression and reflex vasoconstriction do not occur. It is therefore indeed appropriate to refer to pindolol's PAA on the heart as intrinsic sympathomimetic activity.

Hemodynamic consequences of PAA.
During the night, when arterial pressure is already low, this drug does not further reduce perfusion pressure, and this contrasts with beta-adrenoceptor antagonists lacking PAA (13). This is an effect of PAA on cardiac beta adrenoceptors by which a fall in cardiac

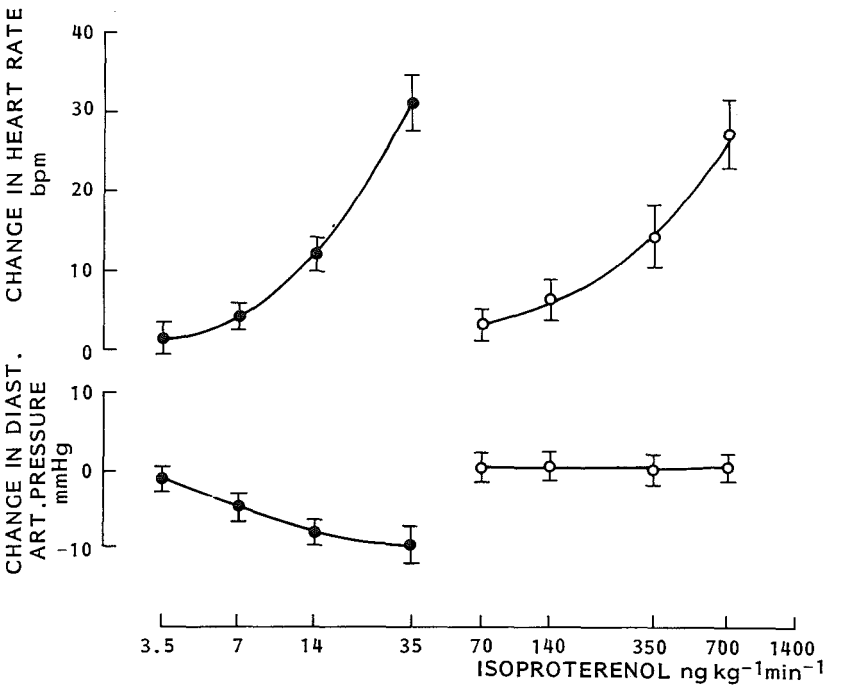

Fig. 5. Isoproterenol dose-response curves for changes in heart rate and diastolic arterial pressure during placebo (●—●) and after pindolol (○—○).

output during the night is prevented. A second example of the dual action of partial agonists, that is, receptor blockade and receptor stimulation at the same time, is demonstrated by the fact that pindolol increased heart rate during the night, whereas it decreased heart rate during the day (Fig. 1). Third, this study shows that

pindolol, unlike propranolol, does not increase cardiac filling pressures and pulmonary artery pressure (12). This suggests that, in contrast to beta-adrenoceptor antagonists devoid of PAA, pindolol does not exert a negative inotropic action.

Mechanism of action.

This antihypertensive mechanism of beta-adrenoceptor antagonists is often explained by the theory of whole-body autoregulation (1, 11, 15, 16). This theory suggests that systemic vascular resistance is readjusted to a lower level on these drugs in response to cardiodepression and reduced nutritional flow. Since cardiac output rose during pindolol treatment, such a theory can hardly be applied to its antihypertensive action. Suppression of renin release by the kidney could be an alternative explanation for the vasodilator and anti-hypertensive action of pindolol. Most studies, however, have shown that pindolol does not decrease or may even increase plasma renin activity (2). It has been suggested that direct stimulation of vascular beta-2 adrenoceptors by pindolol could explain its vasodilator properties (17,18). A strong argument against this view is the present observation that arterial pressure is not lowered by pindolol during the night, when sympathetic tone and circulating catecholamines are low. Furthermore, the beta-2 adrenoceptor agonist salbutamol does not further reduce arterial pressure in hypertensive patients treated with the beta-1 adrenoceptor antagonist practolol (19). More important, the beta-1 adrenoceptor antagonist practolol, with considerable PAA, also lowers arterial pressure by reducing vascular resistance (2), whereas it does not cause direct vasodilation (18). Finally, pindolol increases arterial pressure and it does not lower vascular resistance in patients with chronic autonomic failure (20).

Thus, neither blockade of cardiac and juxtaglomerular beta receptors nor stimulation of vascular beta-2 adrenoceptors can be held responsible for the vasodilator and antihypertensive effects of pindolol. A remaining possibility to explain pindolol's antihypertensive action is that its vasodilator effect depends on blockade of central and/or peripheral presynaptic beta adrenoceptors. The reduction of plasma norepinephrine during treatment with pindolol

is compatible with this hypothesis (21). Reduced release of norepinephrine from sympathetic nerve endings and, consequently, attenuated alpha-adrenoceptor-mediated vasoconstrictor tone could adequately explain the vasodilator effect of pindolol, in particular, if not the antihypertensive action of all beta-adrenoceptor antagonists, in general.

3.6 References

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4. Acute and long-term effects of acebutolol on systemic and renal hemodynamics, body fluid volumes, catecholamines, active renin, aldosterone, and lymphocyte β-adrenoceptor density

4.1 Summary

Acebutolol is a relatively new β-adrenoceptor blocking antagonist, possessing both β_1-adrenoceptor selectivity and partial agonist activity (PAA). Its acute (24 h, 400 mg, twice daily) and long-term effects (3 weeks) on systemic and renal hemodynamics, body fluid volumes, hormones, and β-adrenoceptor density on lymphocytes were studied in a single-blind placebo-controlled trial, in 10 hypertensive patients. The initial response to acebutolol (1-2 h) was a fall in heart rate (HR) (—9.6 $\pm$ 2.7%), cardiac output (—16.0 $\pm$ 3%), and stroke volume (SV) (—10.7 $\pm$ 0.2%), and an increase in systemic vascular resistance (SVR) (18.0 $\pm$ 3.9%). Mean arterial pressure (MAP) began to fall 2-3 h after dosing in parallel with a decrease in SVR. At the end of the acute study, MAP and SVR were decreased by 18.1 $\pm$ 2.7% and 15.6 $\pm$ 5.6%, respectively. By that time, HR and SV had returned to control values despite blockade of β-adrenoceptors. After 3 weeks of treatment (mean dose of acebutolol 480 mg twice daily), the fall in MAP was 10.1 $\pm$ 2.7% and HR was decreased by 13.0 $\pm$ 2.3%. Renal blood flow and glomerular filtration rate did not change. Acute and long-term treatment had no effect on the density of lymphocyte-membrane β-adrenoceptors. This could be explained by acebutolol's β_1 selectivity or, alternatively, this could be due to the drug's PAA.

4.2 Introduction

Acebutolol is one of the newer β-adrenoceptor antagonists. In humans, the drug is transformed for ~50% into its major metabolite diacetolol (1,2). Studies in vivo and in vitro have shown that both the parent compound and diacetolol are β_1-selective and have a modest degree of partial agonist activity (PAA) (3,4). Acebutolol, in contrast to diacetolol, also possesses membrane-stabilizing activity, but this property is not of clinical significance in the range of plasma concentrations attained during normal use (5).

It is generally agreed that β-adrenoceptor blocking agents are equally effective in lowering an elevated blood pressure, irrespective of their ancillary properties such as β_1 selectivity, PAA, or hydrophilicity (6). These ancillary properties, however, may be important with regard to safety, side effects, and the hemodynamic effects of these drugs. The PAA of β-adrenoceptor blocking agents may be relevant in three ways: (a) as a determinant of their hemodynamic profile (6-8); (b) for the occurrence of the β-blocker withdrawal syndrome, which has been related to "upregulation" of β-adrenoceptors (9,10); and (c) for changes in plasma lipids during long-term therapy (11,12).

The efficacy of acebutolol in lowering blood pressure has been well established in several studies (13-15). Detailed studies on the time course of its acute and long-term hemodynamic effects are lacking. β-adrenoceptors on lymphocytes are a good and easily accessible model for the study of drug- or disease-induced changes in human cardiovascular β-adrenoceptors (10,16-18). Studies on the effect of acebutolol on lymphocyte β-adrenoceptors have also not been reported in the literature. Therefore, we compared in the present study the acute changes in hemodynamics, hormones, catecholamines, and changes in lymphocyte β-adrenoceptors after the first oral dose of acebutolol with its effects after 3 weeks of treatment.

4.3 Materials and methods

Ten male subjects, aged 48 ± 3 years (mean ± SEM), with mild to moderate hypertension were recruited from the outpatient hypertension clinic. Eight of the 10 patients were previously treated for hypertension, 6 with a β-adrenoceptor antagonist (atenolol, pindolol or propranolol) and 2 with captopril. Hypertension was well controlled in all but one of these treated patients. When untreated, all patients had sitting diastolic blood pressures of >95 mm Hg on three separate visits to the outpatient clinic. Secondary forms of hypertension were excluded by clinical and laboratory evaluation, including isotope renography. None of the subjects had a history or clinical signs of ischemic heart disease, cerebrovascular disease, or chronic obstructive lung disease. Blood urea nitrogen (BUN), serum creatinine, and electrolytes were all within the normal range of our laboratory. The purpose and procedures of the study were explained to the patients, who all gave their consent to participate. The study protocol was approved by the Hospital Ethical Review Committee.

Study protocol

The study was designed as a single-blind placebo-controlled trial and was divided into an acute and long-term study. Antihypertensive medication, if any, was discontinued at least 3 weeks prior to the study. After this washout period, placebo capsules, twice daily, were provided for 3 weeks. At the end of the first and second week of this placebo period, patients were hospitalized for 2 days. During their stay in the hospital, the patients were restricted to bed. Measurements were started after an overnight rest and a light breakfast. Light meals were provided 5, 9, and 24 h after measurements were started.

When patients were first admitted to the hospital, arterial pressure was continuously monitored for 24 h. Cardiac output, renal plasma flow, glomerular filtration rate, and body fluid volumes were measured, and blood was sampled for determination of plasma concentrations of catecholamines, active renin, aldosterone, and the density of β-adrenoceptors on lymphocyte membranes (B_{max}).

Responsiveness of cardiac and vascular β-adrenoceptors was assessed by isoproterenol infusion after completion of measurements.

During the patients' second admission to the hospital, acebutolol 400 mg twice daily, was given for the first time and its hemodynamic effects were followed for 24 h after 2 h of baseline readings. Arterial pressure was again monitored continuously. Cardiac output and central pressures were determined at hourly intervals, with the patient in a strictly horizontal position for at least 15 min. Measurements of cardiac output and central pressures were interrupted from 11 p.m. to 7 a.m. Catecholamines, active renin, and aldosterone were determined just before and 2, 6, 12, and 24 h after the first dose of the drug. B_{max} was measured before dosing and 24 h later. Then blockade of cardiac and vascular β-adrenoceptors was assessed by isoproterenol infusion.

For the long-term study, patients were seen at weekly intervals in the outpatient clinic. At each visit, blood pressure and heart rate were measured between 8 and 10 a.m. Dose-titration with acebutolol was carried out during weeks 4-6. Acebutolol was given 200 mg and 400 mg twice a day during weeks 4 and 5, respectively. In two patients, 800 mg twice daily was given during week 6 because diastolic arterial pressure was >95 mm Hg. At the end of week 6, measurements of cardiac output, renal plasma flow, glomerular filtration rate, body fluid volumes, catecholamines, active renin, aldosterone, and B_{max} were repeated. B_{max} was also measured at the end of week 5. All these measurements were performed during the morning hours after patients had been recumbent for at least 1 h. After week 6, patients were withdrawn from active treatment and placebo was reinstituted. B_{max} was measured 1 and 2 weeks after withdrawal, and isoproterenol infusion was repeated 1 week after withdrawal from active treatment.

Invasive hemodynamic measurements

All catheterizations were performed after local anesthesia with lidocaine 2%. Arterial pressure was continuously measured in the brachial artery of the nondominant arm by means of the Oxford technique (19). The intraarterial cannula was connected to a

perfusion-transducer device (Northwick Park Hospital, London, England) suspended in front of the chest. The transducer signal was recorded on magnetic tape by means of an analogue tape-recorder (Medilog Recorder II, Oxford Medical Instruments, Oxford, England). The analogue signal was digitized during replay of the tape at 60 times real time with a sampling frequency of $33^1/_3$ samples/s. Traces were analyzed beat by beat and were scrutinized for beat loss, clipping of the amplifier, damping, and movement artefacts by means of a computer system. These events amounted to <2% of all data and were excluded from analysis. Hourly means of systolic and diastolic arterial pressure and heart rate (HR) were calculated.

Cardiac output and central pressures were measured by means of a 7-F Swan-Ganz, flow-directed, thermodilution catheter (Edwards Laboratories). The catheter was introduced percutaneously into an antecubital vein. Cardiac output was determined in triplicate by bolus injections of ice-cold dextrose 5%. Systemic arterial pressure and pulmonary artery pressure were monitored by means of Gould Statham P231D transducers, with zero reference at midaxillary level. Right atrial pressure and pulmonary capillary pressure were measured shortly before cardiac output determinations. HR was derived from a continuously recorded electrocardiogram (ECG). Mean values of pressures were obtained by electronic integration of the analogue signals.

The following hemodynamic variables were derived: SVR = 80(MAP—RAP)/CO, PVR = 80(MPAP—PCWP)/CO, and SV = CO/HR, where MAP = mean arterial pressure (mm Hg), RAP = mean right atrial pressure (mm Hg), CO = cardiac output (l/min), MPAP = mean pulmonary artery pressure (mm Hg), PCWP = mean pulmonary capillary wedge pressure (mm Hg), SVR = systemic vascular resistance (dyn · s · cm^{-5}), PVR = pulmonary vascular resistance (dyn · s · cm^{-5}), SV = stroke volume (ml), and HR = heart rate (beats/min).

Noninvasive hemodynamic measurements

In the outpatient clinic, supine blood pressure and HR were measured every 5 min for 1 h by an oscillometric device (Datascope,

Accutorr 1, Datascope Corp, Paramus, NJ, U.S.A.) Values of blood pressure and HR obtained during 1 h were averaged.
Supine cardiac output was measured by an isotope dilution technique (20,21) at the end of the blood pressure measurements. ^{99m}Technetium-labeled human serum albumin (^{99m}TcHSA) (Technescan HSA, Mallinckrodt Diagnostica BV, Petten, The Netherlands), 100-200 μCi, was used as the indicator. After a rapid intravenous (i.v.) injection of the isotope, time — concentration curves were recorded for 2 min by precordial counting of radioactivity with the use of a single probe. Additional recordings were made after 5 and 10 min when blood was sampled for measurement of radioactivity. CO was calculated by means of the Stewart-Hamilton formula. The method yields a good correlation with the dye-dilution technique ($n = 57$, $r = 0.92$), and the coefficient of variation of duplicate measurements with the isotope dilution technique is 6% ($n = 38$) (21). SVR was calculated as SVR = 80 X MAP/CO and MAP as MAP = DAP + (SAP — DAP)/3, where SAP = systolic arterial pressure and DAP = diastolic arterial pressure.
For renal function studies, a constant infusion technique was used (22). Effective renal plasma flow and GFR were estimated by means of the clearance of [^{131}I-] hippuran and [^{125}I-] thalamate (Amersham, England). The priming doses for hippuran and thalamate were 0.3-0.4 and 0.08-0.1 μCi/kg body weight, and the sustaining infusion rates were 0.2 and 0.05 μCi/min, respectively. The clearance of isotopes was determined at steady state after 90 and 105 min. Renal blood flow (RBF) was calculated by means of central venous packed cell volume and assuming 75% renal extraction of hippuran (23). Renal vascular resistance (RVR) was calculated as RVR = 80 MAP/RBF.

Isoproterenol infusions
During isoproterenol infusions, arterial pressure was measured directly and heart rate was derived from a continuously recorded ECG. Isoproterenol was infused through a cannula in a forearm vein. Baseline values were obtained during continuous infusion of 0.9% saline solution (2 ml/min) for 20 min. The infusion was

then switched to isoproterenol, 3.5, 7, 14, and 35 ng kg^{-1} min^{-1} during placebo and 1 week after withdrawal of acebutolol, and 17.5, 35, 70, and 175 ng kg^{-1} min^{-1} at the end of the acute study. The dosage of isoproterenol was increased every 10 min until a rise in heart rate of at least 25 beats/min was obtained. The average values of HR and DAP during the last 2 min of each dose-step were used to construct log dose-response curves by linear regression analysis. The dosage of isoproterenol to increase HR by 25 beats/min (CD25) was then calculated.

Body fluid volumes

The same indicator as used for the measurement of CO (i.e., ^{99m}TcHSA) was also used for determination of plasma volume. Plasma samples were taken 10, 20, and 30 min after the i.v. injection. Extracellular volume was estimated by measuring the distribution volume of i.v. injected sodium [^{35}S] sulphate (50-60 μCi) with blood sampling at 0, 30, 60, 80, 100, and 120 min.

Analytical procedures

For determination of active plasma renin concentration (APRC) and plasma aldosterone (PA) venous blood was collected in tubes containing EDTA in a final concentration of 2 mg/ml of blood. Samples were centrifugated immediately at 0°C and stored at —20°C until assay. APRC and PA were measured by a radioimmunoassay as described previously (24,25). For measurement of plasma catecholamines 10 ml (central) venous blood was collected in chilled tubes containing 19 mg EGTA and 12 mg gluthathione. After centrifugation at 0°C, samples were stored at —70°C until assay. Plasma norepinephrine (PNE) and epinephrine (PE) were measured by a radioenzymatic method, according to Peuler and Johnson (26). For determination of β-adrenergic receptors on lymphocyte membranes, 50 ml fresh heparinized venous blood was withdrawn. Blood was always taken 12 h after the last dose of acebutolol and before isoproterenol infusion was given. Lymphocytes were collected by a modification of the technique of Böyum (27). The lymphocytes were lysed

mechanically and by exposure to hypotonic media, and the membranes were collected by centrifugation. Binding studies were carried out with the ligand [^{125}I] iodopindolol ([^{125}I]IPIN) as has been described in detail before (28). The data were analyzed by the method of Scatchard (29) to provide a value for the maximal density of specific binding sites of [^{125}I]IPIN and the apparent K_d value of the ligand.

Statistical analysis
Data are presented as mean values ± SEM. Because plasma values of active renin were not distributed normally, mean values were calculated after logarithmic transformation. Hemodynamic parameters relevant to body size were corrected for 1.73 m^2 body surface area.
The mean body surface area was 2.10 ± 0.05 m^2. For comparison, Student's *t* test for paired observations was used; p values <0.05 were considered to indicate a significant difference.

4.4 Results

Acute hemodynamic effects
Twenty-four hour trend plots based on the hourly means of SAP, DAP, and HR are shown in Fig. 1. SAP, DAP, and HR before the first oral dose of acebutolol was given did not differ from the corresponding placebo values 1 week previously. One hour after the first dose of acebutolol HR was reduced from 72 ± 3 beats/min to 63 ± 3 beats/min ($p < 0.01$). The reduction in HR was significant during the day, but not during the night from 10 p.m. to 6 a.m. As compared with placebo, SAP and DAP were reduced within 4 h after dosing. The reduction of DAP was maintained during the whole 24-h period. The reduction of SAP was not significant during the night from 1 to 6 a.m. At the end of the 24-h period, HR was reduced from 81 ± 3 beats/min on placebo to 65 ± 2 beats/min on active treatment ($p < 0.001$). SAP fell from 142 ± 5 to 125 ± 3 mm Hg ($p < 0.001$), and DAP fell from 92 ± 4 to 76 ± 2 mm Hg ($p < 0.01$). Baseline values of MAP, CO, SV, and SVR prior to

the first dose of acebutolol were 117 ± 3 mm Hg, 5.0 ± 0.2 L/min, 76 ± 2 ml and 1935 ± 85 dynes · s · cm^{-5}, respectively. A maximal effect on CO and SV was observed within 2 h after dosing (Fig. 2). By that time, the CO was reduced by 16.0 ± 3.0% ($p < 0.01$) and SV by 10.7 ± 0.2% ($p < 0.01$). The decrease in CO was associated with an increase in TPR (18.0 ± 3.9%, $p < 0.01$), so that MAP did not change initially. Three hours after dosing, MAP was reduced. This decrease in MAP was associated with a parallel decrease of SVR toward or below baseline. At the end of the acute study, MAP and SVR were reduced by 18.1 ± 2.7% ($p < 0.001$) and 15.6 ± 5.6% ($p < 0.01$), respectively. At that time, CO and SV had returned to baseline. Cardiac filling pressures, PAP, and PVR increased after administration of acebutolol (Fig. 3).

Chronic hemodynamic effects

Values of SAP, DAP, and HR at the end of the first and third placebo week did not differ (Fig. 4). SAP, DAP, and HR were reduced after 1-week treatment with acebutolol, 200 mg twice daily. Doubling the dose of acebutolol caused a further significant reduction of SAP but not of DAP. After 3 weeks of treatment, when the dose of acebutolol was 480 ± 60 mg twice daily, arterial pressure was decreased from 145 ± 4 to 132 ± 3 mm Hg systolic (—10 ± 4%, $p < 0.01$), and from 95 ± 3 to 85 ± 2 mm Hg diastolic (—12 ± 3%, $p < 0.01$). HR was reduced from 75 ± 3 to 64 ± 3 bpm ($p < 0.001$).

MAP was decreased from 115 ± 3 to 104 ± 3 mm Hg ($p < 0.01$) after 3 weeks of treatment. This long-term antihypertensive effect of acebutolol was not associated with significant changes in CO, SV, or SVR (Table 1). However, in patients in whom MAP fell by >10% a fall in MAP of 17.8 ± 2.7% was associated with a reduction in SVR of 20.3 ± 3.9%, and CO increased by 4.2 ± 3.5%. This hemodynamic profile contrasted with that in nonresponders, in whom MAP fell by 1.9 ± 1% ($n = 5$), SVR increased by 15.5 ± 10.1%, and CO decreased by 10.6 ± 7%. One and 2 weeks after with-drawal from active treatment, when the subjects were put on placebo again, values of SAP,

DAP, and HR did not differ from the values of these parameters during the initial placebo period.

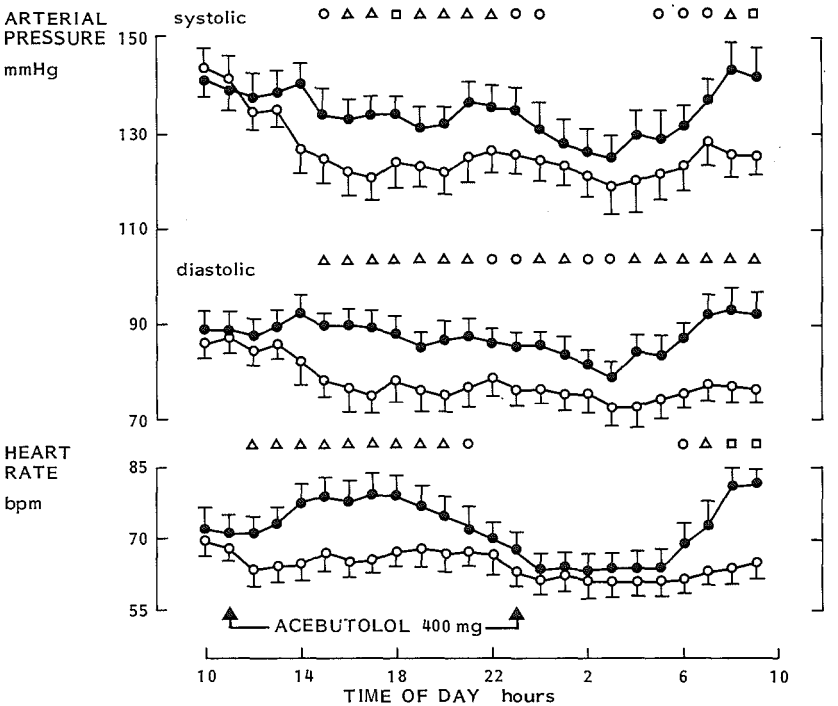

Fig. 1. Twenty-four hour trend plots of hourly mean values of systolic and diastolic arterial pressure and heart rate during placebo (●—●) and after administration of the first doses of acebutolol (○—○). $p < 0.05$ (○); $p < 0.01$ (Δ); $p < 0.001$ (□) for differences between acebutolol and placebo.

GFR and RBF did not change on acebutolol. Renal vascular resistances tended to decrease. This reduction, however, was not significant (Table 1). Plasma volume, extracellular fluid volume, and body weight did not change during long-term treatment with acebutolol (Table 1).

Isoproterenol infusions

The CD25 during placebo was 20 ± 2 ng kg^{-1} min^{-1}. DAP at this infusion rate was decreased by 7 ± 2 mm Hg ($p < 0.001$).

Twenty-four hours after the first dose of acebutolol, the HR log-dose-response curve was shifted to the right in a parallel way (Fig. 5). The CD25 was increased to 119 ± 11 ng kg^{-1} min^{-1} ($p < 0.001$). At this infusion rate, DAP fell by 4 ± 1 mm Hg ($p < 0.01$), which was not different from the fall in DAP at the CD25 of isoproterenol during placebo. One week after withdrawal from long-term treatment, the isoproterenol dose-response curves for changes in HR and DAP did not differ from the dose-response curves during the initial placebo period (Fig. 5).

Effects on catecholamines, APRC, and PA

Values of PNE, PE, APRC, and PA at the end of the first and second week on placebo did not differ (Table 2). Two hours after the first dose of acebutolol, PNE was increased by 28 ± 11% ($p < 0.01$). At the end of the acute study and during long-term treatment, PNE was not different from placebo values. Acute and long-term administration of acebutolol had no effect on PE. APRC was suppressed maximally (—61 ± 12%; $p < 0.01$) 2 h after the first dose of acebutolol. The suppression of APRC was maintained throughout the acute study as well as during long-term treatment (Table 2). PA decreased after acute and long-term treatment with acebutolol by 44 ± 12% ($p < 0.01$) and 23 ± 9% ($p< 0.01$), respectively. Neither the fall in MAP at the end of the acute study nor the antihypertensive effect during long-term treatment was related to baseline values of PNE or APRC.

Effect on β-adrenoceptor density on lymphocyte-membranes

Values of B_{max} and of the K_d for specific binding of [^{125}I]IPIN to lymphocyte-membranes are summarized in Table 3. B_{max} values at the end of the first and second week on placebo were not different. Acute and long-term treatment with acebutolol had no effect on B_{max} or on K_d values.

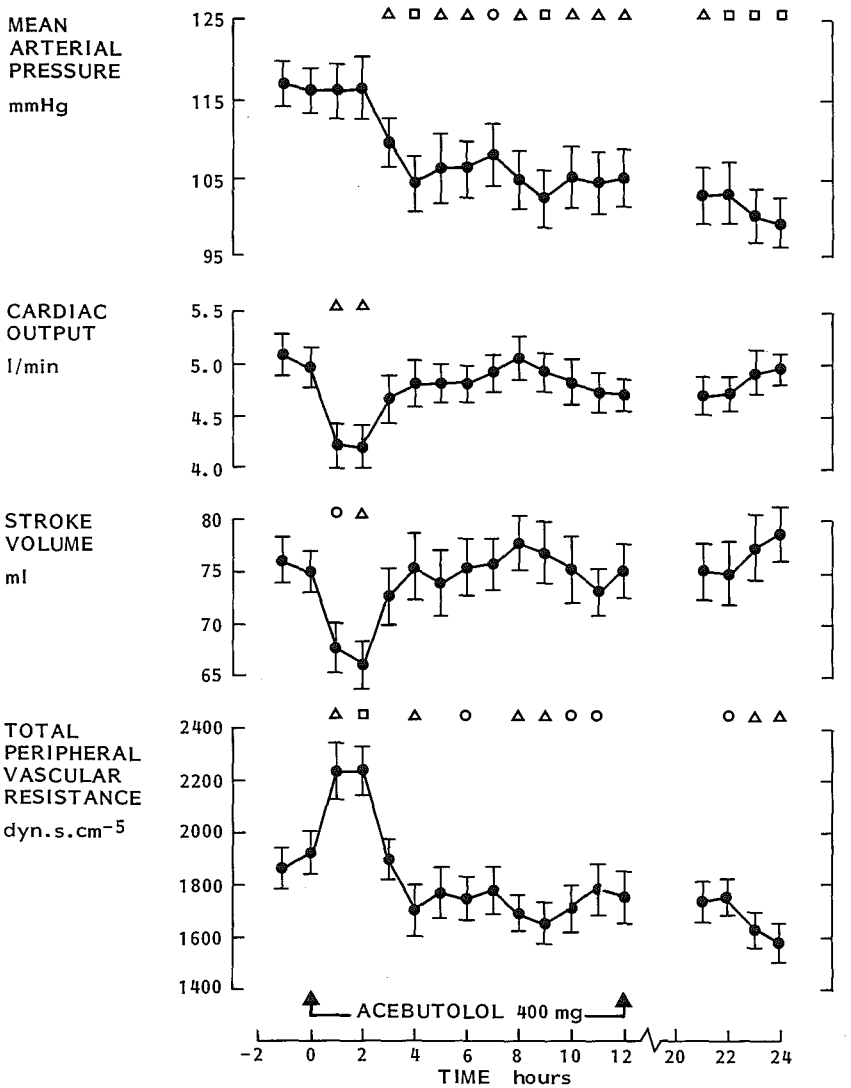

Fig. 2. Time course of the acute effects of acebutolol on systemic hemodynamics. $p < 0.05$ (○); $p < 0.01$ (△); $p < 0.001$ (□).

4.5 Discussion

Hemodynamic changes

The initial hemodynamic response to the first oral dose of acebutolol was a rapid fall in HR, SV, and CO, followed by an increase of SVR. A similar initial hemodynamic response has been reported previously in patients with hypertension and coronary heart disease after an i.v. dose of acebutolol (30-32). A gradual reduction in blood pressure occurred 2-3 h after dosing. This decrease in pressure was associated with a parallel decrease of the initial increased vascular resistance, which had increased further in the first 2 h. When the drug exerted its full antihypertensive action, vascular resistance was below control values and HR, SV, and CO had returned to pretreatment values. This restoration of an initially depressed cardiac function is probably independent of acebutolol's PAA, because it has been observed also during the initial hypotensive effects of timolol and atenolol, two β-adrenoceptor blocking agents devoid of PAA (33,34). Since β-adrenoceptors were blocked, this restoration of cardiac function can not be due to sympathetic stimulation. Therefore, withdrawal of vagal tone, reduction in afterload, and/or an increase in venous return are alternative explanations for this hemodynamic adaptation. This view is supported by the observation that increments in HR and CO after vasodilatation during β-adrenoceptor blockade are mediated by vagal inhibition (35). The present finding that changes in either HR or SV were inversely correlated with changes in blood pressure and vascular resistance (Fig. 6) lends further support to this view. The long-term hemodynamic effects of acebutolol have been reported by Dreslinski and colleagues (36) and by Tsukiyama and co-workers (37). In both studies, the hypotensive action of the drug in the long run was accompanied by a decrease in HR, whereas CO and vascular resistance remained unchanged. These findings are confirmed in the present study, but they are clearly different from the long-term effects of pindolol, a β-adrenoceptor antagonist with a relatively high degree of PAA. The antihypertensive effect of this drug is associated with a decrease in vascular resistance and an unchanged or even increased CO

(38, 39).

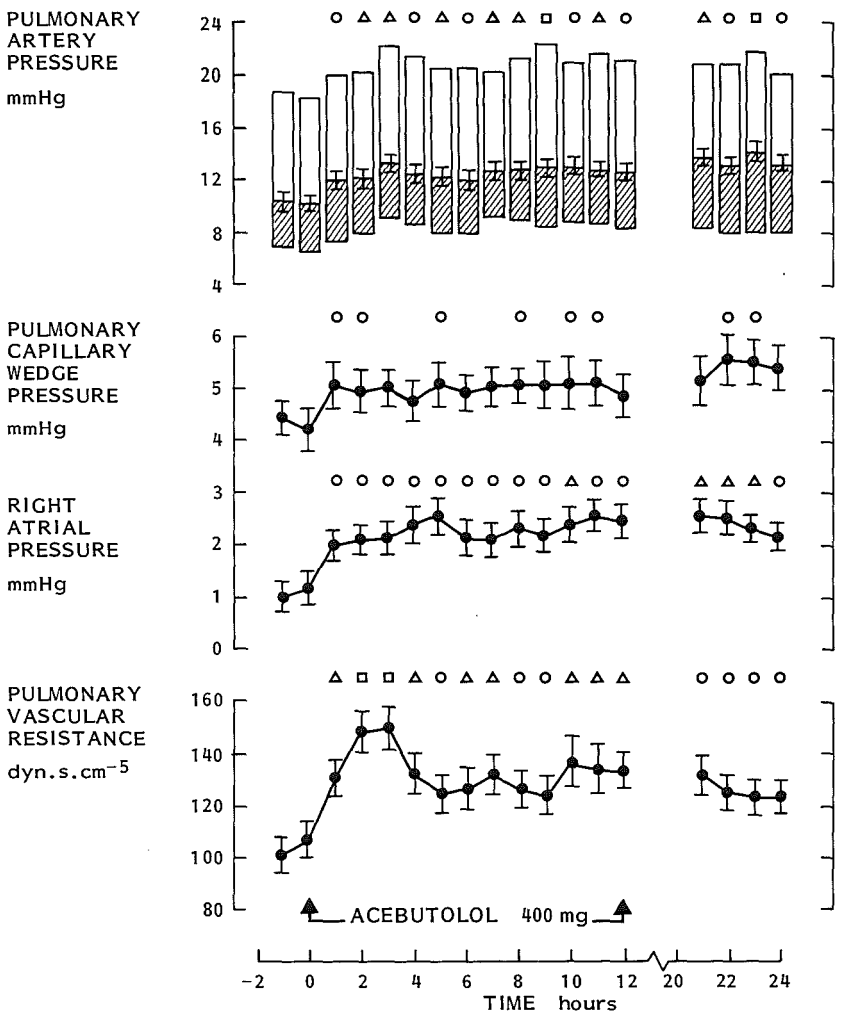

Fig. 3. Time course of acute effects of acebutolol on central hemodynamics. $p < 0.05$ (○); $p < 0.01$ (△); $p < 0.001$ (□).

Table 1. *Effects of long-term treatment with acebutolol on systemic and renal hemodynamics, body fluid volumes, and body weight*

Parameter	Placebo	Acebutolol	Percentage change	p
Mean arterial pressure (mm Hg)	115 ± 3	104 ± 3	—10.1 ± 2.7	<0.01
Heart rate (beats/min)	71 ± 3	61 ± 2	—13.0 ± 2.3	<0.01
Cardiac output (L/min)	5.7 ± 0.5	5.3 ± 0.3	—3.7 ± 7.4	NS
Stroke volume (ml)	81 ± 5	87 ± 5	14.3 ± 7.4	NS
Systemic vascular resistance (dynes/s/cm^{-5})	1,690 ± 120	1,600 ± 110	—4.1 ± 7.1	NS
Glomerular filtration rate (ml)	96 ± 4	96 ± 3	0.7 ± 3.4	NS
Renal blood flow (ml)	1,020 ± 60	1,040 ± 60	2.2 ± 3.4	NS
Renal vascular resistance (dynes/s/cm^{-5})	9,310 ± 690	8,170 ± 530	—10.3 ± 5.2	NS
Plasma volume (L)	3.6 ± 0.2	3.6 ± 0.1	1.3 ± 3.2	NS
Extracellular fluid volume (L)	15.6 ± 0.7	15.9 ± 0.8	2.5 ± 1.6	NS
Body weight (kg)	90.9 ± 2.9	91.3 ± 2.9	0.4 ± 0.4	NS

NS, not significant.

RBF and GFR did not change during long-term treatment with acebutolol. Thus, the fall in blood pressure did not compromise renal perfusion, which contrasts with observations on β-blockers devoid of PAA (40).

Partial agonist activity

Pharmacologic experiments in animals and clinical observations in humans indicate that acebutolol possesses a modest degree of PAA (3,4,31,41—43). The present study shows that from a hemodynamic point of view acebutolol's PAA is indeed rather weak, and considerably less than the PAA of pindolol. On pindolol, cardiodepression and reflex-vasoconstriction are absent and the fall in blood pressure in the long run is due to a decrease of vascular resistance below pretreatment values (38,42,44). Furthermore, also in contrast with the effects of acebutolol, central pressures on pindolol do not rise and, at night, when sympathetic tone is low, HR is increased on this drug (39,45).

Effects on β-adrenergic receptor density

Administration of the nonselective β-adrenoceptor antagonist propranonol, which is devoid of PAA, results in an increase in the density of β-adrenoceptors on lymphocytes (28,46-47). Conversely, pindolol with considerable PAA, causes a decrease

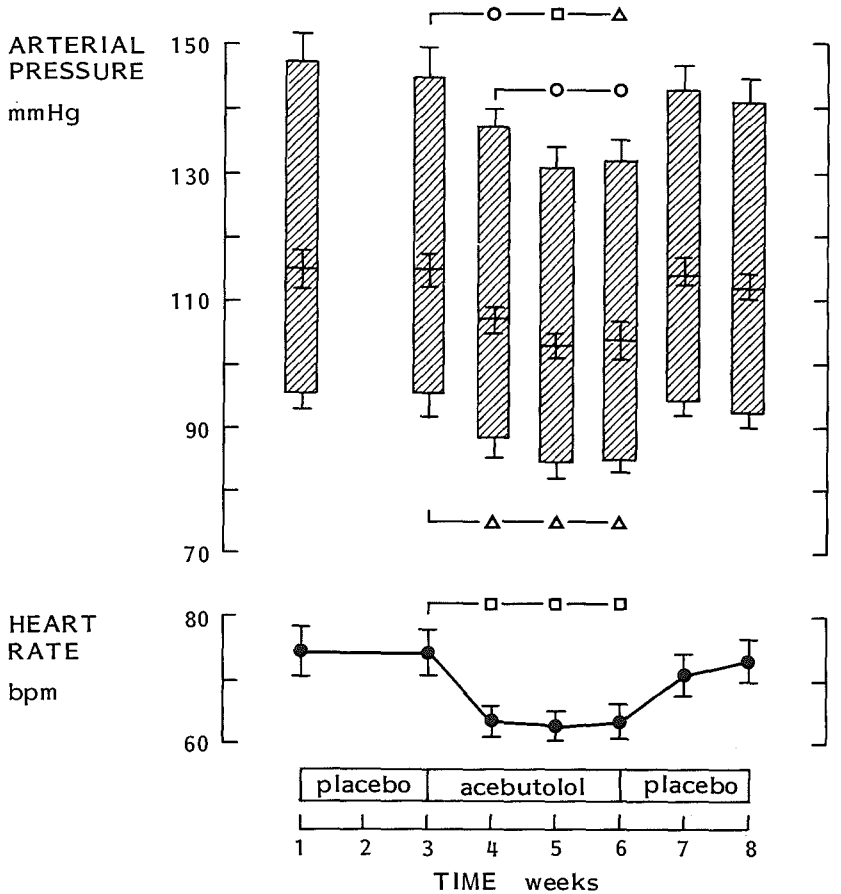

Fig. 4. Long-term effects of acebutolol on systolic and diastolic arterial pressure and heart rate. Dose of acebutolol in week 4, 200 mg twice daily; in week 5, 400 mg twice daily in week 6, 480 ± 60 mg twice daily. $p < 0.05$ (○); $p < 0.01$ (△); $p < 0.001$ (□).

in the density of these receptors (28,47,48). Acute and long-term administration of acebutolol had no effect on the density of β-adrenergic receptors on lymphocyte membranes. This might be related to its β_1 selectivity, since β-adrenergic receptors on lymphocyte membranes are of a homogeneous β_2-subtype population (46,49). However, a recent study showed that administration of the β_1-selective antagonist celiprolol, with a relatively high degree

of PAA, also results in "downregulation" of lymphocyte β_2-adrenoceptors, whereas bisoprolol, a β_1-selective antagonist devoid of PAA, did not affect β-adrenoceptor density (48). These findings indicate that the PAA of a selective β_1-adrenoceptor antagonist probably also possesses a β_2-agonistic component. The absence of any effect of acebutolol on lymphocyte β-adrenoceptor density could therefore be interpreted as a consequence of the drug's β_1-adrenoceptor selectivity, but with only relatively weak PAA.

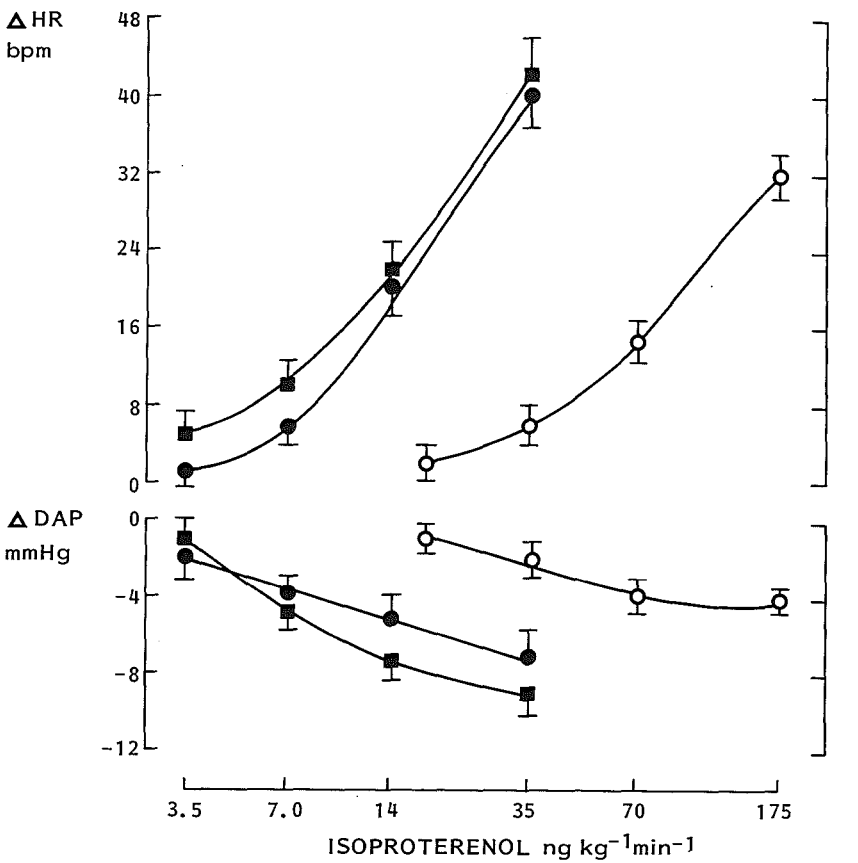

Fig. 5. Isoproterenol dose-response curves for changes in heart rate and diastolic arterial pressure during placebo (●—●, 24 h after the first dose of acebutolol (○—○), and 1 week after withdrawal from longterm treatment (■—■).

Table 2. *Effects of acute and long-term treatment with acebutolol on plasma concentrations of PNE, PE, APRC, and PA*

	Time (weeks)						
		2					
	1	0 h	2 h	6 h	12 h	24 h	6
PNE (pg/m)	221 ± 25	194 ± 23	249 ± 34 *a*	207 ± 31	212 ± 39	226 ± 40	189 ± 35
PE (pg/ml)	63 ± 11	46 ± 6	61 ± 8	30 ± 4	31 ± 8	53 ± 10	34 ± 8
APRC (μU/ml)	8.5 ± 2.5	9.7 ± 2.5	4.2 ± 1.2 *b*	3.3 ± 0.8 *b*	2.4 ± 0.4 *b*	4.1 ± 1.3 *a*	1.8 ± 0.9 *b*
PA (pg/ml)	105 ± 18	122 ± 18	109 ± 11	99 ± 15	85 ± 14	68 ± 9 *a*	81 ± 13 *a*

PNE, plasma norepinephrine; PE, plasma epinephrine; APRC, active plasma renin concentration; PA, plasma aldosterone.
Values of week 6 were compared with placebo values in week 1. Values of week 2 (acute study) at time points 2, 6, 12, and 24 h were compared with baseline values at time point 0.
a $p < 0.05$; *b* $p < 0.01$.

Neurohumoral effects

Plasma catecholamines remained unchanged during the acute and long-term antihypertensive action of acebutolol. NE was increased by 28% during the initial vasoconstrictor response after the first dose of the drug, when blood pressure was not yet changed. This rise in PNE does not necessarily reflect increased sympathetic vasoconstrictor nerve activity. The plasma concentration of NE is determined by the rates of NE release into and removal from plasma. The clearance of NE from plasma is diminished after β-adrenoceptor blockade, probably concomitant with a decrease in cardiac output (50-52). Since cardiac output was reduced during the initial vasoconstrictor phase, it is conceivable that the observed increase in PNE was at least in part due to a diminished clearance rate.

Table 3. *Effects of acute and long-term treatment with acebutolol on density of β-adrenergic receptors on lymphocyte membranes*

	Placebo			Acebutolol		Withdrawal	
Time (weeks)	1	2	2*a*	5	6	7	8
B_{max} (fmol/mg prot)	29 ± 2	25 ± 2	26 ± 2	25 ± 2	28 ± 2	31 ± 3	28 ± 2
K_d (p*M*)	51 ± 5	58 ± 9	57 ± 8	59 ± 10	59 ± 8	46 ± 6	54 ± 8

a Twenty-four hours after starting β-blockade with acebutolol.

Active renin was markedly suppressed after acebutolol, which is in agreement with previous studies (13,53). The suppression

of active renin was already maximal (—61%) 2 h after the first dose of the drug, thus before there was any fall in blood pressure. This dissociation in time between renin suppression and fall in blood pressure after β-adrenoceptor blockade has been noted before (54,55) and argues against the hypothesis that fall in blood pressure after β-adrenoceptor blockade is predominantly caused by renin suppression. Moreover, baseline values of active renin and blood pressure responses to acute as well as long-term treatment with acebutolol were not correlated. The decrease in plasma aldosterone levels might have contributed to acebutolol's antihypertensive effect, although during long-term treatment body fluid volumes and body weight remained unchanged.

β_1 selectivity

The β_1 selectivity or cardioselectivity of acebutolol has been demonstrated in a number of animal studies (3,4,56). A few early studies in humans raised doubts about the cardioselectivity of acebutolol (57,58). More recent studies have shown that at doses equipotent in antagonizing isoproterenol- or exercise-induced tachycardia, acebutolol has less effect than propranolol on vascular β-adrenoceptors (59,60). The selectivity of the drug in antagonizing cardiac or β_1-adrenoceptors relative to vascular or β_2-adrenoceptors was confirmed by the isoproterenol infusions in the present study. At infusion rates that increase HR by 25 beats/min, the isoproterenol-induced fall in DAP 12 h after the second dose of acebutolol, 400 mg, was not different from the fall in pressure during placebo (Fig. 5).

In conclusion, the findings of this study confirm that acebutolol is an effective antihypertensive agent. In the long run, the drug has no adverse effects on renal perfusion or GFR. In the dose range of 200-400 mg b.i.d., the drug is cardioselective but exerts only a modest degree of PAA, as assessed by its hemodynamic effects and the effects on lymphocyte β-adrenoceptors.

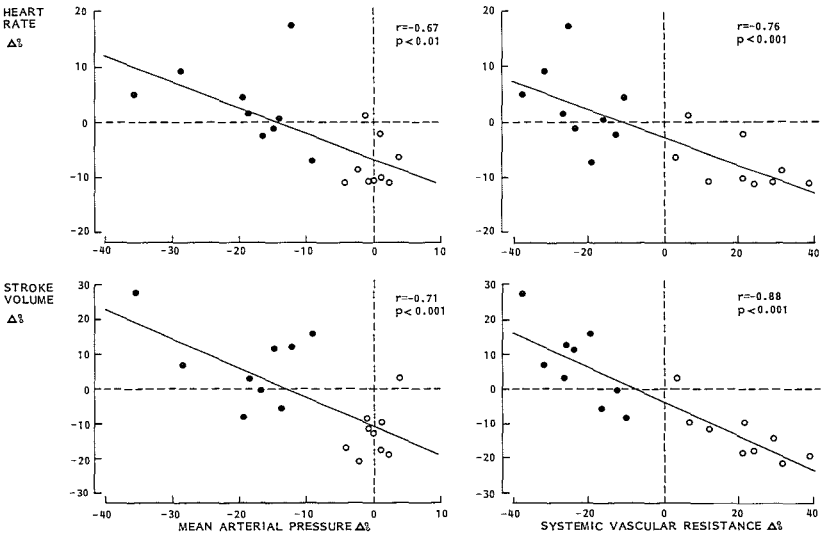

Fig. 6. Correlations between percentage changes in mean arterial pressure and heart rate or stroke volume **(left panel)** and between percentage changes in systemic vascular resistance and heart or stroke volume **(right panel)**. Percentage change 2 h after the first dose of acebutolol as compared to baseline (○). Percentage change 24 h after dosing as compared with baseline (●).

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5. The clinical pharmacology of bopindolol, a new long-acting beta-adrenoceptor antagonist, in hypertension

5.1 Summary

Bopindolol is a new long-acting, nonselective β-adrenoceptor antagonist with partial agonist activity. Its acute (24 hours, 2 mg, administered orally) and long-term (3 weeks, 2 to 4 mg) hemodynamic and hormonal effects were studied in a single-blind placebo-controlled trial in 10 hypertensive subjects. The initial response (mean ± SE) to bopindolol was a fall in cardiac output (—12% ± 2%) and heart rate (—11% ± 2%). Mean arterial pressure began to fall 3 to 4 hours after administration in parallel with a decrease in systemic vascular resistance, which had increased initially. Twenty-four hours after administration, mean arterial pressure and systemic vascular resistance were reduced by 12% ± 2% and 12% ± 5%, respectively. By that time heart rate and cardiac output did not differ from baseline values despite β-blockade. After 3 weeks of treatment mean arterial pressure had fallen by 9% ± 2% and renal blood flow and glomerular filtration rate were not changed. One week after withdrawal from treatment mean arterial pressure and heart rate were no longer reduced, but β-blockade could still be demonstrated, establishing the long duration of action of the drug.

5.2 Introduction

The clinical usefulness of β-adrenoceptor blocking agents in the treatment of hypertension and coronary heart disease has been well established. Bopindolol is a new nonselective β-adrenoceptor blocking drug with partial agonist activity. It is a prodrug that has to be hydrolyzed to an active metabolite before it can exert

its β-adrenoceptor blocking activity (1). The conversion of bopindolol into its active metabolite occurs rapidly; peak plasma levels of hydrolyzed bopindolol are reached within 1 to 2 hours after oral administration (2,3).
On a weight-for-weight basis, bopindolol is about 10 times as potent as pindolol and 200 times as potent as propranolol in antagonizing exercise- or isoproterenol-induced tachycardia (4). Although the plasma half-life of the active metabolite of bopindolol is 4 to 5 hours, the drug appears to have a far longer duration of action, which makes it suitable for once-daily or even less frequent administration (5). This long duration of action has been related to the high affinity of the drug to the β-adrenoceptor. The estimated apparent affinity constant of the active metabolite of bopindolol for the β-adrenoceptor is 100 to 400 times less than the k_D of propranolol (6).
There is some evidence that bopindolol possesses a moderate degree of partial agonist activity (7,8). Because detailed hemodynamic studies have not been performed in humans, it is not known whether the drug's partial agonist activity is hemodynamically relevant. We therefore studied the time course of bopindolol's antihypertensive effect in relation to other hemodynamic changes, by using invasive techniques, in 10 subjects with uncomplicated essential hypertension.

5.3 Subjects and methods

Ten male subjects with mild to moderate essential hypertension were recruited from the outpatient hypertension clinic. The age of the subjects was 48 $\pm$ 4 years (mean $\pm$ SE). When untreated, all patients had sitting diastolic blood pressures of $>$ 95 mm Hg on three separate visits to the outpatient clinic. Secondary forms of hypertension were excluded by clinical and laboratory evaluation, including isotope renography. None of the patients had a history or clinical signs of ischemic heart disease, cerebrovascular disease, or chronic obstructive lung disease. BUN, serum creatinine, and electrolyte values were within the normal range of our laboratory.

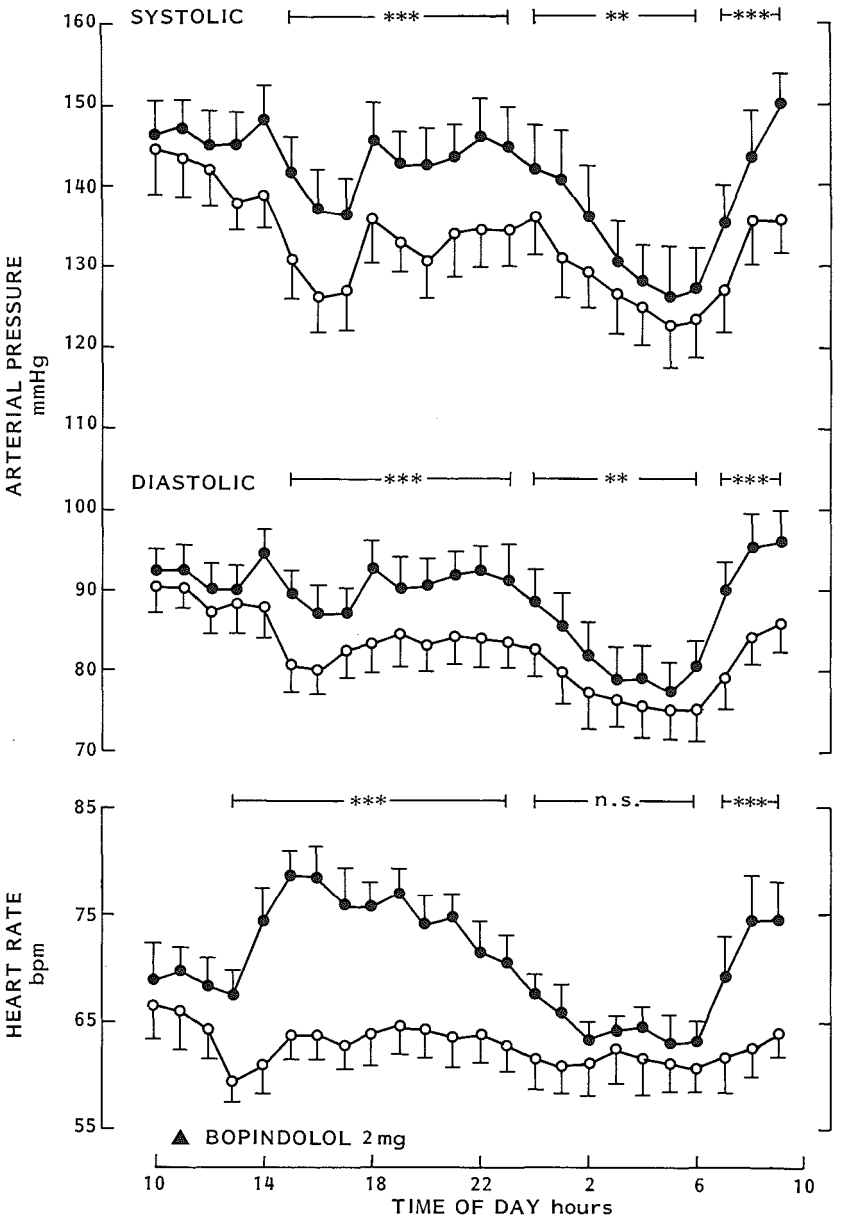

Fig. 1. Comparison of 24-hour trend plots of hourly mean values of SAP and DAP and heart rate during placebo (●—●) and after administration of the first dose of bopindolol (○—○). $^{**}P < 0.01$; $^{***}P < 0.001$ for differences between bopindolol and placebo.

The purpose and procedures of the study were explained to the patients, who gave their consent to participate. The study protocol was approved by the Local Hospital Ethical Review Committee.

Study design

The study was designed as a single-blind, placebo-controlled trial that lasted for 8 weeks. Antihypertensive medication, if any, was discontinued at least 3 weeks before the study. After this washout period placebo capsules, administered once daily, were provided for 3 weeks. At the end of the first and second week of this placebo period all patients were hospitalized for 2 days. During their stay in the hospital the patients had strict bed rest.

At the first hospital admission, arterial pressure was monitored continuously for 24 hours. Cardiac output, renal plasma flow, glomerular filtration rate, and body fluid volumes were measured and blood was sampled for determination of plasma concentrations of catecholamines, active renin, and aldosterone. After completion of measurements an isoproterenol infusion was given to assess the responsiveness of cardiac and vascular β-adrenoceptors.

During the second stay in the hospital, bopindolol, 2 mg, was given by mouth for the first time and the acute hemodynamic effects were studied for 24 hours after 1 to 2 hours of baseline readings. Arterial pressure was monitored continuously and cardiac output and central pressures were determined at hourly intervals. Measurements of cardiac output were interrupted from 11 p.m. to 7 a.m. Just before and 2, 6, 12, and 24 hours after the first dose of bopindolol, blood was sampled for catecholamines, active renin, and aldosterone. At the end of the 24-hour observation period blockade of cardiac and vascular β-adrenoceptors was assessed by isoproterenol infusion. After this study the patients were seen at weekly intervals in the outpatient clinic. At each visit blood pressure and heart rate were measured between 8 a.m. and 10 a.m. Dose titration with bopindolol was carried out during weeks 4, 5, and 6. Bopindolol was given, 1 mg once daily in week 4 and 2 mg once daily in week 5. In five subjects 4 mg, once daily, was given during week 6 because diastolic arterial pressure was $>$95 mm Hg. At the end of week 6 (dose

of bopindolol 3.0 ± 0.3 mg) measurements of cardiac output, renal plasma flow, glomerular filtration rate, and body fluid volumes were repeated and blood was again sampled for catecholamines, renin, and aldosterone. All measurements were performed during the morning hours after the patients had been recumbent for at least 1 hour. After the sixth week placebo was reinstituted for 2 weeks. After 1 week placebo isoproterenol infusion was repeated.

Invasive hemodynamic measurements. Arterial pressure was monitored continuously by means of the Oxford technique (9). After local anesthesia with lidocaine, 2%, the brachial artery of the nondominant arm was cannulated with a 1.0 mm diameter Teflon catheter (Seldicath, Plastimed, Saint-Leu-La-Foret, France). The intra-arterial cannula was connected to a perfusion-transducer device (Northwick Park Hospital, London, U.K.) suspended in front of the chest. The transducer signal was recorded on magnetic tape by means of an analog tape recorder (Medilog II recorder, Oxford Medical Instruments, Oxford, U.K.). The analog signal was digitized during replay of the tape with a sample frequency of 33 1/3 samples per second real time. The full digital trace was stored on magnetic disk. Traces were analyzed beat by beat and scrutinized for beat loss, clipping of the amplifier, damping, movement, and other artefacts by means of a computer. These events accumulated to $< 2\%$ of all data and were excluded from analysis. On the basis of beat-by-beat analysis, hourly means of the systolic and diastolic arterial pressure and heart rate were calculated. The values of these parameters obtained after active treatment was started were compared with the corresponding values during placebo 1 week before. Cardiac output and central pressures were measured by means of a 7F Swan-Ganz, flow-directed, thermodilution catheter (Edwards Laboratories Añasco, Puerto Rico). The catheter was introduced percutaneously into an antecubital vein. Cardiac output was measured in triplicate by bolus injections of ice-cold dextrose, 5%. Systemic arterial pressure and pulmonary artery pressure were monitored by means of Gould Statham P23ID transducers (Gould Inc., Cleveland, Ohio), with zero reference at midaxillary level. Right atrial pressure and pulmonary capillary wedge pressure were measured immediately

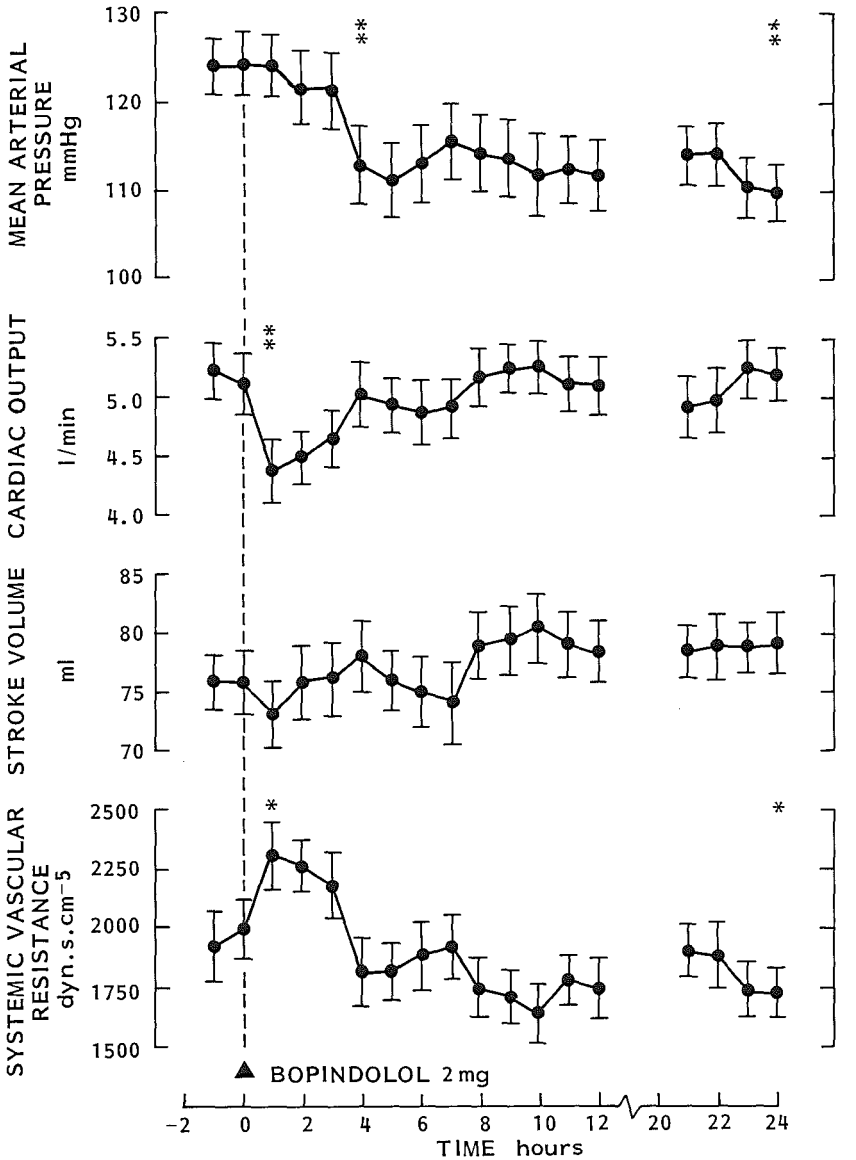

Fig. 2. Time course of the acute effects of bopindolol on systemic hemodynamics. *P < 0.05; **P < 0.01.

before and after cardiac output determinations. The heart rate was derived from the continuously recorded ECG. Mean values of pressures were obtained by electronic integration of the analog signals.

The following hemodynamic variables were derived: SVR = 80 × (MAP — MRAP)/CO, PVR = 80 × (MPAP — MPCWP)/CO, and SV = CO/HR, where MAP = mean arterial pressure (in millimeters of mercury), MRAP = mean right atrial pressure (in millimeters of mercury), CO = cardiac output (in liters per minute), MPAP = mean pulmonary artery pressure (in millimeters of mercury), MPCWP = mean pulmonary capillary wedge pressure (in millimeters of mercury), SVR = systemic vascular resistance (dyne · sec^{-1} · cm^{-5}), PVR = pulmonary vascular resistance (dyne · sec^{-1} · cm^{-5}), SV = stroke volume (in milliliters), and HR = heart rate (in beats per minute).

Noninvasive hemodynamic measurements. In the outpatient clinic supine blood pressure and heart rate were measured every 5 minutes for 1 hour by an oscillometric device (Datascope, Accutorr I, Datascope Corp, Paramus, N.J.). Values of blood pressure and heart rate obtained during 1 hour were averaged.

Supine cardiac output was measured by an isotope dilution technique (10,11) at the end of the blood pressure measurements. ^{99m}Tc-labeled human serum albumin (Technescan HSA, Mallinckrodt Diagnostica BV, Petten, The Netherlands), 100 to 200 μCi, was used as the indicator. After a rapid intravenous injection of the isotope, time-concentration curves were recorded for 2 minutes by precordial counting of radioactivity with the use of a single probe. Additional recordings were made after 5 and 10 minutes when blood was sampled for measurement of radioactivity. Cardiac output was calculated by means of the Stewart-Hamilton formula. The method yields a good correlation with the dye-dilution technique ($n = 57$; $r = 0.92$), and the coefficient of variation of duplicate measurements with the isotope dilution technique is 6% ($n = 38$) (10). The heart rate was measured from a simultaneously recorded ECG. Systemic vascular resistance was calculated as SVR = MAP/CO × 80 and MAP as MAP = DAP + (SAP — DAP)/3, where SAP = systolic arterial

pressure and DAP = diastolic arterial pressure.
For renal function studies a constant-infusion technique was used (12). Effective renal plasma flow and glomerular filtration rate were estimated by means of the clearance of ^{131}I-hippuran and ^{125}I-thalamate (Amersham, U.K.). The priming dose for hippuran was 0.3 to 0.4 μCi/kg body weight and for thalamate, 0.08 to 0.1 μCi/kg body weight. The sustaining infusion rates were 0.2 and 0.05 μCi/min, respectively. The clearance of the isotopes was determined at steady state after 90 and 105 minutes. Renal blood flow (RBF) was calculated by means of central venous packed cell volume, assuming 75% renal extraction of hippuran (13). Renal vascular resistance (RVR) was derived as RVR = MAP/RBF $\times$ 80.
Plasma volume and extracellular fluid volume were measured by ^{99m}Tc-labeled human serum albumin and sodium ^{35}S-sulfate, respectively (10-12). The interstitial fluid volume was calculated by subtracting plasma volume from extracellular fluid volume.
Isoproterenol infusions. During isoproterenol infusions arterial pressure was measured directly in a brachial or radial artery and the heart rate was derived from a continuously recorded ECG. Isoproterenol was infused through an indwelling cannula in a forearm vein. After baseline values of heart rate and arterial pressure had been obtained during infusion of saline solution (2 ml/min) for 20 minutes, the infusion was switched to isoproterenol. The doses of isoproterenol during placebo and 1 week after withdrawal were 3.5, 7, 14, 35, and 70 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}. At the end of the acute study the doses were 35, 70, 140 and 350 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}. The dose of isoproterenol was increased every 10 minutes until a rise in heart rate of 25 bpm was obtained. The average value of heart rate in the last 2 minutes of each dose step was used to construct log-dose response curves. For every patient the best fit line of the linear portion of the log-dose response curve was estimated by linear regression analysis. The dose of isoproterenol required to increase the heart rate by 25 bpm (CD25) was calculated.

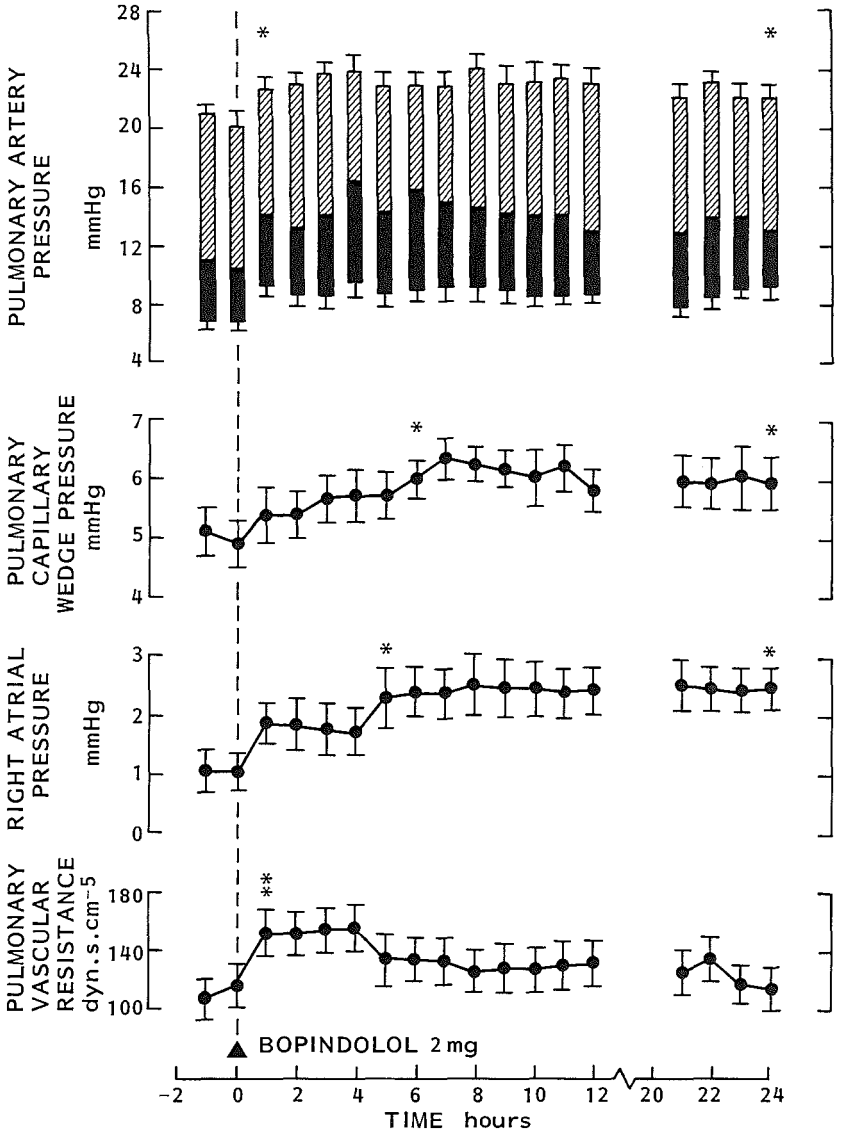

Fig. 3. Time course of acute effects of bopindolol on central hemodynamics. *P < 0.05; **P < 0.01.

Analytic procedures. For determination of active renin and plasma aldosterone, venous blood was collected in tubes containing EDTA in a final concentration of 2 mg/ml blood. The samples were centrifugated immediately at 0°C and stored at —20°C. All samples of one patient were measured in a single assay. Active renin was measured by RIA as described previously (13); the normal range is 5 to 40 μU/ml. Plasma aldosterone was also measured by RIA (14); the normal range is 30 to 180 pg/ml. For determination of catecholamines, 10 ml venous blood was collected in chilled tubes containing 19 mg EGTA and 12 mg glutathione. After centrifugation at 0°C samples were stored at —70°C. Plasma norepinephrine and epinephrine were measured by a radioenzymatic method, according to Peuler and Johnson (15).

Statistical analysis. Data are presented as mean values $\pm$ SE. Plasma values of active renin were not distributed normally, and mean values were calculated after logarithmic transformation. Systemic, central, and renal hemodynamics and body fluid volumes were expressed per 1.73 m^2. The 24-hour blood pressure recordings were analyzed by means of a linear regression model with the SPSS/PC+ program (SPSS Inc., Chicago, Ill.). As dependent variable the within-patient difference of SAP, DAP, and heart rate between the corresponding hours of weeks 2 and 1 was taken. As independent variables the recording of the first week was included to correct for a regression effect, and a dummy variable, indicating the periods before and after the first dose of bopindolol, was taken to evaluate the effect of the drug. The effects of bopindolol on SAP, DAP, and heart rate during the day and night were evaluated separately. Single Student paired t-tests were used for remaining comparisons. P values < 0.05 were considered to indicate a statistically significant difference.

5.4 Results

Acute hemodynamic effects. Twenty-four-hour trend plots based on the hourly means of SAP, DAP, and heart rate are shown in Fig. 1. Values of SAP, DAP, and heart rate of week 2 before the first dose of bopindolol was given were lower than the cor-

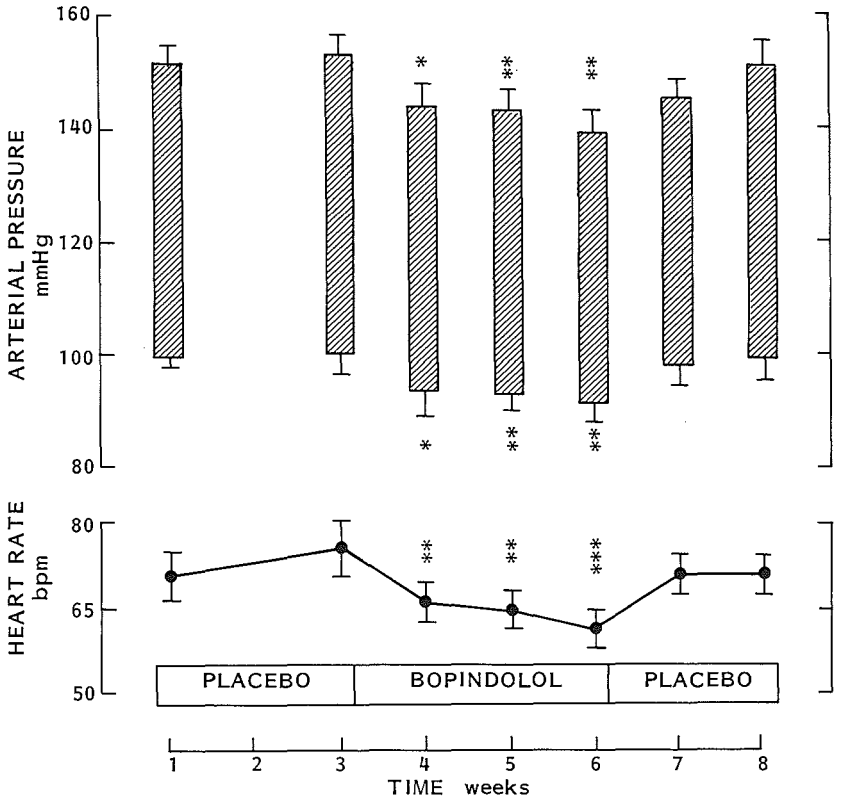

Fig. 4. Long-term effects of bopindolol on SAP and DAP and heart rate. Dose of bopindolol during week 4: 1 mg once daily; during week 5: 2 mg once daily; during week 6: 3 ± 0.3 mg once daily. *P < 0.05; **P < 0.01; ***P < 0.001.

Table 1. *Effects of long-term treatment with bopindolol on systemic and renal hemodynamics*

	Placebo	Bopindolol	P Value
MAP (mm Hg)	120 ± 3	110 ± 4	< 0.01
Heart rate (bpm)	74 ± 2	61 ± 2	< 0.001
Cardiac output (L/min)	5.5 ± 0.5	4.7 ± 0.3	NS
Stroke volume (ml)	74 ± 6	78 ± 5	NS
SVR (dyne · sec^{-1} · cm^{-5})	1890 ± 170	1970 ± 140	NS
Glomerular filtration rate (ml/min)	103 ± 4	97 ± 4	NS
Renal blood flow (ml/min)	1012 ± 77	976 ± 84	NS
RVR (dyne · sec^{-1} · cm^{-5})	10105 ± 860	9575 ± 750	NS

responding placebo values of week 1. However, this difference was not significant. After administration of bopindolol, compared with placebo, heart rate was reduced within 1 hour ($P < 0.05$). This decrease in heart rate, was maintained during the day but not during the night (11 p.m. to 6 a.m.). Compared with placebo SAP and DAP did not change within the first 3 hours after administration. After this period SAP and DAP were reduced significantly during both the day ($P < 0.001$) and night ($P < 0.01$).

Before the first oral dose of bopindolol, 2 mg, baseline values of MAP, heart rate, cardiac output, stroke volume, and SVR were 124 ± 3 mm Hg, 67 ± 2 bpm, 5.1 ± 0.3 L/min, 76 ± 4 ml, and 1990 ± 130 dyne · sec^{-1} · cm^{-5}, respectively. Already 1 hour after administration a pronounced effect of bopindolol on cardiac output and heart rate was observed (Fig. 2). By that time cardiac output was reduced by 12% ± 2% ($P < 0.01$) and heart rate by 11% ± 2% ($P < 0.01$), whereas stroke volume, was not changed. The decrease in cardiac output was associated with an increase in SVR (11% ± 4%; $P < 0.05$). MAP did not change initially. However, 4 hours after administration MAP was reduced by 9% ± 2% ($P < 0.01$). This fall in MAP was the result of a fall of SVR toward, and later even below, baseline. The reduction in MAP was maintained during the entire 24-hour observation period. After 24 hours MAP was reduced by 12% ± 2% ($P < 0.01$). By that time SVR was reduced by 12% ± 5% ($P < 0.05$) whereas cardiac output and stroke volume, did not differ from pretreatment values.

Cardiac filling pressures and MPAP were increased after administration of bopindolol. An increase of PVR was evident only during the first few hours (Fig. 3).

Long-term hemodynamic effects. SAP and DAP were reduced after 1 week of treatment with bopindolol, 1 mg once daily, compared with placebo values at weeks 1 and 2 (Fig. 4). Increasing the dose of bopindolol during the following 2 weeks did not cause any further significant fall in pressure. After 3 weeks of treatment SAP and DAP were reduced from 153 ± 4 to 139 ± 4 mm Hg ($P < 0.01$) and from 100 ± 2 to 91 ± 2 mm

Hg ($P < 0.01$), respectively. Heart rate was decreased from 74 $\pm$ 2 to 61 $\pm$ 2 bpm ($P < 0.001$). One and 2 weeks after withdrawal, SAP, DAP, and heart rate did not differ from the initial placebo values at weeks 1 and 2. During long-term treatment, MAP was reduced by 9% $\pm$ 2% ($P < 0.01$). This reduction in MAP was not associated with significant changes in cardiac output (—10% $\pm$ 6%), stroke volume (8% $\pm$ 6%), or SVR (5% $\pm$ 6%) (Table I).

Glomerular filtration rate, renal blood flow and renal vascular resistance did not change during chronic treatment with bopindolol (Table I).

Isoproterenol infusions. The CD25 during placebo was 21 $\pm$ 3 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}. At this infusion rate of isoproterenol DAP fell by 7 $\pm$ 2 mm Hg ($P < 0.001$). Twenty-four hours after the first dose of bopindolol, 2 mg, the CD25 was increased to 265 $\pm$ 55 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} ($P < 0.001$), indicating significant blockade of cardiac β-adrenoceptors (Fig. 5). In spite of the much higher infusion rate the effect on DAP was completely abolished, indicating blockade of vascular β-adrenoceptors as well. One week after withdrawal from chronic bopindolol treatment the CD25 was still increased (51 $\pm$ 9 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}; $P < 0.01$), and the fall in DAP was still blunted.

Effects on catecholamines, active renin, aldosterone, and body fluid volumes. The placebo values of plasma norepinephrine, epinephrine, active renin, and plasma aldosterone during the first and second weeks did not differ (Table II). Acute and chronic treatment with bopindolol had no effects on plasma catecholamines. Active renin was decreased by 50% $\pm$ 9% ($P < 0.01$) 2 hours after the first dose of bopindolol. At the end of the acute study active renin was still decreased by 59% $\pm$ 9% ($P < 0.01$). A similar fall in active renin was observed during chronic treatment (—62% $\pm$ 10%; $P < 0.01$). Plasma aldosterone was decreased at the end of the acute and long-term studies by 40% $\pm$ 9% ($P < 0.01$) and 20% $\pm$ 6% ($P < 0.05$), respectively. The fall in MAP at the end of the acute and long-term studies was not related to baseline values of active renin.

Plasma volume, extracellular fluid volume, and interstitial fluid

volume and the ratio plasma volume/interstitial fluid volume did not change; mean values of these parameters before treatment were 2.5 ± 0.1 L, 13.7 ± 0.4 L, 11.3 ± 0.4 L, and 0.22 ± 0.01, respectively.

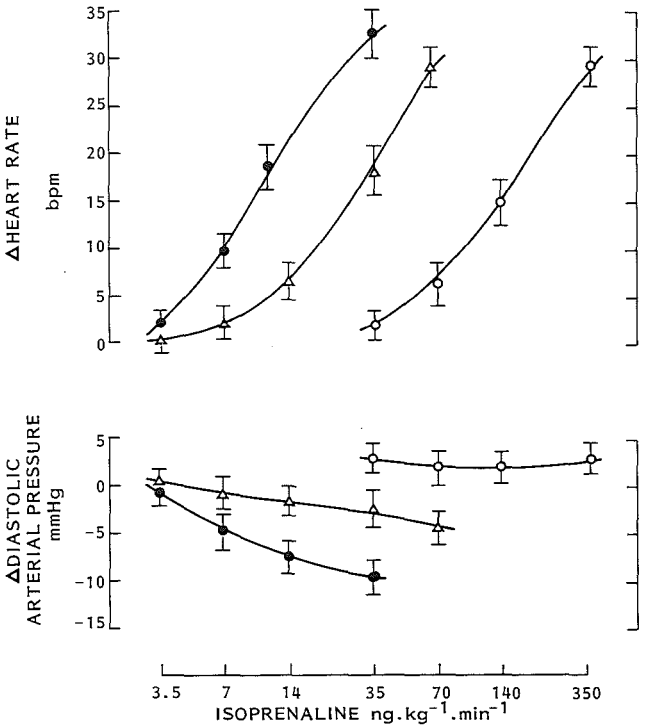

Fig. 5. Isoproterenol dose-response curves for changes in heart rate and DAP during placebo (●—●), 24 hours after the first dose of bopindolol, 2 mg, (○—○), and 1 week after withdrawal from long-term treatment (△—△).

5.5 Discussion

Hemodynamic changes. The present study confirms earlier observations that monotherapy with bopindolol, once daily, is efficacious in lowering blood pressure for 24 hours (5,16). The initial hemodynamic response 1 hour after bopindolol was a fall in cardiac output and an increase in SVR. A gradual decrease in blood pressure was seen 3 to 4 hours after administration.

It occurred in parallel with a decrease of SVR to pretreatment values and even lower (Fig. 2). Interestingly, cardiac output then also returned toward control values. Because β-adrenergic receptors were blocked, this restoration of cardiac function cannot be the result of sympathetic stimulation. Therefore withdrawal of vagal tone, reduction in afterload, or increase in venous return to the heart are alternative explanations for this hemodynamic adaptation to β-blockade. Indeed, it has been shown that changes in heart rate and cardiac output after vasodilatation during β-adrenoceptor blockade are mediated by vagal withdrawal and increase in venous return (17). Furthermore the significant inverse correlations of MAP and SVR with either heart rate or stroke volume support this view (Fig. 6).

In contrast to the early hemodynamic changes, the long-term effect of bopindolol on MAP was associated with a decrease in cardiac output and a slight increase in SVR, although these changes were not statistically significant. A difference between the acute and long-term hemodynamic profile as observed for bopindolol has also been described for the β_1-selective antagonist atenolol and the nonselective β-adrenoceptor antagonist timolol, both of which are devoid of partial agonist activity (18,19). The mechanism responsible for this secondary fall in cardiac output during long-term treatment with β-adrenoceptor blocking drugs is not known. It has been speculated that reduction of the plasma-to-interstitial-volume ratio may play a role (19). In the present study this could not be demonstrated.

During chronic treatment with bopindolol, renal blood flow and glomerular filtration rate were not adversely affected. This is in contrast to observations with propranolol (20,21).

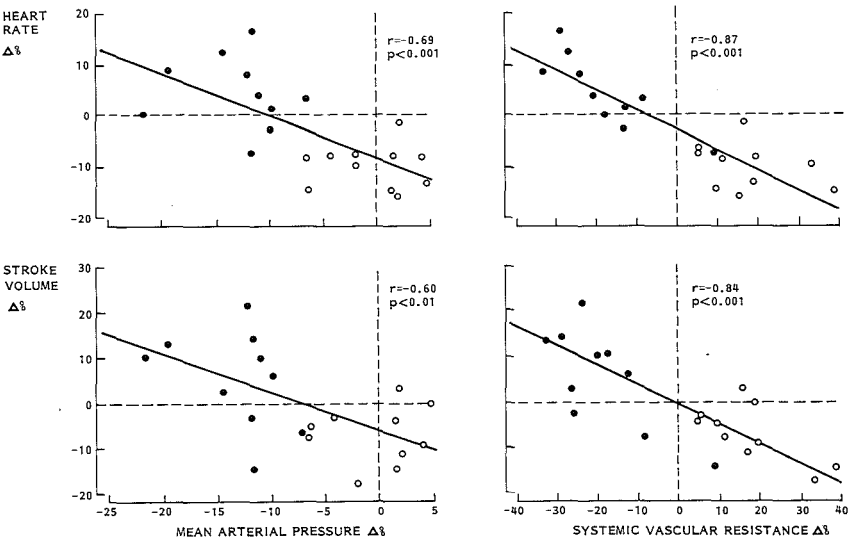

Fig. 6. Correlations between percentage changes in MAP and heart rate or stroke volume *(left panel)* and between percentage changes in SVR and heart rate or stroke volume *(right panel).* ○, percentage change compared with baseline 2 hours after the first dose of bopindolol; ●, percentage change compared with baseline 24 hours after the first dose of bopindolol.

Table II. *Effects of acute and long-term treatment with bopindolol on plasma concentrations of norepinephrine, epinephrine, active renin, and aldosterone*

		2					
Time (wk)	*1*	*oh*	*2h*	*6h*	*12h*	*24h*	*6*
PNE (pg/ml)	261 ± 30	258 ± 36	269 ± 17	308 ± 29	248 ± 15	324 ± 33	264 ± 39
PE (pg/ml)	49 ± 9	46 ± 13	67 ± 29	62 ± 27	40 ± 10	62 ± 13	41 ± 13
APRC (μU/ml)	8.1 ± 2.8	9.2 ± 2.5	4.1 ± 1.0*	3.0 ± 0.8	2.3 ± 0.6	3.4 ± 1.0*	2.9 ± 0.6*
PA (pg/ml)	74 ± 14	69 ± 10	52 ± 10*	69 ± 12	44 ± 4	34 ± 4*	45 ± 10†

PNE norepinephrine; PE, epinephrine; APRC, active renin; PA, plasma aldosterone.
Values of week 6 (long-term treatment) were compared with placebo values of week 1. Values of week 2 (acute study) at time points 2, 6, 12, and 24 hours were compared with baseline values at time point 0. *$P < 0.01$; †$P < 0.05$.

Hemodynamic interrelations. All β-adrenoceptor blocking agents are approximately equally effective in lowering blood pressure (22). It has been suggested that this antihypertensive effect is always caused by vasodilatation, irrespective of the ancillary properties

of these drugs (23). However, this assertion was based on interpolation of the compiled results of studies, concerning either the acute or long-term effects of β-blockers. In the present study we have shown that indeed within 24 hours after starting β-blocker treatment the fall in blood pressure evolves in parallel with a reduction in SVR. Moreover, both acutely (24 hours) and chronically (3 weeks) MAP and SVR were positively correlated ($r = 0.81$; $P < 0.001$ and $r = 0.77$; $P < 0.01$, respectively), whereas MAP and cardiac output were not.

Bopindolol's partial agonist activity. Although studies in healthy volunteers (8) and animals (7) have shown that bopindolol possesses a moderate degree of partial agonist activity, our present findings do not indicate that this property has any clinical relevance for its hemodynamic profile. First, as already discussed, the hemodynamic profile of bopindolol, both acutely and chronically, is comparable to that of β-adrenoceptor antagonists devoid of partial agonist activity. Consequently its hemodynamic profile contrasts markedly with the hemodynamic effects of β-adrenoceptor blocking drugs with a considerable degree of partial agonist activity (23,25). With these drugs there is neither cardio-depression nor reflex vasoconstriction, and the change in MAP, both acutely and chronically, is the result of a decrease of SVR below pretreatment level. Second, also in contrast to the present findings, after administration of β-adrenoceptor blocking drugs with pronounced partial agonist activity, cardiac filling pressures do not rise (25,26). Third, we and others have shown that at night, when sympathetic tone is low, heart rate with these drugs is increased compared with placebo values, (27,28) which also contrasts with the effects of bopindolol on heart rate in the present study.

Catecholamines. In agreement with an earlier report, catecholamines remained unchanged during long-term treatment with bopindolol (29). Unexpectedly, plasma norepinephrine also did not change during the initial vasoconstrictor response after the first dose of the drug. Animal studies have shown that this initial vasoconstriction in response to the fall in cardiac output is mediated by the arterial baroreflex (30,31). Apparently central venous plasma norepinephrine does not reflect this baroreflex-mediated increase

of the vasoconstrictor nerve activity.

Renin and aldosterone. Renin suppression by β-adrenoceptor blocking drugs has been proposed as an important mechanism in their blood pressure-lowering effect (32). Administration of bopindolol was associated with a marked suppression of active renin. However, this reduction in active renin was already maximal 2 hours after the first dose, thus before any fall in blood pressure was observed. This dissociation in time between renin suppression and the antihypertensive effect has been noted before (33,34). This finding argues against the hypothesis that blood pressure reduction by β-adrenoceptor blocking drugs is achieved predominantly through renin suppression. Moreover, the blood pressure response to acute and long-term treatment with bopindolol was not related to baseline values of active renin, which is in agreement with a previous study (16). The reduction in plasma aldosterone levels might have contributed to the antihypertensive action of bopindolol, although we could not find any change in body fluid volumes.

Duration of action. The extremely high β-adrenoceptor blocking potency of bopindolol and its long duration of action were confirmed by the isoproterenol infusion studies. Twenty-four hours after the first single oral dose of bopindolol, 2 mg, the heart rate dose-response curve was shifted to the right by a factor of almost 12. One week after withdrawal from long-term treatment, blockade of cardiac and vascular β-adrenoceptors could still be demonstrated by the isoproterenol infusions. This reduced β-adrenoceptor responsiveness could be the result of either residual β-blockade, related to bopindolol's high potency, or down regulation of β-adrenoceptors by its partial agonist activity. A decrease in β-adrenoceptor density after withdrawal of bopindolol treatment has indeed been described recently (35). However, the hemodynamic profile of bopindolol is not consistent with such a degree of partial agonist activity (see above).

It is generally agreed that abrupt discontinuation of β-blocker treatment may lead to a hyperadrenergic state and supersensitivity of β-adrenergic receptors for catecholamines (36). The gradual offset of the β-adrenoceptor blocking activity of bopindolol suggests

that this hyperadrenergic state does not occur after discontinuation of this drug. If this proves to be true, bopindolol may be especially advantageous in patients with angina pectoris, in whom discontinuation of β-blocker treatment is not without hazards (37).

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6. Hemodynamic and hormonal adaptations to β-adrenoceptor blockade. A 24-hour study of acebutolol, atenolol, pindolol, and propranolol in hypertensive patients

6.1 Summary

Comparison of the hemodynamic and hormonal effects of β-adrenoceptor antagonists with different ancillary properties may help to clarify the antihypertensive mechanism of these drugs. Under strict basal conditions, the effects of acebutolol (400 mg b.i.d.), atenolol (100 mg b.i.d.), pindolol (10 mg b.i.d.), and propranolol (80 mg t.i.d.), were studied for the first 24 hours in 40 hypertensive patients. With pindolol, mean arterial pressure was reduced ($p < 0.05$) 1 hour after administration, whereas the cardiac index and the systemic vascular resistance index did not change. With the other three drugs, the fall in mean arterial pressure was delayed 2-3 hours. With these drugs, the fall in mean arterial pressure was preceded by a rise in the resistance index, which compensated for the initial fall in cardiac index. With each drug, the decrements in mean arterial pressure were associated with parallel decrements in the resistance index, and percent changes in mean arterial pressure and the resistance index were always significantly ($p < 0.001$) correlated. At the end of the 24-hour period, the four drugs shared an equal antihypertensive effect, which varied 14-17%. This was associated with a return of the cardiac index toward control values by acebutolol, atenolol, and propranolol treatment and a moderately increased cardiac index above pretreatment values (13%, $p < 0.01$) with pindolol. The secondary rise in the cardiac index was inversely correlated ($p < 0.001$) with the fall in mean arterial pressure with all four drugs. Plasma renin was maximally suppressed 2 hours after

treatment, thus before any change in mean arterial pressure had occurred with acebutolol, atenolol, and propranolol. Pretreatment values of active renin and the reduction of mean arterial pressure 24 hours after administration were not correlated in any of the four groups.
Despite the "vasodilator" action of the four drugs, plasma norepinephrine did not rise. Our data show that the main hemodynamic change that occurs at the time blood pressure falls after β-adrenoceptor antagonism is vasodilation. Neither autoregulation of blood flow nor renin suppression can explain this vasodilator action. The absence of an increase in norepinephrine, despite vasodilation, suggests that β-adrenoceptor antagonism interferes with sympathetic vasoconstrictor nerve activity. This effect may explain the vasodilator and antihypertensive potential of β-adrenoceptor antagonists.

6.2 Introduction

More than 20 years after the original observation by Prichard (1) that β-adrenoceptor antagonists lower blood pressure in hypertension, the mechanism of this action remains a quandary. The short- and long-term effects of β-adrenoceptor antagonists on systemic hemodynamics have been well characterized (2). After oral administration of a β-adrenoceptor antagonist, heart rate and cardiac output fall in the absorption phase of the drug, but owing to compensatory mechanisms, total peripheral vascular resistance rises, and blood pressure does not change. During long-term treatment, the fall in cardiac output is maintained, so when blood pressure is reduced, it is done so through attenuation or abolishment of the initial vasoconstrictor response.
Several theories offer explanations for the effect of β-adrenoceptor antagonists on blood pressure, such as adjustment of the resistance vessels to reduced cardiac output through autoregulation of tissue perfusion, (3,4) "resetting" of the arterial baroreflex, (5,6) renin suppression, (7,8) or attenuation of α-adrenoceptor-mediated vasoconstrictor nerve activity through a central action (9,10) or through blockade of presynaptic β-adrenoceptors on postganglionic

sympathetic nerve terminals (11-13).
The introduction of β-adrenoceptor antagonists with different so-called "ancillary" pharmacological properties has challenged some of these theories. For example, cardiac output and renin are suppressed to a lesser extent by β-adrenoceptor antagonists with a relatively high degree of partial agonist activity as compared with β-adrenoceptor antagonists lacking this property, yet the antihypertensive efficacy of these drugs is the same (2,14,15). Furthermore, for a central mode of action, the drug's penetration of the blood-brain barrier is a prerequisite. This occurs only to a minimal extent, if any, with highly hydrophilic β-adrenoceptor antagonists like atenolol, yet with these drugs, blood pressure reduction is no less than with lipophilic β-adrenoceptor antagonists (2,12,16). Thus, comparison of the hemodynamic and hormonal effects of β-adrenoceptor antagonists with different ancillary properties may help to clarify their antihypertensive mechanism. In particular, the responses that occur at the time that blood pressure begins to fall may be informative.
In this study, we compared the effects of four orally administered β-adrenoceptor antagonists, each with different ancillary properties, for the first 24 hours after beginning treatment in 40 patients with uncomplicated essential hypertension. The following drugs were chosen: acebutolol, which is β_1-selective with a moderate degree of partial agonist activity; atenolol, which is β_1-selective, highly hydrophilic, and devoid of partial agonist activity; pindolol, which is nonselective with strong partial agonist activity; and propranolol, which is highly lipophilic, nonselective, and devoid of partial agonist activity. Because partial agonist activity is expressed most distinctly when sympathetic tone is low, all measurements were performed while patients were restricted to bed.

6.3 Patients and Methods

Patients
Forty male patients with mild-to-moderate essential hypertension were studied. They were recruited from the outpatient hypertension clinic if their untreated sitting diastolic blood pressure was over

95 mm Hg on three separate occasions. The mean age of the patients was 45.5 years (range, 27-64 years). Routine clinical and laboratory investigations did not reveal causes of their hypertension. A history of or clinical signs of coronary or valvular heart disease, congestive heart failure, cerebrovascular disease, or chronic obstructive lung disease were all negative. After the purpose and the procedures of the study had been explained, all patients gave their consent to participate. The study protocol was approved by the local Hospital Ethical Review Committee.

Table 1. *Clinical Characteristics of Four Groups of Patients Treated With Acebutolol, Atenolol, Pindolol, or Propranolol*

	Treatment group			
	Acebutolol (400 mg b.i.d.)	Atenolol (100 mg b.i.d.)	Pindolol (10 mg b.i.d.)	Propranolol (80 mg t.i.d.)
Patients *(n)*	10	10	10	10
Age (yr)	48 ± 3	42 ± 3	47 ± 3	42 ± 4
Body weight (kg)	86.1 ± 2.6	75.9 ± 3.0	82.8 ± 3.3	78.3 ± 4.5
Height (cm)	179 ± 2	171 ± 2	179 ± 2	172 ± 3
Body surface area (m^2)	2.09 ± 0.04	1.90 ± 0.04	2.04 ± 0.05	1.95 ± 0.07
SAP (mm Hg)	161 ± 4	163 ± 4	165 ± 3	166 ± 4
DAP (mm Hg)	106 ± 2	109 ± 3	105 ± 2	108 ± 3
MAP (mm Hg)	124 ± 3	127 ± 3	125 ± 3	126 ± 4
Heart rate (beats/min)	76 ± 4	66 ± 3	71 ± 3	67 ± 2

Data are mean ± SEM.
SAP, DAP, and MAP, systolic, diastolic, and mean arterial pressure, respectively. MAP, DAP + (SAP-DAP)/3.

Study Protocol

Antihypertensive and other types of medication, if any, were discontinued at least 3 weeks before the study. Blood pressure was measured in triplicate with a blind sphygmomanometer (London School of Hygiene Sphygmomanometer, Cinetronics, Middenhall, UK) (17). After this washout period, the patients were given placebos for 2 weeks. The placebo tablets were matched with the active medication with regard to appearance and number of tablets taken each day. After 1 week and after 2 weeks of placebo administration, all patients were hospitalized for 2 days.

During both hospitalizations, the patients stayed in a single room and were restricted to bedrest. Catheterizations to perform he-

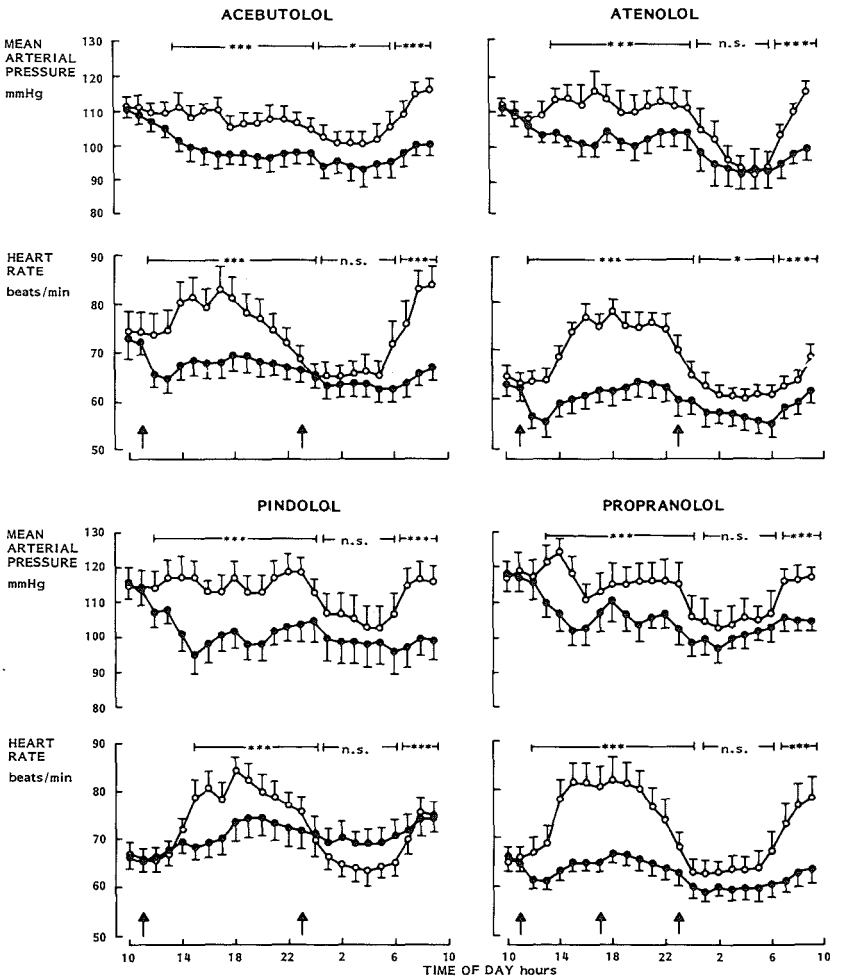

Fig. 1. Twenty-four hour trend plots of hourly mean values of mean arterial pressure and heart rate during placebo administration (○) and after beginning β-adrenoceptor blockade (●) with either acebutolol, 400 mg b.i.d., atenolol, 100 mg b.i.d., pindolol, 10 mg b.i.d., or propranolol, 80 mg t.i.d. Arrows indicate the times at which the drugs were administered.

modynamic measurements were started after an overnight bedrest and a light beakfast (one cup of tea and one sandwich), additional light meals were provided 4, 8, and 22 hours after beginning the measurements. Until the second meal, the patients were required to stay in the recumbent position; talking was limited to a minimum, and reading, eating, or drinking was forbidden. After the second meal, these restrictions became less rigid. The patients were then allowed to read, to drink a cup of tea, and to listen to the radio; they remained, however, restricted to bed. The patients were allowed visitations from 7 to 8 a.m. During the first admission, intra-arterial pressure was continuously monitored for 24 hours during placebo administration, and blood was sampled for determinations of catecholamines and renin. During the second stay in the hospital, either acebutolol, 400 mg b.i.d., atenolol, 100 mg b.i.d., pindolol, 10 mg b.i.d., or propranolol, 80 mg t.i.d., was given for the first time, and the effects of these drugs were followed for 24 hours after 2 hours of baseline readings. Intra-arterial pressure was again monitored continuously. Cardiac output and central pressures were determined at hourly intervals with the patient in a horizontal position for at least 15 minutes. Measurements of cardiac output and central pressures were discontinued from 11 p.m. to 7 a.m. because a pilot study had shown that these measurements disturbed sleep. Catecholamines and active renin were measured just before and 2, 6, 12, and 24 hours after the first dose of the β-adrenoceptor antagonist.

Hemodynamic Measurements

All catheterizations were performed after local anesthesia with 2% lidocaine. Arterial pressure was measured in the brachial artery of the nondominant arm by means of the Oxford technique (17,18). The intra-arterial cannula was connected to a miniature perfusion-transducer device (Northwick Park Hospital, London, UK), suspended in front of the chest. The pressure signal was continuously recorded on magnetic tape (Oxford Medical Recorder II, Oxford Medical Instruments, Oxford, UK). The analog signal was digitized during replay of the tape at 60 times real time with a sampling frequency of $33^1/_3$ samples/sec real time. The

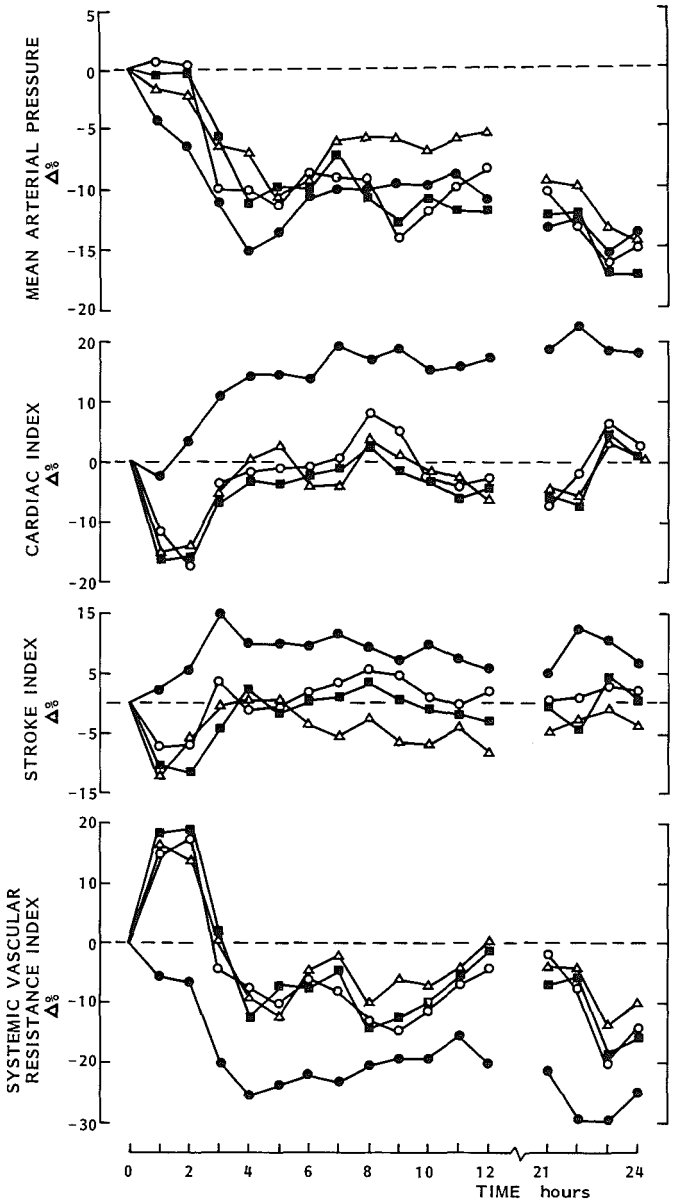

Fig. 2. Plots of time course of percent changes in systemic hemodynamics after the first oral doses of either acebutolol (■), atenolol (△), pindolol (●), or propranolol (○). First oral dose was given at time 0. For times at which subsequent doses were given, see Figure 1.

quantifiable level of this procedure is 0.3-0.5 mm Hg. Traces were analyzed beat by beat, and they were scrutinized for beat loss, clipping of the amplifier, damping, and movement artifacts by means of a computer system. These events accumulated to less than 2% of all data and were excluded from analysis. The mean values for integrated mean arterial pressure and heart rate were computed during hourly periods. The values of these variables in the 24-hour period in which active treatment was started were compared with the corresponding values during placebo.

Cardiac output and central pressures were measured by means of a 7F Swan-Ganz, flow-directed, thermodilution catheter (Edwards Laboratories, Anasco, Puerto Rico). The catheter was introduced percutaneously into an antecubital vein. Cardiac output was determined in triplicate by bolus injections of ice-cold dextrose 5%. Systemic arterial pressure and pulmonary artery pressure were monitored by means of Gould-Statham P231D transducers (Hato Rey, Puerto Rico), with zero reference at midaxillary level. Right atrial pressure and pulmonary capillary wedge pressure were measured shortly before the cardiac output determinations. Heart rate was derived from a continuously recorded electrocardiogram. Mean values of pressures were obtained by electronic integration of the analog signals. The following hemodynamic variables were derived: cardiac index (CI) = CO/body surface area (1/min/m^2), systemic vascular resistance index (SVRI) = 80 × (MAP — MRAP)/CI (dyne · sec · cm^{-5}/m^2), pulmonary vascular resistance index (PVRI) = 80 × (MPAP — MPCWP)/CI (dyne · sec · cm^{-5}/m^2), and stroke index (SI) = CI/HR (ml/m^2), where CO is cardiac output (l/min), MAP is mean arterial pressure (mm Hg), MRAP is mean right atrial pressure (mm Hg), MPAP is mean pulmonary artery pressure (mm Hg), MPCWP is mean pulmonary capillary wedge pressure (mm Hg), and HR is heart rate (beats/min).

Analytical Procedures

For determination of active plasma renin concentration, venous blood was collected in chilled tubes containing ethylenediaminetetraacetic acid in a final concentration of 2 mg/ml of blood.

Samples were centrifuged immediately at 0°C and stored at —20°C until assay. Active plasma renin concentration was measured indirectly by a radioimmunoassay of angiotensin I as described previously (19). For measurement of plasma catecholamines, 10 ml mixed venous blood was collected in chilled tubes containing 19 mg ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and 12 mg gluthathione. After centrifugation at 0°C, samples were stored at —70°C until assay. Plasma norephrinephrine and epinephrine were measured by a high-performance liquid chromatography system with electrochemical detection (20).

Statistical Analysis

Data are presented as mean values ± SEM. Because plasma values of active renin were not distributed normally, mean values were calculated after logarithmic transformation. The 24-hour blood pressure recordings were analyzed by a linear regression model with the SPSS/PC program (SPSS, Chicago, Illinois). The within-patient difference of MAP and heart rate between the corresponding hours of week 2 and week 1 was taken as the dependent variable. For independent variables, the recording of week 1 was included to correct for a regression effect, and a dummy variable that indicated the periods before and after the first dose of each β-adrenoceptor antagonist was taken to evaluate the effect of each respective drug. The effects of the four drugs were analyzed separately for the daytime and the night-time periods. For comparison of the other data, Student's *t* test for paired and unpaired data was used. Values of p less than 0.05 were considered statistically significant.

6.4 Results

Effects on Systemic Hemodynamics

Clinical characteristics of the four groups of patients at the end of the washout period are summarized in Table 1. Age, body weight, height, and blood pressures did not differ between the four groups. Although heart rate in the acebutolol group compared with the other three groups was higher, this difference was not

significant.

Table 2. *Effects of Acebutolol, Atenolol, Pindolol, and Propranolol on Systemic Hemodynamics 2 and 24 Hours After the First Oral Doses of β-Adrenoceptor Antagonists*

Drug	Baseline	2 Hours	*p*	24 Hours	*p*
Acebutolol					
MAP (mm Hg)	116 ± 3	117 ± 4	NS	96 ± 4	< 0.001
HR (beats/min)	66 ± 3	61 ± 2	< 0.01	66 ± 3	NS
CI (l/min/m²)	2.9 ± 0.1	2.3 ± 0.1	< 0.01	2.9 ± 0.7	NS
SI (ml/m²)	43 ± 2	38 ± 1	< 0.01	43 ± 1	NS
SVRI (dyne·sec·cm⁻⁵/m²)	3,285 ± 150	3,932 ± 183	< 0.01	2,619 ± 117	< 0.01
Atenolol					
MAP (mm Hg)	117 ± 2	115 ± 2	NS	101 ± 3	< 0.01
HR (beats/min)	60 ± 3	56 ± 2	< 0.01	63 ± 2	NS
CI (l/min/m²)	2.6 ± 0.1	2.2 ± 0.1	< 0.001	2.6 ± 0.1	NS
SI (ml/m²)	44 ± 2	39 ± 2	< 0.01	42 ± 2	NS
SVRI (dyne·sec·cm⁻⁵/m²)	3,583 ± 190	4,188 ± 156	< 0.01	3,107 ± 272	< 0.05
Pindolol					
MAP (mm Hg)	118 ± 3	111 ± 3	< 0.05	102 ± 3	< 0.001
HR (beats/min)	63 ± 2	64 ± 2	NS	73 ± 4	< 0.05
CI (l/min/m²)	2.8 ± 0.1	2.9 ± 0.2	NS	3.3 ± 0.2	< 0.01
SI (ml/m²)	44 ± 1	46 ± 2	NS	46 ± 1	NS
SVRI (dyne·sec·cm⁻⁵/m²)	3,427 ± 162	3,100 ± 158	NS	2,543 ± 142	< 0.001
Propranolol					
MAP (mm Hg)	120 ± 3	120 ± 4	NS	102 ± 4	< 0.01
HR (beats/min)	63 ± 2	57 ± 2	< 0.01	63 ± 3	NS
CI (l/min/m²)	2.6 ± 0.1	2.2 ± 0.1	< 0.001	2.7 ± 0.1	NS
SI (ml/m²)	42 ± 2	39 ± 2	< 0.05	42 ± 2	NS
SVRI (dyne·sec·cm⁻⁵/m²)	3,626 ± 152	4,131 ± 262	< 0.01	3,101 ± 223	< 0.01

Data are mean ± SEM.

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SI, stroke index; SVRI, systemic vascular resistance index.

Twenty-four hour trend plots of the hourly means of MAP and heart rate during placebo and after administration of acebutolol, atenolol, pindolol, or propranolol are shown in Figure 1. Despite the fact that all patients were fully restricted to bed, a circadian rhythm of blood pressure and heart rate was maintained in all four groups. During placebo administration, heart rate increased after 2 a.m. by approximately 10 beats/min in all four groups. This increment in heart rate coincided with the second meal and with the somewhat less rigid circumstances of the protocol, although the patients remained in the recumbent position. Values of MAP

and heart rate shortly before administration of the β-adrenoceptor antagonists did not differ from the corresponding values during placebo administration. After administration of acebutolol, atenolol, and propranolol, heart rate decreased within 1 hour compared with both placebo and baseline values, whereas no decrease in heart rate as compared with baseline occurred with pindolol.

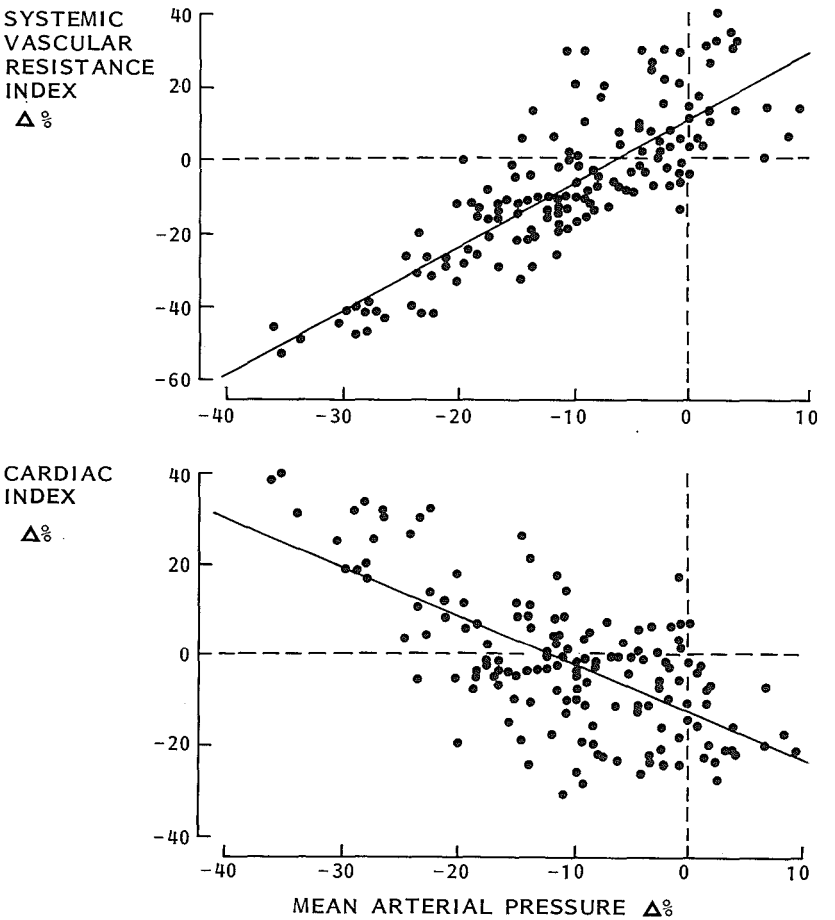

Fig. 3. Plots of relations between percent changes in mean arterial pressure and percent changes in either systemic vascular resistance index or cardiac index during onset of the antihypertensive effect of propranolol. All corresponding hourly changes of the three variables of the 10 patients are shown.

During the day, compared with placebo administration, heart rate was reduced by all four drugs. During the night, heart rate was reduced significantly only during acebutolol treatment, whereas it was increased, although not significantly, by pindolol. MAP was reduced ($p < 0.05$) by 1 hour after pindolol administration. In contrast, the fall in MAP was delayed 2-3 hours during treatment with the other three drugs.

Baseline values of MAP, heart rate, cardiac index, stroke index, and the systemic vascular resistance index in the four groups did not differ (Table 2).

Administration of acebutolol, atenolol, and propranolol caused a rapid fall in the cardiac and stroke indexes (Figure 2). Two hours after drug treatment, the cardiac and stroke indexes were reduced, respectively, by 16 ± 3% ($p < 0.01$) and 10 ± 2% ($p < 0.01$) during acebutolol, 14 ± 3% ($p < 0.001$) and 7 ± 2% ($p < 0.01$) during atenolol, and 17 ± 3% ($p < 0.001$) and 7 ± 3% ($p < 0.05$) during propranolol treatment, whereas the cardiac and stroke indexes did not change after pindolol. Because MAP did not change initially, the decrements in cardiac index during acebutolol, atenolol, and propranolol treatment were associated with a proportional rise in the resistance index of 18 ± 4% ($p < 0.01$), 14 ± 4% ($p < 0.001$), and 18 ± 4% ($p < 0.01$), respectively. The rapid fall in MAP on pindolol was entirely due to a decrease in the resistance index. The more gradual decrease of MAP on acebutolol, atenolol, and propranolol was associated with a parallel decrease of the resistance index toward, and later even below, baseline values. During the gradual development of their full antihypertensive action, the cardiac and stroke indexes returned to control values, whereas these variables tended to increase above baseline on pindolol (Figure 2). At the end of the 24-hour period, MAP was decreased to the same degree by the four drugs (Table 2 and Figure 2). At this time, MAP and the resistance index were reduced, respectively, by 18 ± 3% ($p < 0.001$) and 16 ± 6% ($p < 0.01$) with acebutolol, 14 ± 2% ($p < 0.01$) and 11 ± 4% ($p < 0.05$) with atenolol, 15 ± 2% ($p < 0.001$) and 25 ± 4% ($p < 0.001$) with pindolol, and 15 ± 4% ($p < 0.01$) and 15 ± 5% ($p < 0.01$) with propranolol.

At the end of the 24-hour period, the cardiac index was moderately increased during pindolol treatment ($13 \pm 6\%$, $p < 0.01$), whereas the cardiac index was not different from control values on the other three drugs (Table 2).

Hemodynamic Interrelations

With all four drugs, there were strong positive correlations between the percent changes of MAP and the resistance index, whereas the percent changes between MAP and the cardiac index were inversely correlated (Figure 3 and Table 3). The regression lines as defined by the equations listed in Table 3 are depicted in Figure 4. The slopes of the lines (MAP vs. the cardiac index or MAP vs. the resistance index) did not differ between the four groups. The rise in the cardiac index (toward baseline on acebutolol, atenolol, and propranolol and above baseline on pindolol) that accompanied the "vasodilator" action of the four drugs was due to an increase in both heart rate and the stroke index. Significant inverse correlations between the percent changes of the resistance index and the percent changes in either heart rate or stroke index were present with all four drugs (Figure 5 and Table 4).

Table 3. *Regression Equations and Correlation Coefficients of the Relations Between the Percent Changes in Mean Arterial Pressure and the Percent Changes in Either Systemic Vascular Resistance Index or Cardiac Index*

Drug	Systemic vascular resistance index	Cardiac index
Acebutolol	$\Delta\%SVRI = 1.2 \times \Delta\%MAP + 6.2$ $r = 0.66$	$\Delta\%CI = -0.5 \times \Delta\%MAP - 9.8$ $r = -0.33$
Atenolol	$\Delta\%SVRI = 1.3 \times \Delta\%MAP + 7.0$ $r = 0.56$	$\Delta\%CI = -0.5 \times \Delta\%MAP - 8.2$ $r = -0.31$
Pindolol	$\Delta\%SVRI = 1.5 \times \Delta\%MAP - 4.7$ $r = 0.64$	$\Delta\%CI = -1.4 \times \Delta\%MAP + 1.1$ $r = -0.42$
Propranolol	$\Delta\%SVRI = 1.8 \times \Delta\%MAP + 10.7$ $r = 0.82$	$\Delta\%CI = -1.1 \times \Delta\%MAP - 13.9$ $r = -0.67$

SVRI, systemic vascular resistance index; MAP, mean arterial pressure; CI, cardiac index.
$p < 0.001$ in all cases.

Effects on Cardiac Filling Pressures, Pulmonary Arterial Pressure, and Pulmonary Vascular Resistance

Baseline values of mean right atrial pressure, mean pulmonary capillary wedge pressure, mean pulmonary artery pressure, and pulmonary vascular resistance index between the four groups did

not differ (Figure 6 and Table 5). Cardiac filling pressures and mean pulmonary artery pressure increased during acebutolol, atenolol, and propranolol treatment. The increase in the resistance index on these drugs was no longer significant at the end of the 24-hour period. Central pressures did not rise during pindolol treatment, and the resistance index during treatment with this drug tended to decrease.

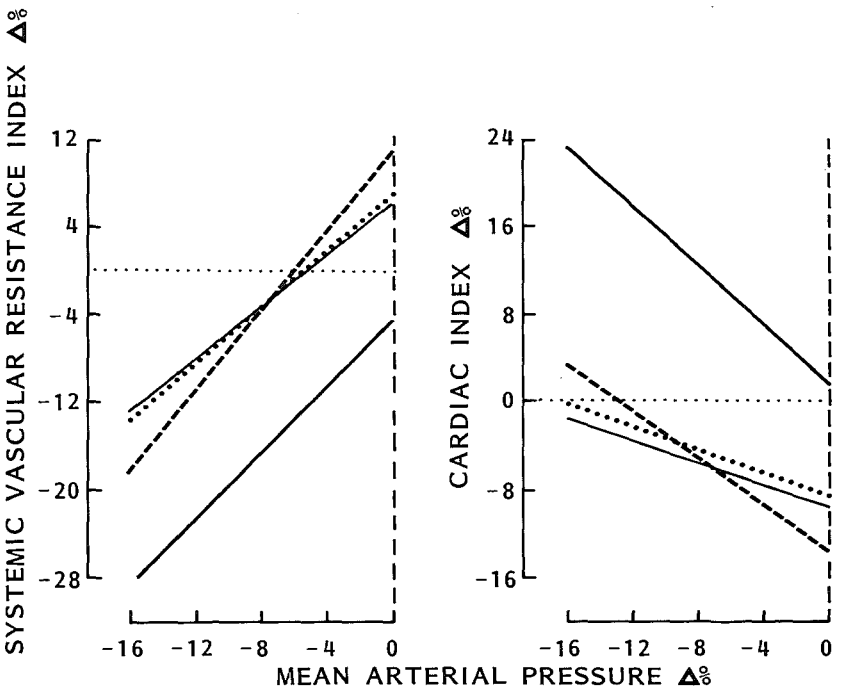

Fig. 4. Plots of relations between percent fall in mean arterial pressure and percent change in either systemic vascular resistance index or cardiac index during onset of the antihypertensive action of acebutolol (———), atenolol (· · · · ·), pindolol (_____), and propranolol (- - - -). Regression equation lines are summarized in Table 3.

Effects on Plasma Catecholamines and Active Renin

Plasma norepinephrine was increased 2 hours after administration of acebutolol and propranolol, a phenomenon that coincided with the initial vasoconstrictor response to these drugs (Table 6).

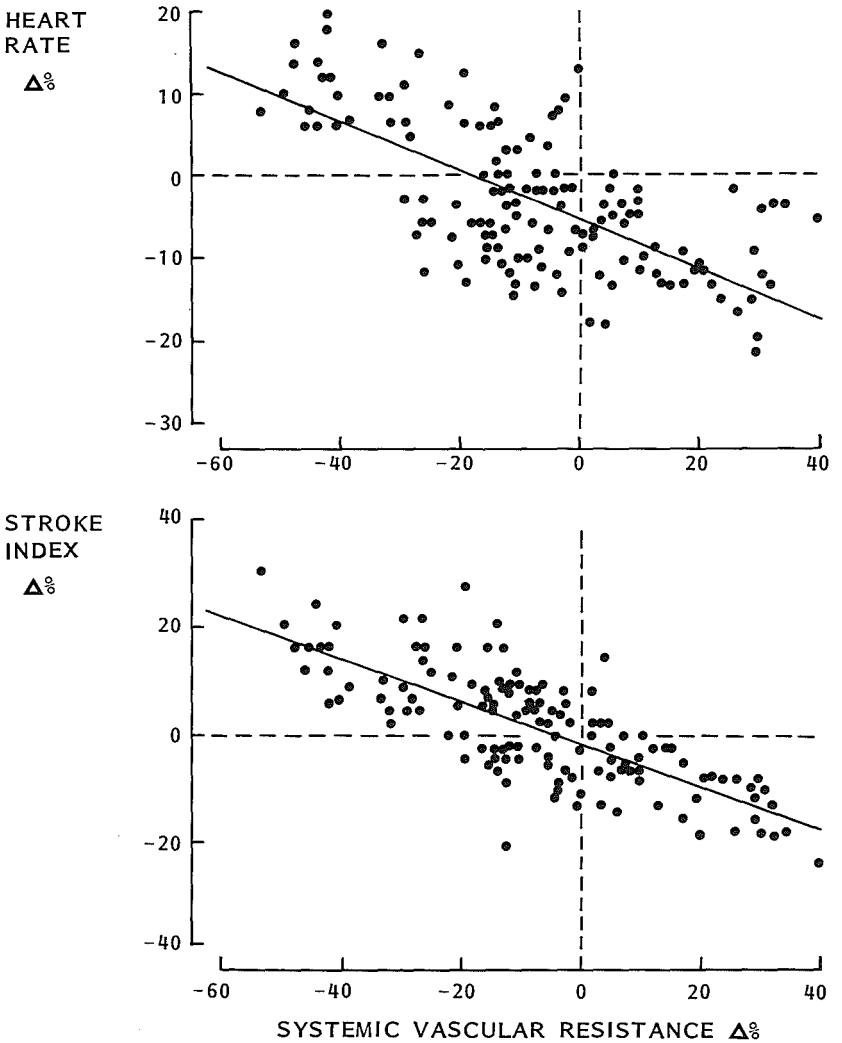

Fig. 5. Plots of relations between percent changes in systemic vascular resistance index and percent changes in heart rate or stroke index during onset of the antihypertensive effect of propranolol. All corresponding hourly changes of the three variables of the 10 patients are shown.

Plasma norepinephrine was no longer increased 6, 12, and 24 hours after administration of these drugs. Plasma norepinephrine

did not rise during atenolol and pindolol treatment. Baseline values of plasma norepinephrine and the fall in blood pressure 24 hours after treatment were not correlated in either of the four groups. Plasma epinephrine did not change during treatment with the four drugs. Active plasma renin concentration decreased markedly in all groups. A near maximal decrease in active renin was already present 2 hours after treatment, at which time active renin was decreased by 56 ± 4% ($p < 0.01$) during acebutolol, 52 ± 7% ($p < 0.01$) during atenolol, 39 ± 9% ($p < 0.01$) during pindolol, and 56 ± 3% ($p < 0.001$) during propranolol treatment.
On all drugs, except pindolol, a significant decrease in active renin was maintained during the 24-hour period (Table 6). Baseline values of active renin and the fall in blood pressure 24 hours after treatment were not correlated in any of the four groups.

6.5 Discussion

Hemodynamic Effects
The hemodynamic responses to the four β-adrenoceptor blocking agents in the first hours after the initial oral doses largely agree with previously reported hemodynamic responses to these drugs when administered intravenously (2,21,22). A rapid fall in cardiac output, heart rate, and stroke volume was observed after administration of acebutolol, atenolol, and propranolol, whereas no initial changes in these variables occurred with pindolol. Earlier studies with intravenous doses of acebutolol have shown that the magnitude of the fall in cardiac output of this drug is intermediate between that of pindolol and propranolol (2,21,22). In the present study, the cardiodepressant effect of acebutolol was approximately the same as that of atenolol and propranolol. The somewhat higher baseline values of heart rate and cardiac output in the acebutolol group compared with the other three groups may partly explain the present findings.
The fall in cardiac output after administration of acebutolol, atenolol, and propranolol was associated with a rise in systemic vascular resistance. This vasoconstrictor response was proportional to the fall in cardiac output, both with the nonselective β-adrenoceptor

antagonist propranolol as with β_1-selective antagonists acebutolol and atenolol. This confirms earlier suggestions that this vasoconstriction does not reflect blockade of vasodilator vascular β_2-adrenoceptors (unopposed α-adrenergic stimulation) but represents a reflex circulatory adjustment (23,24). This is further substantiated by the absence of a vasoconstrictor response to pindolol.

Table 4. *Regression Equations and Correlation Coefficients of the Relations Between Percent Changes in Systemic Vascular Resistance Index and Percent Changes in Either Heart Rate or Stroke Index*

Drug	Heart rate		Stroke index	
Acebutolol	$\Delta\%HR = -0.26 \times \Delta\%SVRI - 3.3$	$r = -0.40$	$\Delta\%SI = -0.46 \times \Delta\%SVRI - 4.8$	$r = -0.65$
Atenolol	$\Delta\%HR = -0.31 \times \Delta\%SVRI - 5.1$	$r = -0.48$	$\Delta\%SI = -0.42 \times \Delta\%SVRI - 4.9$	$r = -0.61$
Pindolol	$\Delta\%HR = -0.53 \times \Delta\%SVRI - 1.3$	$r = -0.59$	$\Delta\%SI = -0.44 \times \Delta\%SVRI - 1.2$	$r = -0.61$
Propranolol	$\Delta\%HR = -0.32 \times \Delta\%SVRI - 5.3$	$r = -0.68$	$\Delta\%SI = -0.41 \times \Delta\%SVRI - 1.9$	$r = -0.78$

HR, heart rate; SVRI, systemic vascular resistance index; SI, stroke index.
$p < 0.001$ in all cases.

A latency of several hours after starting treatment and the fall in blood pressure is a well-know, yet unexplained, feature of β-adrenoceptor antagonists. A similar latency was also observed in this study with acebutolol, atenolol, and propranolol.

With pindolol, however, blood pressure was significantly reduced by 1 hour after administration, which confirms an early study by Anavekar et al (25). Absence of cardiodepression and reflex vasoconstriction is a major contrast between pindolol and the other three drugs. It could be argued therefore, that the initial vasoconstrictor response per se accounts for the delayed onset of the blood pressure-lowering effect of β-adrenoceptor antagonists devoid of a significant degree of partial agonist activity. Experiments in the spontaneously hypertensive rat support this view (26).

With all four drugs, the onset of the blood pressure-lowering effect coincided with a gradual decline in systemic vascular resistance. The identical slopes of the regression lines depicting the relation between the fall in blood pressure and the fall in vascular resistance in the four groups (Figure 4) show that the mechanism behind this relation is independent of ancillary properties

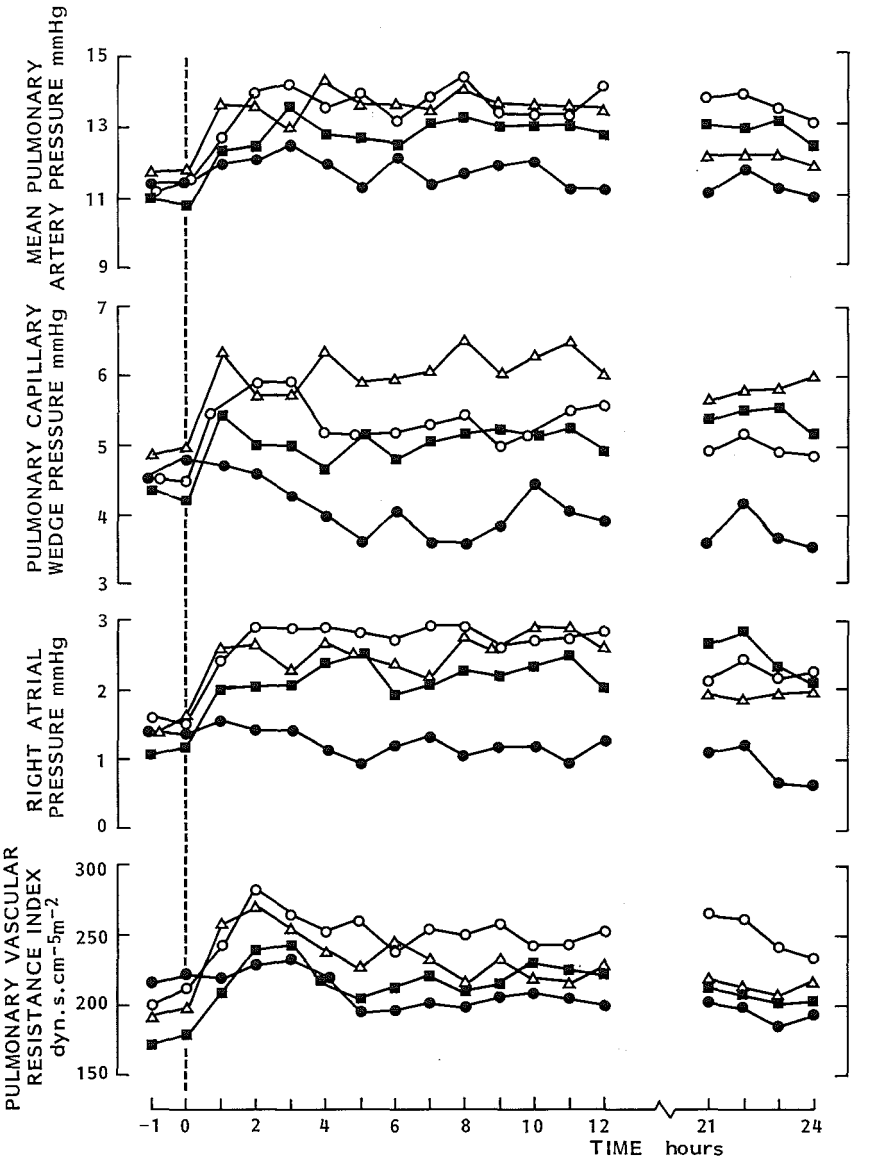

Fig. 6. Plots of time course of changes in mean pulmonary artery pressure, cardiac filling pressures, and pulmonary vascular resistance index after the first oral dose of either acebutolol (■), atenolol (△), pindolol (●), or propranolol (○). First oral dose was given at time 0. For times at which subsequent doses were given, see Figure 1.

such as β_1-selectivity or partial agonist activity.

The long-term antihypertensive response to β-adrenoceptor antagonists lacking partial agonist activity has been reported to be invariably associated with a reduction in cardiac output (2). The current data are at variance with these reports. The onset of blood pressure reduction after administration of acebutolol, atenolol, and propranolol was associated with a return of the initially reduced cardiac output, stroke volume, and heart rate to baseline values. Moreover, with pindolol treatment, cardiac output, stroke volume, and heart rate even rose above baseline. With all four drugs, the magnitude of these changes in cardiac output was inversely correlated with the magnitude of the fall in blood pressure, suggesting that this phenomenon reflects an adjustment to the "vasodilator" action of these drugs. Restoration of an initially depressed cardiac function at the onset of their hypotensive action has also been noted in other studies with atenolol and the nonselective β-adrenoceptor antagonist timolol (3,4). Because this increment in cardiac output occurs despite blockade of β-adrenoceptors, withdrawal of vagal tone, an increase in venous return to the heart, and reduction of afterload rather than sympathetic stimulation are the factors most likely to be involved in this hemodynamic adaptation. This contention is supported by a previous study that showed that withdrawal of vagal tone contributes considerably to the increments in heart rate and cardiac output during vasodilatation after β-adrenoceptor blockade (27). The inverse correlations between changes in systemic vascular resistance and either changes in heart rate or changes in stroke volume in the present study support this view.

The difference in hemodynamic profile that is associated with the initial and more long-term blood pressure-lowering effect of β-adrenoceptor antagonists could be explained by the strict basal conditions in the relatively long-lasting hemodynamic observation periods in the short-term protocols of this study and previous studies (2-4) as opposed to the short-lasting observation periods (1 hour) in the long-term, previous studies (2,12,28). β-Adrenoceptor antagonists possess the unique feature, as compared

Table 5. *Effects of Acebutolol, Atenolol, Pindolol, and Propranolol on Mean Right Atrial Pressure, Mean Pulmonary Capillary Wedge Pressure, Mean Pulmonary Artery Pressure, and Pulmonary Vascular Resistance Index 2 and 24 Hours After the First Oral Dose of the β-Adrenoceptor Antagonists*

Drug	Baseline	2 Hours	*p*	24 Hours	*p*
Acebutolol					
MRAP (mm Hg)	1.2 ± 0.4	2.1 ± 0.5	NS	2.1 ± 0.4	NS
MPCWP (mm Hg)	4.2 ± 0.3	5.0 ± 0.4	NS	5.0 ± 0.3	< 0.05
MPAP (mm Hg)	10.7 ± 0.4	12.4 ± 0.4	< 0.01	12.0 ± 0.4	< 0.05
PVRI (dyne·sec·cm^{-5}/m^2)	185 ± 14	251 ± 15	< 0.001	210 ± 14	NS
Atenolol					
MRAP (mm Hg)	1.6 ± 0.4	2.7 ± 0.5	< 0.01	2.1 ± 0.4	NS
MPCWP (mm Hg)	5.1 ± 0.3	5.8 ± 0.2	< 0.05	6.0 ± 0.5	<0.05
MPAP (mm Hg)	11.7 ± 0.5	13.6 ± 0.6	< 0.01	13.1 ± 0.7	NS
PVRI (dyne·sec·cm^{-5}/m^2)	208 ± 16	284 ± 21	< 0.001	229 ± 24	NS
Pindolol					
MRAP (mm Hg)	1.4 ± 0.6	1.4 ± 0.6	NS	0.7 ± 0.4	NS
MPCWP (mm Hg)	4.8 ± 0.6	4.1 ± 0.7	NS	3.2 ± 0.4	NS
MPAP (mm Hg)	11.5 ± 0.7	12.0 ± 0.7	NS	10.7 ± 0.5	NS
PVRI (dyne·sec·cm^{-5}/m^2)	227 ± 22	239 ± 16	NS	202 ± 17	NS
Propranolol					
MRAP (mm Hg)	1.5 ± 0.3	2.9 ± 0.5	< 0.01	2.2 ± 0.4	< 0.05
MPCWP (mm Hg)	4.5 ± 0.3	5.9 ± 0.4	< 0.05	4.7 ± 0.3	NS
MPAP (mm Hg)	11.7 ± 0.5	13.9 ± 0.9	< 0.01	12.6 ± 0.7	< 0.05
PVRI (dyne·sec·cm^{-5}/m^2)	223 ± 22	294 ± 35	< 0.01	241 ± 23	NS

Data are mean ± SEM.
MRAP, mean right atrial pressure; MPCWP, mean pulmonary capillary wedge pressure; MPAP, mean pulmonary artery pressure; PVRI, pulmonary vascular resistance index.

with other antihypertensive agents, to counteract cardiac sympathetic drive, which triggers a baroreflex-mediated vasoconstrictor response. As a consequence, also in contrast to other antihypertensive agents, the cardiodepressant effect of β-adrenoceptor antagonists depends on cardiac sympathetic drive through which the hemodynamic profile of the drugs is modified (28). Thus, by minimizing cardiac sympathetic drive, the vasodilator effect of β-adrenoceptor antagonists can be unmasked. Moreover, during heavy physical exercise, the sympathetic drive to the heart is very high and para-sympathetic tone is minimal. Blockade of cardiac β-adrenoceptors in this situation, located either presynaptically or postsynaptically, irrespective of whether the drug possesses partial agonist activity, will always result in a lower cardiac output as

compared with placebo. Initially, the systemic vascular resistance may be increased as compared with pretreatment values, but it should be noted that during exercise in the presence of β-adrenoceptor blockade the fall in vascular resistance with increasing levels of oxygen consumption is more pronounced than in the absence of β-adrenoceptor blockade (28). Thus, despite a permanently depressed cardiac output during physical exercise, a vasodilator response is still responsible for the maintenance of the antihypertensive action of β-adrenoceptor blockers.
Several studies, mostly in patients with coronary heart disease, have noted an increase in cardiac filling pressures and pulmonary artery pressure after administration of β-adrenoceptor antagonists (29). These increments in central pressures seem to be related to the negative inotropic effects of these drugs because they are observed on β-adrenoceptor antagonists devoid of partial agonist activity, both β_1-selective and nonselective, but not on β-adrenoceptor antagonists with a relatively high degree of partial agonist activity (29,30). In line with these observations are our present data; central pressures increased both with atenolol and propranolol but not with pindolol. The observation that these pressures also rose with acebutolol can be interpreted as evidence of the relatively weak partial agonist activity of this drug.

Mechanism of Vasodilator Effect and Antihypertensive Action
Whole-body autoregulation, that is, a decrease in systemic vascular resistance as an adaptation to the fall in cadiac output has been cited as the hypotensive mechanism of β-adrenoceptor antagonists (3,4,31,32). This mechanism has been advocated recently by Colfer et al[3] in a detailed study of the hemodynamic effects of timolol. According to these researchers, the observation that the magnitude of the initial reduction in blood pressure (7-8 hours after the first dose) was significantly correlated with the immediate reduction in cardiac output (1 hour after treatment) was advanced as major evidence supporting the theory of whole-body autoregulation. In the present study, such a correlation could not be established for acebutolol, atenolol, or propranolol. Moreover, the finding that the hypotensive action of pindolol was not preceded by a

fall, but was associated in fact with a moderate increase in cardiac output, is also a major challenge to this theory. Whether local autoregulation of blood flow plays a role in the maintenance of the antihypertensive action of β-adrenoceptor blockers during heavy physical exercise cannot be concluded from the present study.

Table 6. *Effects of Acebutolol, Atenolol, Propranolol, and Pindolol on Plasma Catecholamines and Active Plasma Renin Concentration After the First Oral Dose*

	Time (hr)				
Drug	0	2	6	12	24
Norepinephrine (pg/ml)					
Acebutolol	202 ± 22	249 ± 34*	207 ± 31	212 ± 39	226 ± 40
Atenolol	238 ± 16	233 ± 15	248 ± 22	236 ± 15	310 ± 25
Pindolol	238 ± 38	225 ± 26	272 ± 43	253 ± 36	266 ± 32
Propranolol	247 ± 21	306 ± 44*	273 ± 23	279 ± 28	297 ± 31
Epinephrine (pg/ml)					
Acebutolol	51 ± 7	46 ± 6	61 ± 8	30 ± 4	53 ± 10
Atenolol	39 ± 6	41 ± 9	30 ± 5	26 ± 4	46 ± 9
Pindolol	79 ± 16	77 ± 16	73 ± 11	55 ± 7	76 ± 7
Propranolol	79 ± 12	94 ± 19	93 ± 3	74 ± 15	85 ± 10
Active renin (μU/ml)					
Acebutolol	9.7 ± 2.7	4.2 ± 1.2†	3.3 ± 0.8	2.4 ± 0.9	4.1 ± 1.4*
Atenolol	10.1 ± 2.1	4.0 ± 0.9†	2.4 ± 0.7	1.6 ± 0.5	2.3 ± 0.8*
Pindolol	9.8 ± 1.6	4.9 ± 0.9†	3.0 ± 1.0	3.0 ± 0.8	6.5 ± 1.8
Propranolol	14.5 ± 3.6	6.5 ± 1.6†	4.8 ± 1.4	3.3 ± 1.0	3.5 ± 0.9*

Data are mean ± SEM.
Values at time 0 before treatment are averages of two measurements.
*$p < 0.05$, †$p < 0.01$.

A decrease in plasma renin through blockade of juxtaglomerular β-adrenoceptors is another often proposed mechanism to explain the antihypertensive action of β-adrenoceptor blocking agents (7,8). Some evidence suggests that this mechanism may indeed contribute to the fall in blood pressure in the relatively rare forms of hypertension associated with a high plasma renin activity (7,8,33). Whether it also plays a role in the majority of hypertensive patients with normal or low plasma renin activity remains controversial (8,14,15,33). Although plasma renin was significantly reduced with all four drugs in the present study, several findings suggest

that this renin-suppressing effect was unimportant for their antihypertensive action. First, a near-maximal reduction in active renin was achieved by 2 hours after treatment, thus before any fall in blood pressure had occurred during acebutolol, atenolol, or propranolol treatment. Second, notwithstanding a similar antihypertensive effect, suppression of active renin was less marked with pindolol than with the other three agents. Moreover, 24 hours after treatment, suppression of renin during treatment with this drug was no longer significant, whereas its full antihypertensive effect was still maintained. Third, baseline values of active renin were not related to the fall in blood pressure or to the fall in vascular resistance in any of the four groups.

Several investigators, including us, have proposed that a decrease in sympathetic vasoconstrictor nerve activity may be involved in the antihypertensive mechanism of β-adrenoceptor antogonists (10-13,34-37). Ostensibly, in contradiction with this view is the knowledge that the antihypertensive effect of most β-adrenoceptor blocking agents is associated with unchanged or even an increased plasma norepinephrine. One has to take into account, however, that the clearance of norepinephrine from plasma by the lungs and liver is impaired during β-adrenoceptor blockade (38-40). Thus, unchanged or even increased plasma norepinephrine might still be compatible with a reduced sympathetic tone.

In the present study, the vasodilator action of the four drugs was not associated with a decrease in plasma norepinephrine; one should emphasize, however, that the vasodilator action was not associated with an increase in plasma norepinephrine either. This contrasts with the effects of direct-acting vasodilators like hydralizine, minoxidil, and sodium nitroprusside. Vasodilatation during administration of these drugs is associated with a marked increase in plasma norepinephrine, reflecting baroreflex-mediated activation of the sympathetic nervous system (41-43). In this regard, the absence of a rise in plasma norepinephrine during the vasodilator action of β-adrenoceptor antagonists can be considered inappropriate and could therefore suggest interference of these drugs with the sympathetic nervous system.

Conclusions

The data from this study clearly show that the most important hemodynamic change during the onset of the hypotensive action of β-adrenoceptor antagonists is vasodilatation. Our data do not support an important role for autoregulation of blood flow or renin suppression in the vasodilator action of β-adrenoceptor antagonists. Indirect evidence suggests that β-adrenoceptor antagonists, have the ability to interfere with the activity of the sympathetic nervous system. Reduced vasoconstrictor nerve activity through blockade of central β-adrenoceptors or through blockade of presynaptic β-adrenoceptors could be an explanation for our findings. The lack of a substantial difference between the hemodynamic responses to highly lipophilic propranolol and highly hydrophilic atenolol seems to favor the latter possibility.

During moderately elevated levels of sympathetic tone, the apparent vasodilator effect of β-adrenoceptor antagonists is masked. In this situation, the sympathetic tone to the heart is high, and β-adrenoceptor antagonism will result in a relatively greater reduction in cardiac output than in blood pressure. On the basis of the data obtained under strict basal conditions, we also believe that vasodilatation, that is, a fall in vascular resistance from a higher to a lower level, underlies the anti-hypertensive effect of β-adrenoceptor antagonists.

This view is supported by the fact that the fall in vascular resistance with the increasing levels of physical exercise is enhanced during β-adrenoceptor blockade.

Thus, β-adrenoceptor antagonists may or may not increase systemic vascular resistance in hypertension, depending on a number of factors: 1) the duration of β-adrenoceptor blockade, 2) the magnitude of the antihypertensive response, 3) their degree of partial agonist activity, and 4) the level of (cardiac) sympathetic drive. Under all circumstances, however, it is the ability of these drugs to interfere with sympathetic vasoconstrictor nerve activity that is likely to be responsible for their blood pressure-lowering efficacy.

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7. Hemodynamic and β-adrenergic receptor adaptations during long-term β-adrenoceptor blockade. Studies with acebutolol, atenolol, pindolol and propranolol in hypertensive patients.

7.1 Summary

In an attempt to further clarify the mechanism of the maintenance of the antihypertensive effect of β-adrenoceptor antagonists, the effects of four antagonists with different ancillary properties (acebutolol, atenolol, pindolol, and propranolol) on systemic and renal hemodynamics, body fluid volumes, hormones and lymphocyte β-adrenoceptor density were studied in four groups of 10 hypertensive patients. The patients were followed for three weeks during active treatment and for two weeks after withdrawal of treatment. At the end of the three week treatment period the four drugs shared an equal antihypertensive effect (fall in mean arterial pressure 10-13%). Although renin was suppressed (60 to 70%) on all four drugs, changes in renin or pretreatment values of renin were not correlated with the fall in blood pressure. The drugs had no effects on plasma catecholamines or body fluid volumes. Notwithstanding a similar antihypertensive effect the changes in flow and resistance underlying the fall in blood pressure differed considerably. With pindolol the fall in blood pressure was associated with a fall in vascular resistance (26 ± 6%), whereas with propranolol it was predominantly associated with a fall in cardiac output (11 ± 7%). No significant changes in vascular resistance or cardiac output occurred with atenolol or acebutolol. The changes in renal blood flow and renal vascular resistance ran in parallel with the changes in cardiac output and systemic

vascular resistance. Plasma epinephrine concentration and pretreatment cardiac chronotropic responsiveness to isoproterenol appeared to be inversely correlated with lymphocyte β-adrenoceptor density (B_{max}) ($r = -0.41$ and -0.43, respectively). With pindolol B_{max} decreased by maximally 39 $\pm$ 6%, with propranolol it increased by 51 $\pm$ 17%. On both drugs significant changes in B_{max}were already present 24 hours after treatment. Furthermore, one week after withdrawal of treatment with pindolol, B_{max} was still 'down-regulated' and cardiac chronotropic responsiveness was still decreased, whereas one week after withdrawal of propranolol B_{max} was still 'up-regulated' and cardiac chronotropic responsiveness was still increased. No changes in B_{max} occurred with the β_1-selective antagonists acebutolol and atenolol. Thus, despite an equal antihypertensive effect, the four β-adrenoceptor antagonists appear to have dissimilar effects on cardiac output, renal blood flow and lymphocyte β-adrenoceptors. Changes in cardiac output, the circulating blood volume or angiotensin-mediated vasoconstriction are factors unlikely to be crucial for the antihypertensive effect of β-adrenoceptor antagonists. Diminished α-adrenoceptor mediated vasoconstriction through either blockade of central β-adrenoceptors or peripheral prejunctional β-adrenoceptors remains an attractive alternative to explain their blood pressure lowering potential.

7.2 Introduction

We recently reported the acute hemodynamic effects of four β-adrenoceptor antagonists studied for 24 hours after they had been administered orally for the first time to four groups of 10 subjects with essential hypertension (1). The antagonists were: acebutolol, which is β_1-selective with a moderate degree of partial agonist activity (PAA), atenolol, which is β_1-selective, highly hydrophilic, and devoid of PAA, pindolol, which is non-selective with strong PAA, and propranolol, which is non-selective, highly lipophilic, and devoid of PAA. The experimental conditions in that study were rigidly standardized by restricting the patients to bed for 36 hours. The results led us to conclude that despite the different

ancillary properties the four drugs exerted a similar antihypertensive effect, which was mediated by a vasodilator mechanism. Autoregulatory adjustment of the vasculature to changes in tissue perfusion or suppression of renin were found to be unlikely candidates to explain this vasodilator mechanism.

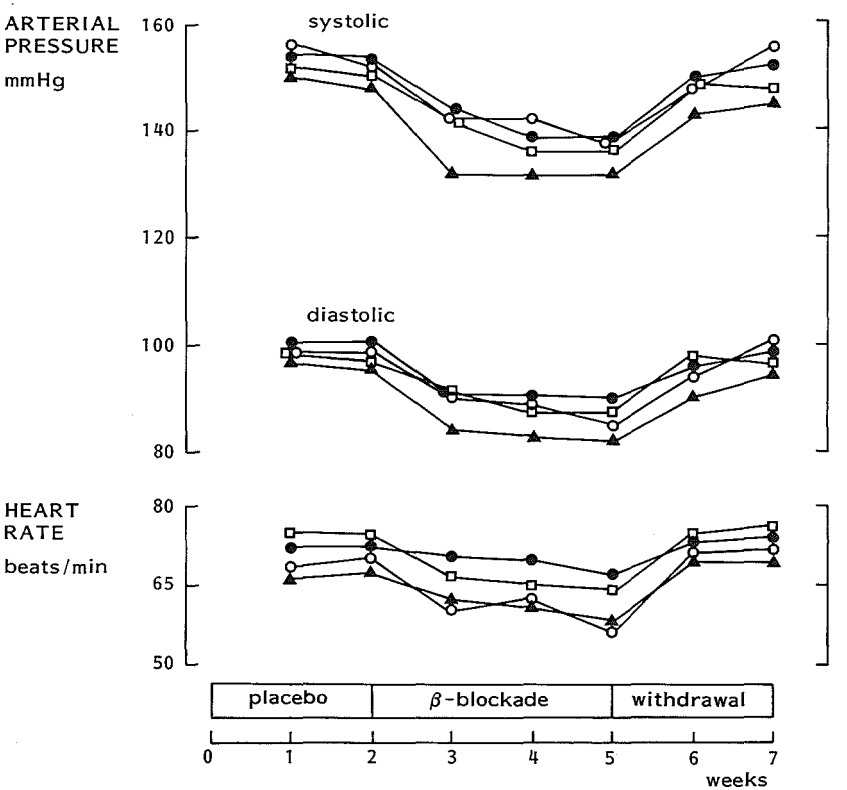

Fig. 1. Changes in blood pressure and heart rate before, during and after propranolol (○), atenolol (▲), acebutolol (□), and pindolol (●).

The present study is an extension of our initial report. The subjects were followed for three weeks during active treatment and for two weeks after withdrawal of treatment. In this period repeated measurements of cardiac output, renal perfusion, plasma catecholamines, renin, aldosterone, body fluid volumes, lymphocyte

β-adrenoceptors and cardiac chronotropic responsiveness to isoproterenol were performed. In this way, by searching for similarities, dissimilarities and eventually common denominators of the antihypertensive effect of the four different β-adrenoceptor antagonists, we hoped to gain additional information on the mechanism of the maintenance of the antihypertensive effect during prolonged treatment.

7.3 Patients and methods

Forty male patients (age 27-64 years, mean age 46 years) with mild-to-moderate essential hypertension were studied. They were recruited from the outpatient hypertension clinic if their untreated sitting diastolic blood pressure was over 95 mm Hg (phase V of Korotkoff sounds) at three separate occasions. Routine clinical and laboratory evaluation did not reveal causes of their hypertension. A history or clinical signs of coronary or valvular heart disease, congestive heart failure, cerebrovascular disease, or chronic obstructive lung disease were all negative. After the purpose and the procedures of the study had been explained, all patients gave their consent to participate. The protocol was approved by the local Hospital Ethical Review Committee.

Study Protocol

The study was single-blind placebo controlled, and lasted for 7 weeks. Antihypertensive and other types of medication, if any, were discontinued at least three weeks before the study. A moderate dietary salt restriction was advised (approximately 8 gr NaCl per day). After the washout period placebo was given for 2 weeks. The placebo tablets were matched with the active medication with regard to the appearance and the number of tablets taken each day. After the placebo period the patients were treated with one of the four β-adrenoceptor antagonists for 3 weeks. The first week of the active treatment propranolol was given 40 mg three times daily, atenolol 50 mg once daily, acebutolol 200 mg twice daily, and pindolol 5 mg twice daily. Depending on the blood pressure response the dose of the β-adrenoceptor antagonists was

doubled during each of the two subsequent weeks. The aim was to obtain blood pressures below 140/90 mm Hg. After 3 weeks active treatment the patient was put on placebo again for 2 weeks. During the entire study patients were seen at weekly intervals in the outpatient clinic. At each visit supine blood pressure and heart rate were measured for one hour. Cardiac output, glomerular filtration rate, effective renal plasma flow and the plasma and extracellular volumes were measured at the end of the initial placebo period and after 3 weeks active treatment. Blood was collected with the patients in the supine position for determination of plasma levels of catecholamines, renin and aldosterone, two times, one week apart, during the placebo period and at the end of the active treatment period. The density of β-adrenoceptors on lymphocyte membranes (B_{max}) was determined two times during the placebo period, 24 hours after the first dose of the β-adrenoceptor antagonists, at the end of the second and third week of active treatment, and at the end of the first and second week after withdrawal of active treatment. Blood was always sampled at the end of the dosing interval of the respective drugs. The chronotropic responsiveness of cardiac β-adrenoceptors to isoproterenol (CD 25) was assessed at the end of the initial placebo period and one week after withdrawal of the β-adrenoceptor antagonists.

Hemodynamic Measurements

Supine blood pressure and heart rate were measured at 5 minute intervals for 1 hour by means of a an automatic oscillometric device (Datascope, Accutorr I, Datascope Corp., Paramus N.J.). Blood pressure readings with this device agree well with intraarterial blood pressure measurements (2). The values of systolic, diastolic, and mean arterial blood pressure and heart rate obtained during one hour were averaged.

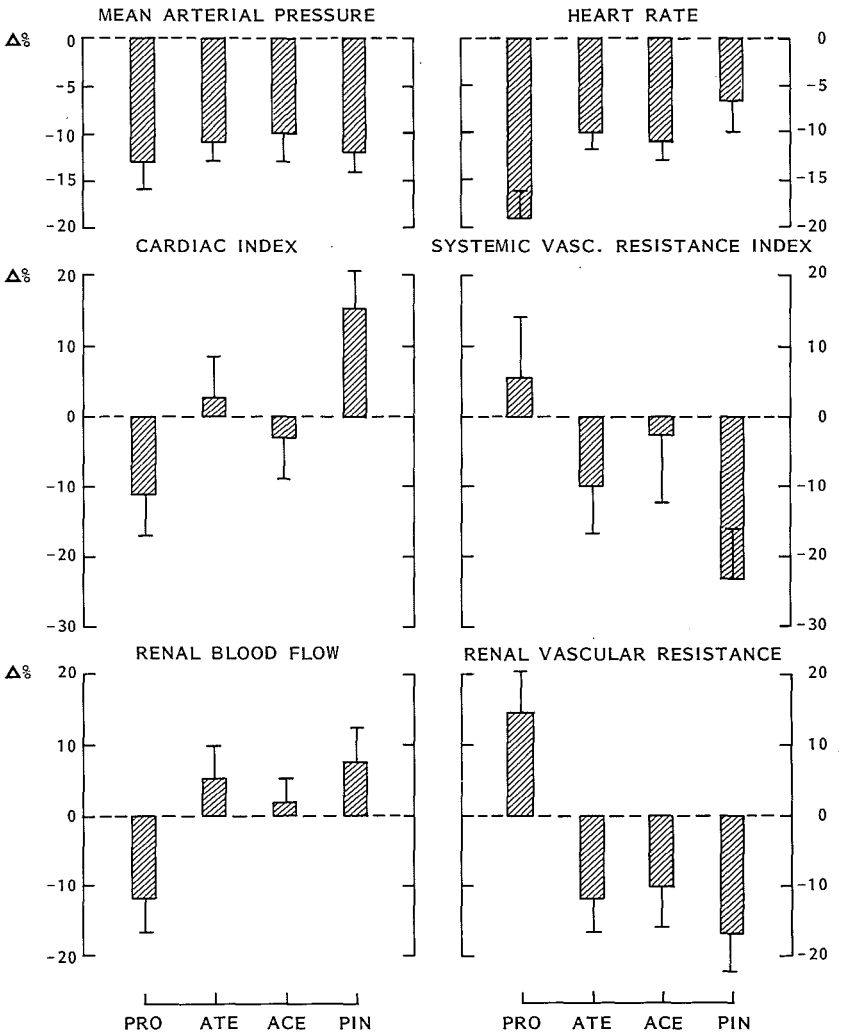

Fig. 2. Percent changes in systemic and renal hemodynamics after 3 weeks β-adrenoceptor blockade with propranolol (PRO), atenolol (ATE), acebutolol (ACE), and pindolol (PIN).

Cardiac output was measured by an isotope dilution technique (3,4) after 2 hours supine rest. ^{99m}Technetium-labeled human serum albumin (Technescan HSA, Mallinckrodt Diagnostica BV, Petten,

The Netherlands), 100 to 200 μCi was used as the indicator. After a rapid intravenous injection of the isotope the time-concentration curve was recorded for two minutes by precordial counting of radioactivity with the use of a single probe. Additional recordings were made after 5 and 10 minutes when blood was sampled for measurement of radioactivity. Cardiac output was calculated according to the formula of Steward Hamilton. In a previous study we have shown that this method correlates well with the dye-dilution technique ($n = 57$, $r = 0.92$) (3). The coefficient of variation for duplicate measurements with this technique is 6% ($n = 38$) (3). During the measurement of cardiac output heart rate was derived from a simultaneously recorded ECG.

Systemic vascular resistance index (dyne · sec · $cm^{-5}m^{-2}$) was calculated as 80 x MAP/CI, and stroke index (ml/m^2) as CI/HR, where MAP = mean arterial pressure (mm Hg), CI = cardiac index ($l\ min^{-1}m^{-2}$), and HR = heart rate (beats/min).

Glomerular Filtration Rate and Renal Plasma Flow

For renal function studies a constant infusion technique was used (5,6). Effective renal plasma flow and glomerular filtration rate were estimated by means of the clearance of ^{131}I-hippuran and ^{125}I-thalamate (Amersham, UK). The priming dose for hippuran was 0.3 to 0.4 μCi/kg body weight and for thalamate, 0.08 to 0.1 μCi/kg body weight. The sustaining infusion rates were 0.2 and 0.05 μCi/min, respectively. The clearance of the isotopes was determined at steady state after 90 and 105 minutes. Renal blood flow was calculated by means of central venous packed cell volume and assuming 75% renal extraction of hippuran (6). Glomerular filtration rate and renal blood flow were corrected for body surface area, and expressed per m^2. Renal vascular resistance index (dyne · sec · $cm^{-5}m^{-2}$) was calculated as MAP x 80/RBF, where RBF = renal blood flow ($l\ min^{-1}m^{-2}$).

Body Fluid Volumes

The same indicator as used for the measurement of cardiac output (i.e. ^{99m}TcHSA) was also used for determination of plasma volume.

Plasma samples were taken 10, 20, and 30 minutes after the intravenous injection. By linear extrapolation of the time-log-concentration-curve the radioactivity at time 0 was calculated (3). Extracellular fluid volume was estimated by measuring the distribution volume of intravenously injected $Na_2(^{35}S)$ Sulphate (50-60 μCi) with blood sampling at 0, 30, 60, 80, 100, and 120 minutes (7).

Isoproterenol Infusions

For determining the chronotropic responsiveness of the heart increasing infusion rates of isoproterenol were used. Heart rate was derived from a continuously recorded ECG. Isoproterenol was infused through an indwelling cannula (Venflon, 18G, Viggo AB, Helsingborg, Sweden) in a forearm vein by means of a infusion pump (Perfusor VI, B Braun Melsungen AG, W.-Germany). Baseline values of heart rate were obtained during a continuous infusion of saline at a flow rate of 22 ml/h for 20 minutes. The infusion was then switched to isoproterenol, 3.5, 7, 14, 35, and 70 ng kg^{-1} min^{-1}. The infusion rate was increased every 10 minutes until a rise in heart rate of at least 25 beats/min was obtained. The rise in heart rate during each dose step was used for constructing a log dose-response curve. From this curve the dose of isoproterenol required to increase the heart rate by 25 beats/min (CD25, ng kg^{-1} min^{-1}) was calculated.

β-Adrenergic Receptor Density on Lymphocyte Membranes

Preparation of Lymphocyes

Blood samples for the preparation of lymphocyte membranes were always taken after 1 h supine rest at the end of the dosing interval, before isoproterenol was given, or isotopes were used for hemodynamic studies.

Lymphocytes were isolated from fresh heparinized blood by a modification of the technique described by Böyum (8). Fresh heparinized blood (50 ml) was diluted with an equal volume of 0.154 M NaCl, and divided into four equal fractions. An aliquot (15 ml) of Isolymph (Gallard-Schlesinger Chemical Manufacturing

Corp., Carle Place, NY, U.S.A.) was carefully layered under 25 ml of the diluted blood using an 18-gauge spinal tap needle. Tubes were centrifuged at 400 g for 40 min at 20°C. After careful removal of the plasma, the lymphocyte band (at least 90% small lymphocytes, < 8% monocytes, < 2% polymorphonuclear leukocytes) was harvested by vacuum aspiration. Lymphocytes prepared from 50 ml whole blood were subdivided into four equal fractions, each of which was diluted with 20 ml of 20 mM Tris-isosaline (pH7.5, 20°C). After centrifugation at 400 g for 40 min at 20°C, the supernatant was carefully removed by vacuum suction and the pellet gently resuspended in 20 ml of solution I (anhydrous D-glucose 1.0 g/l; $CaCl_2$ 0.0056 g/l; $MgCl_26H_2O$ 0.4249 g/l; KCl 0.4026 g/l and Tris 17.565 g/l; pH7.6) using a rubber policeman. Homegenates were centrifuged at 20.000 g for 10 min at 4°C and supernatants were discarded. Pellets resuspended in 10 ml of ice-cold distilled H_2O were then homogenized (Polytron, Kinematica GmbH, Kriens/Luzern, Switserland, setting 6 for 10 s), and samples were centrifuged at 20.000 g for 10 min at 4°C. The supernatants were discarded and the four pellets were resuspended in two tubes containing 4 ml of solution I (4°C) using a Polytron (setting 5.5 for 10 s). The membranes originating from 50 ml of whole blood were stored at —70°C until assay.

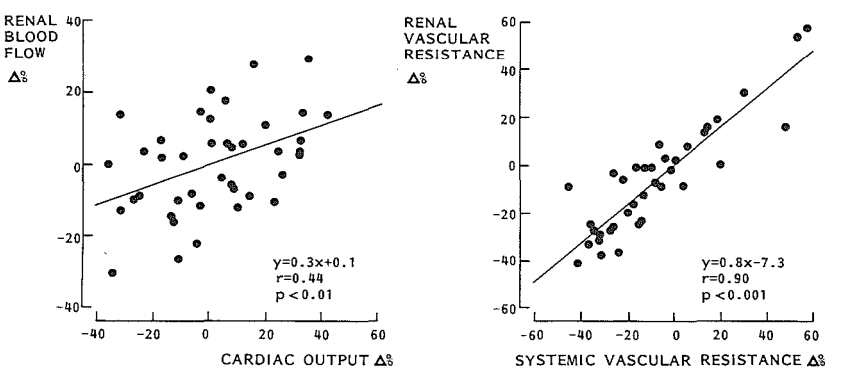

Fig. 3. Correlation between β-adrenoceptor antagonist induced changes in cardiac output and renal blood flow (left panel), and changes in systemic vascular resistance and renal vascular resistance (right panel).

β-Adrenergic Receptor Binding Assay

(-)-Pindolol was iodinated with ^{125}I and ^{125}I-pindolol (^{125}I-PIN) was purified to theoretical specific activity (2.2 Ci/μMol) as described previously (9). Samples were thawed and rehomogenized (Polytron, setting 6 for 8 sec). Aliquots (100 μl) of each homegenate containing 15-25 μg of protein were incubated with the indicated concentrations of ^{125}I-PIN and guanosine-5'-triphosphate (GTP, 100 μM) in a final volume of 0.250 ml. All assays were carried out in 0.01% ascorbic acid, 0.0004% bovine serum albumin, 0.154 M NaCl, and 20 mM Tris at pH 7.4 at 25°C.

Binding assays were routinely carried out in new disposable polypropylene tubes (Sarstedt No. 538). Samples were incubated for 25 min at 37°C. Reactions were stopped by the addition of 10 ml of Tris-isosaline (20°C) and samples were filtered through Schleicher and Schuell glass fiber filters (ZE21). Each filter was washed with an additional 10 ml of 10 mM Tris-isosaline (20°C), and radioactivity remaining on the filter was determined in a gamma counter.

Specific binding of ^{125}I-PIN was defined as the amount of ^{125}I-PIN bound in the absence of a competing ligand minus the amount bound in the presence of 50 μM of (-)-isoproterenol. This concentration is 100 times the K_d of isoproterenol, and with an observed Hill coefficient of 1 corresponds to 99% occupancy of the receptors. The amount of ^{125}I-PIN bound was always less than 10% of the total amount of ^{125}I-PIN in the incubation. Thus, the presence of competing drug did not affect the concentration of free ^{125}I-PIN. The density of specific binding sites for ^{125}I-PIN was determined by Scatchard analysis of the specific binding of ^{125}I-PIN (10). Duplicate samples were incubated with increasing concentrations of ^{125}I-PIN (10,000-250,000 cpm; 10-250 pM) with or without isoproterenol to define specific binding.

Protein Determinations

Protein concentrations were determined according to the method of Lowry et al (11).

Catecholamines, Active Renin and Aldosterone

For determination of plasma catecholamines 10 ml of venous blood was collected in chilled tubes containing 19 mg ethyleneglycol-bis-(β-aminoethyl-ether)-N,N,N',N'-tetraacetic acid and 12 mg gluthathione. After centrifugation at 0°C, samples were stored at —70°C until assay. Plasma norepinephrine and epinephrine were measured by a high-performance liquid chromatography system with electrochemical detection (12). For measurement of active plasma renin concentration 10 ml blood was sampled in chilled tubes containing ethylendiaminetetraacetic acid in a final concentration of 2 mg/ml blood.

Samples were centrifuged immediately at 0°C and stored at —20°C until assay. Active plasma renin concentration was measured indirectly by a radioimmunoassay of formed angiotensin I in the presence of saturating concentrations of sheep plasma renin substrate, as described previously (13). For determination of plasma aldosterone 5 ml of blood was collected in heparinized tubes. Samples were centrifuged immediately and stored at —20°C until assay. Plasma aldosterone was measured by a radioimmunoassay using a commercial kit (Coat-A-Count®, Diagnostic Products corporation, Los Angeles, CA).

Statistics

Data are presented as mean values ± SEM. Plasma values of renin were not distributed normally; they were so after logarithmic transformation and mean values were calculated after such transformation. Linear regression analysis was performed by the method of least squares. Differences between groups were analysed by analysis of variance, and differences within each group by Student's paired t-test. A p-value less than 0.05 was considered to indicate a significant difference.

7.4 Results

Systemic Hemodynamics

With regard to the clinical characteristics of the four groups of patients at the end of the washout period there were no significant

differences (Table 1).

Table 1. *Characteristics of Four Groups of Patients Treated With Propranolol, Atenolol, Acebutolol, or Pindolol*

	Treatment Group			
	Propranolol	Atenolol	Acebutolol	Pindolol
Patients (n)	10	10	10	10
Age (yr)	42 ± 4	42 ± 3	48 ± 3	47 ± 3
Body weight (kg)	78.3 ± 4.5	75.9 ± 3.0	86.1 ± 2.6	82.8 ± 3.3
Height (cm)	172 ± 3	171 ± 2	179 ± 2	179 ± 2
BSA (m^2)	1.95 ± 0.07	1.90 ± 0.04	2.09 ± 0.04	2.04 ± 0.05
SAP (mm Hg)	166 ± 4	163 ± 4	161 ± 4	165 ± 2
DAP (mm Hg)	108 ± 3	109 ± 3	106 ± 2	105 ± 2
MAP (mm Hg)	126 ± 4	127 ± 3	124 ± 3	125 ± 3
Heart rate (beats/min)	67 ± 2	66 ± 3	76 ± 4	71 ± 3

SAP, DAP, and MAP: systolic, diastolic and mean arterial pressure respectively.
MAP = DAP + (SAP-DAP)/3.

The changes in blood pressure and heart rate during administration and after withdrawal of the four β-adrenoceptor blocking agents were very similar (Figure 1). After 3 weeks of treatment the four drugs shared an equal antihypertensive effect.

At this time the dose of acebutolol was 480 ± 60 mg b.i.d., of atenolol 95 ± 12.5 mg once daily, of pindolol 15 ± 5 mg b.i.d., and of propranolol 112 ± 13 mg t.i.d. Systolic and diastolic arterial pressure were reduced by 10 ± 2% ($p < 0.01$) and 9 ± 2% ($p < 0.001$) with acebutolol, 11 ± 3% ($p < 0.01$) and 12 ± 3.1% ($p < 0.01$) with atenolol, 9 ± 1% ($p < 0.01$) and 11 ± 2% ($p < 0.01$) with pindolol, and 13 ± 3% ($p < 0.01$) and 12 ± 3% ($p < 0.01$) with propranolol. Systolic and diastolic arterial pressure one week after cessation of active treatment did not differ from values observed during the initial placebo period. Heart rate decreased during administration of acebutolol, atenolol and propranolol. In contrast, no change in heart rate occurred with pindolol (Table 2). After three weeks of treatment the heart rate on acebutolol (64 ± 2 beats/min) was slightly higher ($p < 0.05$) than the heart rates on atenolol (58 ± 3 beats/min)

or propranolol (57 ± 2 beats/min). After withdrawal from active treatment heart rate did not differ from the values of the initial placebo period in any of the four groups (Figure 1). Mean arterial pressure fell by 10 ± 3% ($p < 0.01$) with acebutolol, 11 ± 2% ($p < 0.001$) with atenolol, 12 ± 2% ($p < 0.01$) with pindolol and 13 ± 3% ($p < 0.01$) with propranolol after three weeks treatment (Figure 2). With pindolol the fall in blood pressure was entirely due to a fall in vascular resistance index (—26 ± 6%, $p < 0.001$), and cardiac index was slightly increased (16 ± 6%, $p < 0.01$) (Table 2, Figure 2). With the other three drugs the fall in mean arterial pressure was not associated with significant changes in either vascular resistance index or cardiac index, although with propranolol, but not with acebutolol or atenolol, cardiac index tended to decrease (—11 ± 7%, $p > 0.05$). Changes in cardiac output were not correlated to changes in blood pressure. Stroke index did not change with either acebutolol or propranolol, whereas it was increased with atenolol (14 ± 7%, $p < 0.01$), and pindolol (26 ± 6%, $p < 0.01$).

Renal Hemodynamics

Changes in renal blood flow and renal vascular resistance ran in parallel with the changes in systemic hemodynamics (Table 2, Figure 2). With pindolol renal blood flow tended to increase (7 ± 5%, $p > 0.05$) and renal vascular resistance decreased (—17 ± 5%, $p < 0.01$), whereas with propranolol renal blood flow decreased (—12 ± 4%, $p < 0.01$) and renal vascular tended to increase (8.1 ± 10%, $p > 0.05$). Renal blood flow did not change with acebutolol or atenolol and renal vascular resistance decreased with 10 ± 6% ($p > 0.05$) and 14 ± 4% ($p < 0.01$) on these two drugs, respectively. The percent changes in cardiac index and renal blood flow were positively correlated ($r = 0.44$, $p < 0.01$) (Figure 3). As a consequence the percent changes in systemic vascular resistance index and renal vascular resistance index were also strongly correlated ($r = 0.90$, $p < 0.001$) (Figure 3). As expected patients with a higher blood pressure had a lower renal blood flow ($r = —0.36$; $p < 0.05$). Changes in blood pressure and renal blood flow during treatment were not correlated.

Table 2. *Effects of Propranolol, Atenolol, Acebutolol, and Pindolol on Systemic and Renal Hemodynamics.*

	Placebo	Propranolol	p-value	Placebo	Atenolol	p-value
MAP (mm Hg)	123 ± 5	107 ± 4	< 0.01	117 ± 3	104 ± 3	< 0.001
HR (beats/min)	65 ± 2	53 ± 2	< 0.001	64 ± 3	57 ± 3	< 0.001
CI (l/min/m²)	3.0 ± 0.1	2.6 ± 0.2	NS	2.6 ± 0.2	2.6 ± 0.2	NS
SI (ml/m²)	46 ± 2	50 ± 3	NS	41 ± 2	46 ± 3	< 0.05
SVRI (dyne·sec·cm⁻⁵/m²)	3440 ± 280	3538 ± 260	NS	3746 ± 230	3299 ± 223	NS
GFR (ml/m²)	58 ± 3	54 ± 1	NS	52 ± 2	57 ± 3	NS
RBF (ml/m²)	630 ± 38	549 ± 28	< 0.01	539 ± 25	563 ± 33	NS
RVR (dyne·sec·cm⁻⁵/m²)	16362 ± 1498	18207 ± 635	NS	17726 ± 938	15170 ± 1000	< 0.01
	Placebo	Acebutolol	p-value	Placebo	Pindolol	p-value
MAP (mm Hg)	115 ± 3	104 ± 3	< 0.01	122 ± 3	106 ± 2	< 0.01
HR (beats/min)	71 ± 3	61 ± 2	< 0.01	68 ± 2	63 ± 3	NS
CI (l/min/m²)	3.3 ± 0.3	3.1 ± 0.2	NS	2.7 ± 0.2	3.1 ± 0.2	< 0.01
SI (ml/m²)	47 ± 3	51 ± 3	NS	40 ± 3	49 ± 3	< 0.01
SVRI (dyne·sec·cm⁻⁵/m²)	2924 ± 193	2776 ± 198	NS	3809 ± 316	2859 ± 228	< 0.001
GFR (ml/m²)	56 ± 2	56 ± 2	NS	56 ± 2	59 ± 2	NS
RBF (ml/m²)	592 ± 36	600 ± 35	NS	558 ± 23	596 ± 30	NS
RVR (dyne·sec·cm⁻⁵/m²)	16106 ± 1287	14134 ± 917	NS	17741 ± 1213	1455 ± 962	< 0.01

Data are mean ± SEM.

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SI, stroke index; SVRI, systemic vascular resistance index; GFR, glomerular filtration rate; RBF, renal blood flow, RVR, renal vascular resistance. GFR, RBF and RVR are indexed per m².

Plasma Catecholamines, Active Renin and Aldosterone

Before treatment plasma norepinephrine and epinephrine concentrations did not differ between the four groups (Table 3). Plasma catecholamines did not change with any of the four β-adrenoceptor antagonists. Renin was suppressed by all four drugs. At the end of the 3 weeks treatment period active renin was decreased by 65 ± 9% ($p < 0.01$) with acebutolol, 66 ± 11% ($p < 0.01$) with atenolol, 62 ± 7% ($p < 0.001$) with pindolol, and 70 ± 5% ($p < 0.001$) with propranolol. Pretreatment values of active renin or the decrease in active renin and the blood pressure responses to the four drugs after 3 weeks treatment were not correlated. Pretreatment values of renin and aldosterone were correlated ($r = 0.32$; $p < 0.05$). Plasma aldosterone was lowered by all four drugs (Table 3). The percent decrements in aldosterone and renin during β-adrenoceptor blockade were not correlated.

Table 3. *Effects of propranolol, atenolol, acebutolol, and pindolol on plasma concentrations of norepinephrine, epinephrine, active renin, and aldosterone and on body fluid volumes.*

	Placebo	Propranolol	Placebo	Atenol
Norepinephrine (pg/ml)	216 ± 19	281 ± 33	264 ± 32	282 ± 85
Epinephrine (pg/ml)	81 ± 15	73 ± 14	38 ± 6	33 ± 6
Active renin (μU/ml)	14.2 ± 3.9	3.5 ± 0.9***	11.3 ± 2.8	2.4 ± 0.6**
Aldosterone (pg/ml)	109 ± 21	45 ± 8**	88 ± 8	61 ± 5**
Plasma Volume (l)	2.9 ± 0.1	3.1 ± 0.2	2.8 ± 0.1	2.9 ± 0.2
Extracellular Fluid Volume (l)	13.0 ± 0.6	13.5 ± 0.6	14.3 ± 0.7	14.8 ± 0.4
	Placebo	Acebutolol	Placebo	Pindolol
Norepinephrine (pg/ml)	221 ± 25	189 ± 35	254 ± 31	257 ± 27
Epinephrine (pg/ml)	63 ± 11	36 ± 8	86 ± 16	74 ± 11
Active renin (μU/ml)	8.5 ± 2.5	1.8 ± 0.9**	10.9 ± 2.0	3.4 ± 0.8***
Aldosterone (pg/ml)	106 ± 22	81 ± 13*	96 ± 13	71 ± 14*
Plasma Volume (l)	3.5 ± 0.1	3.6 ± 0.2	3.4 ± 0.1	3.5 ± 0.2
Extracellular Fluid Volume (l)	15.5 ± 0.9	15.7 ± 0.9	15.4 ± 0.4	14.9 ± 0.5

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, 3 weeks treatment vs. placebo.

Body Fluid Volumes

Before treatment plasma volumes and extracellular fluid volumes were not different between the four groups. No significant changes in these variables occurred during treatment (Table 3).

Lymphocyte β-Adrenoceptor Density, Plasma Epinephrine and Chronotropic Responsiveness to Isoproterenol

During placebo the density of β-adrenoceptors on lymphocyte membranes (B_{max}) and the chronotropic responsiveness to isoproterenol (CD25) did not differ between the four groups (Tables 4 and 5). During placebo B_{max} and CD25 were inversely correlated ($r = -0.43$; $p < 0.01$) (Figure 4). Interestingly, B_{max} and plasma epinephrine concentration were also inversely correlated ($r = -0.41$; $p < 0.01$, Figure 4), but plasma epinephrine and CD25 were not. B_{max} was not correlated with plasma norepinephrine, age, systolic, diastolic or mean arterial pressure.

B_{max} increased by 51 ± 17% ($p < 0.01$) after 3 weeks of treatment with propranolol (Figure 5). Already 24 hours after starting of treatment B_{max} was increased by 30 ± 10% ($p < 0.05$). Furthermore,

1 week, but not 2 weeks, after propranolol withdrawal, lymphocyte β-adrenergic receptor density was still increased. With pindolol B_{max} decreased maximally by 39 ± 6% ($p < 0.01$) after 3 weeks of treatment ((Figure 5). Like the initial increase in B_{max} after propranolol, the fall in B_{max} after pindolol was also seen already 24 hours after beginning of treatment (—35 ± 5%; $p < 0.01$).

Table 4. *Effects of Propranolol, Atenolol, Acebutolol, and Pindolol on Density of β-adrenergic Receptors on Lymphocyte Membranes (B_{max}) and on the affinity constant (K_d) for specific ^{125}I-IPIN binding to Lymphocyte Membranes.*

	Placebo		β-adrenoceptor Blockade			Withdrawal	
Time weeks	1	2	2¶	4	5	6	7
B_{max} (fmol/mg protein)							
Propranolol	23 ± 3	22 ± 3	29 ± 4*	31 ± 4	35 ± 5**	27 ± 3*	26 ± 3
Atenolol	26 ± 3	24 ± 3	28 ± 4	29 ± 4	26 ± 3	25 ± 3	25 ± 2
Acebutolol	29 ± 3	26 ± 2	27 ± 2	25 ± 2	28 ± 2	31 ± 3	28 ± 2
Pindolol	17 ± 2	17 ± 2	11 ± 2*	10 ± 1	9 ± 1**	13 ± 1*	16 ± 2
K_d (pM)							
Propranolol	82 ± 22	87 ± 18	74 ± 10	107 ± 23	133 ± 42*	73 ± 19	70 ± 15
Atenolol	57 ± 12	64 ± 12	63 ± 6	75 ± 19	73 ± 14	68 ± 14	66 ± 11
Acebutolol	51 ± 5	58 ± 9	57 ± 8	59 ± 10	59 ± 8	46 ± 6	54 ± 8
Pindolol	50 ± 6	56 ± 11	61 ± 15	67 ± 9	67 ± 10	59 ± 13	53 ± 5

¶ 24 hours after administration of the first dose of the β-adrenoceptor antagonist.
* $p < 0.05$; ** $p < 0.01$ active treatment or withdrawal versus placebo.

One week after withdrawal of treatment B_{max} was still decreased by 33 ± 9% ($p < 0.05$). No significant changes in B_{max} were seen with the β-adrenoceptor antagonists acebutolol and atenolol. CD25 one week after withdrawal of propranolol and atenolol was significantly decreased ($p < 0.05$). Conversely, CD25 one week after withdrawal of pindolol was increased ($p < 0.01$) (Table 5). Separate analysis of the pindolol and propranolol group showed that both before ($n = 18$, $r = -0.80$, $p < 0.01$), and 1 week after withdrawal ($n = 15$, $r = -0.63$; $p < 0.05$) B_{max} and CD25 were inversely correlated (Figure 6). In agreement with a previous report (14) the apparent K_d for specific ^{125}I-PIN binding to lymphocyte membranes was increased after 3 weeks of treatment with propranolol (Table 4). No change in K_d values were seen with the other β-adrenoceptor antagonists.

Table 5. *Dose of Isoproterenol (ng kg^{-1} min^{-1}) to Increase Heart Rate by 25 beats/min during Placebo and One Week after Withdrawal of β-Adrenoceptor Blockade with Propranolol, Atenolol, Acebutolol, or Pindolol.*

	Placebo	Withdrawal	p-value
	Week 2	*Week 6*	
Propranolol	24 ± 5	17 ± 2	< 0.05
Atenolol	23 ± 2	17 ± 3	< 0.05
Acebutolol	20 ± 2	19 ± 2	NS
Pindolol	30 ± 6	43 ± 5	< 0.01

7.5 Discussion

Systemic Hemodynamics

Previous findings that β-adrenoceptor antagonists, irrespective of their different ancillary pharmacological properties, lower an elevated blood pressure to a similar extent when adequately dosed, were confirmed in the present study (15,16). It was also confirmed that, in relation to these different ancillary properties, the systemic hemodynamic effects, other than their antihypertensive effect, are markedly different. (16,17). With propranolol, a non-selective antagonist devoid of PAA, the fall in blood pressure was predominantly associated with a decrease in cardiac output (Figure 2). Conversely, during treatment with pindolol, a non-selective antagonist with a relatively high degree of PAA, the antihypertensive effect was entirely due to a fall in vascular resistance. At variance with some previous studies the antihypertensive effect of the β_1-selective antagonist atenolol was not associated with a decrease in cardiac output (18,19). The alterations in systemic hemodynamics as caused by acebutolol did not differ from those of atenolol. This reflects the relatively low degree of PAA of this drug.

Comparing the current data with those of previous studies the fall in cardiac output, if present at all, during administration of β-adrenoceptor antagonists devoid of PAA, was relatively small. Moreover, after pindolol with its pronounced PAA cardiac output even moderately increased. Hemodynamic measurements in this study were performed after two hours supine rest. The patients were therefore in a very basal state, and as reflected by their

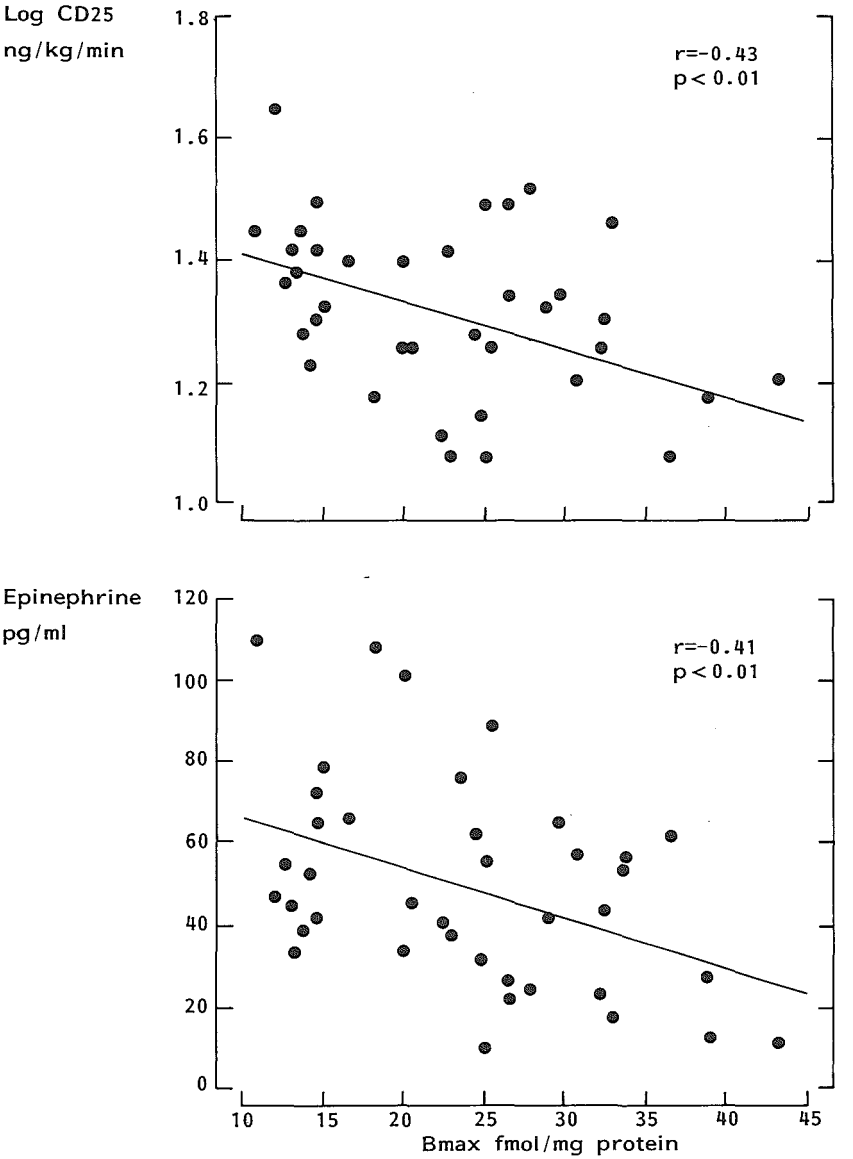

Fig. 4. Correlation between the density of β-adrenoceptors on lymphocyte membranes (B_{max}) and the dose of isoproterenol to increase heart rate by 25 beast/min (log CD25) (upper panel) and the relationship between B_{max} and plasma epinephrine concentration (lower panel).

low heart rates, cardiac sympathetic drive was low. This may explain why on average relatively little cardiodepression was exerted by the β-adrenoceptor antagonists and why the cardiostimulant action of pindolol could be demonstrated.
The hemodynamic data after 3 weeks of treatment with equipotent doses of the four β-adrenoceptor agonists are in close agreement with the hemodynamic profiles of these drugs during the onset of their blood pressure lowering effect within the first 24 hours after their administration (1). Analysis of the hemodynamic interrelations during onset of the antihypertensive effect of these drugs led us to conclude that, after an initial period of cardiodepression and reflex-vasoconstriction, acebutolol, atenolol and propranolol lowered blood pressure through a fall in vascular resistance which was accompanied by return of cardiac output, heart rate and stroke volume to baseline. With pindolol the initial cardiodepression and reflex-vasoconstriction were absent. The fall in blood pressure on this drug was through a reduction in vascular resistance, whereas cardiac output was raised. We suggested, that under the strict basal conditions of our study, despite cardiac β-blockade, withdrawal of vagal tone, afterload reduction, and an increase in venous return to the heart were the factors most likely to be involved in the adaptive changes of cardiac output.

Renal Function
Although deterioration of renal function during administration of β-adrenoceptor antagonists has sporadically been reported (20,21) the adverse effects of these drugs on renal function usually appear to be small and reversible (22,23). This could be confirmed in the present study: glomerular filtration rate did not change significantly after any of the four drugs, although it tended to decrease on propranolol and to increase on pindolol.
Reduction in cardiac output, suppression of renin, blockade of vasodilating β_2-receptors in the kidney, and alterations in plasma volume are all possible factors, which may adversely affect renal perfusion during β-adrenoceptor blockade (22,23). We found the changes in renal perfusion to occur in parallel to the changes in cardiac output, not only when a comparison was made between

the four groups of patients, but also when the data of the individual subjects were compared. Renin was suppressed to a similar degree, and plasma volume remained unchanged. These findings therefore suggest that the changes in renal perfusion, which may occur during β-adrenoceptor antagonism, are mainly a consequence of the β-adrenoceptor antagonist induced changes in systemic hemodynamics.

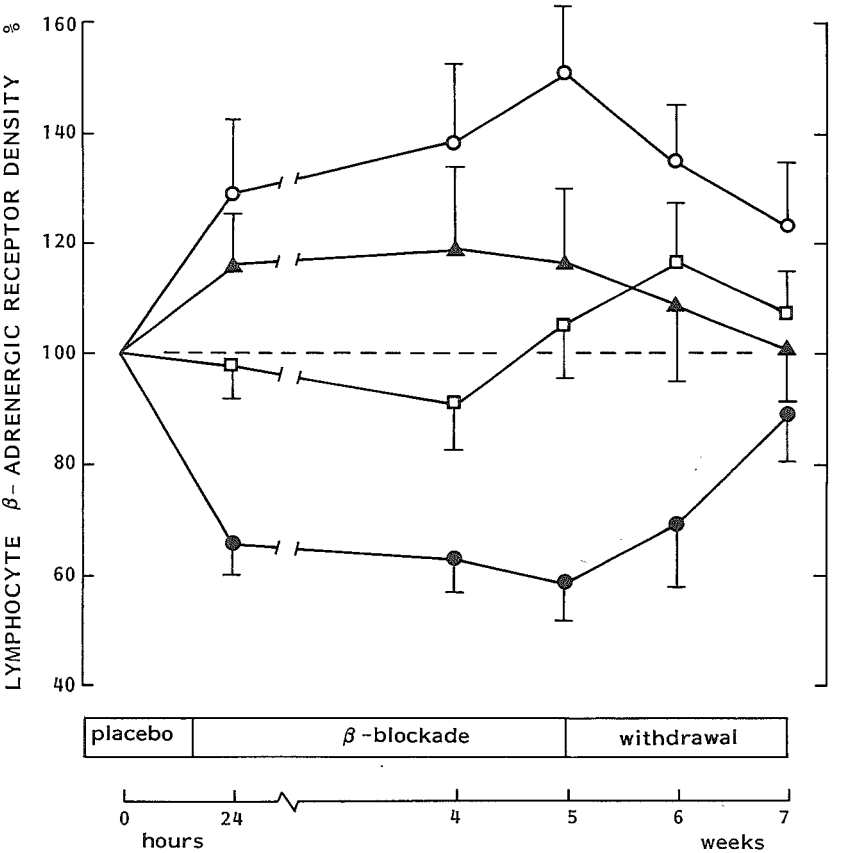

Fig. 5. Percent changes in lymphocyte β-adrenoceptor density during administration and after withdrawal of propranolol (○), atenolol (▲), acebutolol (□), and pindolol (●).

Plasma Catecholamines

No unanimity in the literature exists about the effects of β-adrenoceptor antagonism on plasma levels of norepinephrine and epinephrine. Increments, decrements and no changes in plasma catecholamines during administration of β-receptor antagonists have all been reported (24). The interpretation of plasma levels of norepinephrine during administration of β-blockers is complicated by the fact that these drugs may not only affect the release of this neurotransmitter into plasma, but also its clearance rate from plasma. Norepinephrine is mainly cleared from the circulation by the liver and the lung (25). A decreased flow through these organs, concomitantly with a fall in cardiac output, impairs the clearance of norepinephrine and as a consequence may result in increased plasma levels of the neurotransmitter (26-28). This may explain why in the present study norepinephrine tended to increase with propranolol, but not with the other three drugs, because only on this drug cardiac output tended to decrease. Increments in plasma norepinephrine during β-adrenoceptor antagonism therefore do not necessarily implicate an increased sympathetic tone. In other words, reduced sympathetic activity and diminished release of norepinephrine can easily be masked by this phenomenon. The absence of a rise in norepinephrine during the 'vasodilator' and antihypertensive action of β-adrenoceptor antagonists can be considered inappropriate and could therefore suggest interference of these drugs with the sympathetic nervous system.

Renin Suppression

Suppression of active renin during administration of β-adrenoceptor antagonists has been well documented (16,24). The β-adrenoceptor mediating renin release is probably of the β_1 subtype, since β_1-selective antagonists cause a similar degree of renin supression as non-selective β-adrenoceptor antagonists (29). Whether renin is also suppressed by pindolol is still a matter of controversy (30,31). In the present study renin was markedly suppressed by all four drugs.

Moreover, the degree of renin suppression on pindolol was not

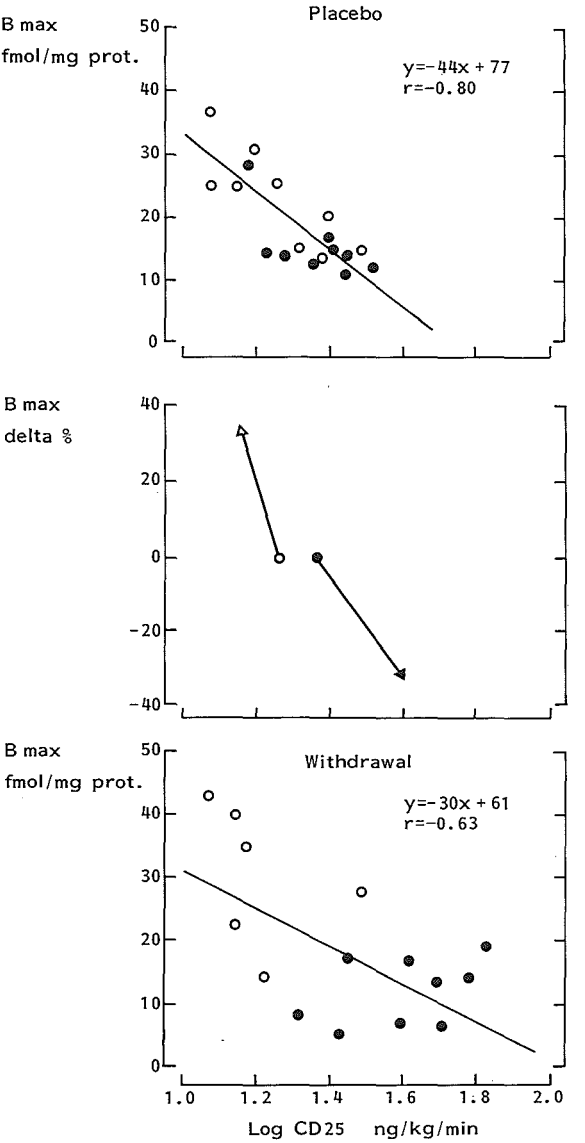

Fig. 6. Correlation between the density of β-adrenoceptors on lymphocyte membranes (B_{max}) and the dose of isoproterenol to increase heart rate by 25 beats/min (log CD25) before (upper panel and middle panel, circles) and after withdrawal (lower panel and middle panel, triangles) of treatment with pindolol (closed symbols) and propranolol (open symbols).

different from that of the other three drugs. This finding seems paradoxical in the light of the effects of pindolol on heart rate and cardiac output, in which the PAA of the drug came clearly to expression. This differential effect of pindolol on renin on the one side and on hemodynamics on the other side supports *in vitro* studies, which suggest that pindolol's agonist activity is predominantly restricted to the β_2-receptor (9,32). This subtype β-receptor coexists with the β_1-receptor in the human heart (33-35). An alternative explanation for our findings is that under resting conditions renal juxtaglomerular β-receptors are subjected to a higher sympathetic tone than the cardiac β-receptors.
The renin suppressant effect of β-adrenoceptor blocking agent has been linked to the antihypertensive mechanism of these drugs (36). Evidence indeed indicates that in high-renin forms of hypertension renin-suppression contributes to the blood pressure lowering effect of these drugs, but it is not very likely that this renin suppressant effect also contributes to the blood pressure lowering effect of these drugs in the majority of patients with normal or low-renin forms of hypertension (37,38). If, in the present study, the fall in blood pressure was due to renin suppression a relationship between pretreatment values of renin and the antihypertensive effect would be expected, however, such a correlation was not found. Furthermore, in our acute study it was shown that 2 hours after administration of acebutolol, atenolol and propranolol renin was already near maximally suppressed whereas blood pressure had not been fallen at all (1). Moreover, in that study it was also shown that pindolol, 24-hours after administration exerted its full antihypertensive effect, whereas renin was no longer reduced at that time.

Lymphocyte β-Adrenoceptor Density and Isoproterenol Sensitivity
Studies in man and *in vitro* studies indicate that the changes in the density and responsiveness of β-adrenoceptors on lymphocytes can be used as a model to study the alterations of these receptors in less accessible tissues such as the heart and the lung (39,40). To some extent our study supports these data. First of all, during placebo an inverse correlation between the density of lymphocyte

β-adrenoceptors and the chronotropic responsiveness of the heart to isoproterenol could be established. An inverse correlation between these two parameters has also been reported by Fraser et al (41). However, in their study this correlation was found after repeated measurements of these parameters in the same group of normotensive subjects under different sodium balances. Second, one week after withdrawal of propranolol and pindolol an inverse correlation between lymphocyte β-adrenoceptor density and cardiac chronotropic responsiveness was still present. Moreover, and more importantly, the "down-regulation" of lymphocyte β-adrenoceptors after withdrawal of pindolol coincided with a decrease in the cardiac chronotropic responsiveness to isoproterenol, whereas the 'up-regulation' of lymphocyte β-adrenoceptors after withdrawal of propranolol was associated with an increased cardiac chronotropic responsiveness.

Lymphocyte β-adrenoceptors are of a homologeous β_2-subtype (42). No change in these receptors would therefore be expected during administration of β_1-selective β-adrenoceptor antagonists, as was seen in the present study with atenolol. During administration of acebutolol lymphocyte β-adrenoreceptors also did not change. Once again this may be related to the β_1-selectivity of this drug. On the other hand it has recently been shown that the partial agonist activity of β-adrenoceptor blocking agents, either β_1-selective or not, possesses a β_2 component (43). Absence of any effect of acebutolol on lymphocyte β-adrenoceptors may therefore indicate that the partial agonist activity of this drug, at least as compared to that of pindolol, is rather weak, which fits well with the hemodynamic effects of this drug. The dose of isoproterenol to increase the heart rate by 25 beats/min one week after withdrawal of atenolol was also significantly decreased, indicating an increased cardiac chronotropic responsiveness of the heart. This increased cardiac chronotropic responsiveness might be explained by an 'up-regulation' of cardiac β_1-receptors.

Another notable finding of this study was the inverse correlation between plasma concentrations of epinephrine and the density of β-adrenoceptors on lymphocytes. This finding suggests that at least under basal conditions endogeneous epinephrine, man's

natural β_2-agonist, is capable of regulating these receptors. In agreement with previous reports plasma concentrations of norepinephrine and lymphocyte β-adrenoceptor density were not correlated (44). This is not surprising, since norepinephrine has no β_2-agonist activity.

In summary it can be concluded from our results that lymphocyte β-adrenoceptors are a suitable model for studying drug-induced changes in cardiovascular β-adrenoceptors as far as these changes are caused by β-adrenergic agents. Studies on lymphocyte β-adrenoceptors may add to the explanation of the so-called β-blocker withdrawal syndrome, which has been reported after discontinuation of propranolol as well as other β-adrenoceptor antagonists, but never after discontinuation of pindolol (45). Studies on lymphocyte β-adrenoceptors may also be helpful for determining the degree of partial agonist activity and/or receptor selectivity of β-adrenoceptor antagonists.

Conclusions

The β-adrenoceptor antagonists propranolol, pindolol, acebutolol and atenolol appeared to exert, when dosed appropriately, an equal antihypertensive effect. Despite this similarity in antihypertensive effect the drugs have dissimilar effects on cardiac output, renal blood flow and lymphocyte β-adrenoceptors. In contrast, their effects on catecholamines, the renin-angiotensin-aldosterone-system and body fluid volumes are not different. Changes in cardiac output, the circulating blood volume or angiotensin mediated vasoconstriction are unlikely to be the crucial mechanism for the antihypertensive effect of β-adrenoceptor antagonists.

Diminished α-adrenoceptor mediated vasoconstriction through either blockade of central β-adrenoceptors or peripheral prejunctional β-adrenoceptors still remains an attractive possibility to explain the vasodilator and antihypertensive effect of these drugs. The absence of a fall in plasma norepinephrine after β-adrenoceptor blockade does not contradict such a contention, since concomitant changes in the clearance of plasma norepinephrine have to be taken into account.

7.6 References

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8. Epinephrine-induced enhancement of sympathetic activity in man; inhibition by non-selective as well as β_1-selective β-adrenoceptor blockade

8.1 Summary

In order to collect further evidence that epinephrine (EPI) facilitates norepinephrine (NE) release in man, the effect of a low-dose intravenous infusion of EPI (20 ng kg^{-1} min^{-1}) on arterial (art) levels of NE and on the local production of NE in the forearm was studied both before and during isometric exercise (IET), cold provocation (CPT), orthostatic stress (OST) and mental stress (CWT) in ten subjects with borderline to mild hypertension. Studies were performed during placebo, during β_1-selective β-adrenoceptor blockade with atenolol, 50 mg once daily, and during non-selective β-blockade with bopindolol, 1 mg once daily. Atenolol and bopindolol were administered for one week, two weeks apart, in a randomized double-blind cross-over design. During infusion of EPI, which raised venous plasma EPI levels within the high physiological range, resting levels of NEart increased from 214 $\pm$ 20 to 263 $\pm$ 26 pg/ml ($p < 0.01$) and resting NE forearm production increased from 296 $\pm$ 48 to 529$\pm$98 pg 100 ml^{-1} min^{-1} ($p < 0.01$). The effect of EPI on these two indices of sympathetic activity was abolished by bopindolol as well as by atenolol. The delta response of NEart, but not of NE forearm production, to IET, CPT and CWT was also increased ($p < 0.05$) by EPI. This increment, once again, was abolished by the two β-adrenoceptor antagonists. Our findings support the hypothesis that physiological levels of EPI are capable of enhancing sympathetic activity in man. This effect is mediated by β-adrenoceptors and can be blocked, not only by non-selective, but also by β_1-selective β-adrenoceptor antagonists.

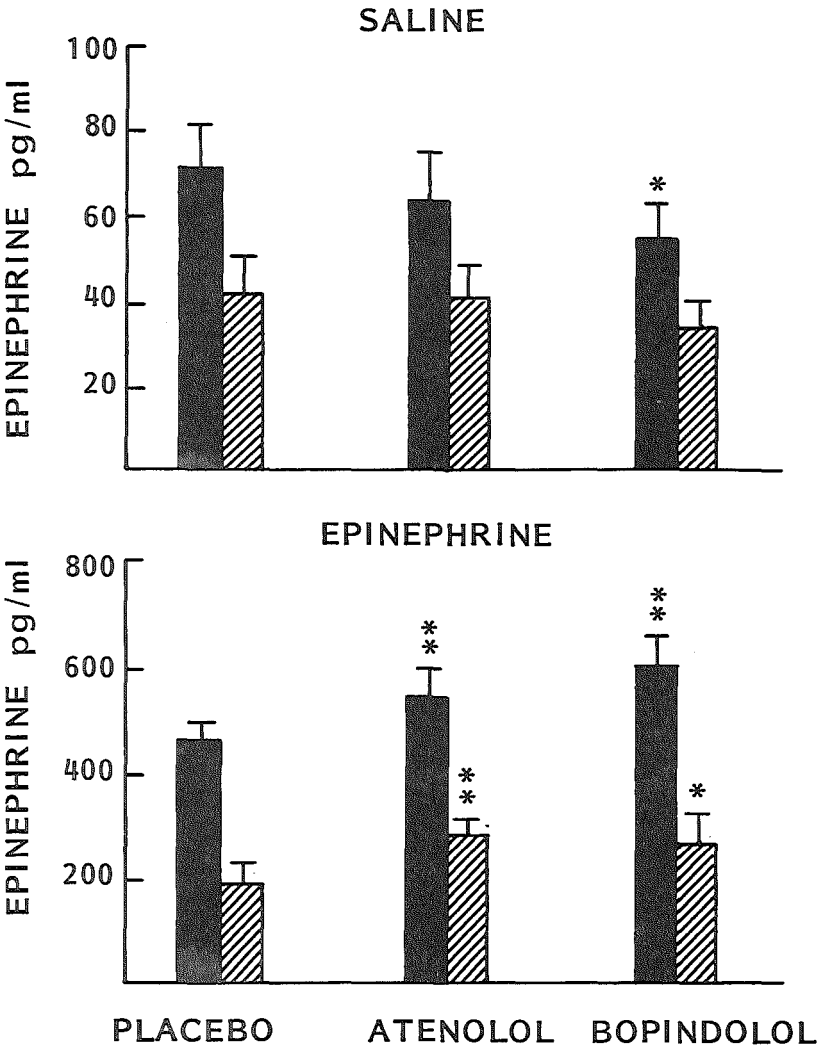

Fig. 1. Arterial (■) and venous (///) plasma levels of epinephrine during placebo, atenolol and bopindolol. Upper panel infusion of saline, lower panel infusion of epinephrine (20 ng kg^{-1} min^{-1}).
* $p < 0.05$; ** $p < 0.01$ atenolol or bopindolol versus placebo.

8.2 Introduction

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

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

The present study was conducted in order to collect more information on the facilitatory effect of EPI on NE release in man. Our aim was twofold. First, to confirm and extend earlier observations that EPI, in concentrations within the physiological range, enhances resting and activated sympathetic activity. Second, to study the effects of non-selective and β_1-selective β-adrenoceptor blockade on this EPI-induced enhancement in sympathetic activity. Arterial plasma levels of NE were used as an index of overall sympathetic activity, whereas the production of NE in the forearm, with exclusion of the hand, was used as an index of local muscle sympathetic activity.

8.3 Subjects and Methods

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

Study Protocol

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

Procedures and Tests

Patients arrived at 8 A.M. in the laboratory after a light breakfast at home. While resting supine on a tilt-lable the brachial artery of the non-dominant arm was cannulated with a 1.0 mm thick, 5 cm long, Teflon® catheter after local anaesthesia with a 2% lidocaine solution. A deep antecubital vein was cannulated in the same arm for venous blood sampling. An indweling catheter in a vein of the contralateral forearm was used for intravenous

infusions. The flow in the non-dominant arm was measured semicontinuously by an ECG triggered venous occlusion plethysmograph (Janssen Scientific Instruments, Beerse, Belgium). A mercury-in-Silastic strain gauge was placed around the mid-forearm. The venous occlusion pressure of 50 mm Hg was intermittently applied for a period of three heart beats, with a recovery period of two heart beats. During flow measurements the arm was slightly elevated above heart level and handflow was excluded from the circulation by a small wrist cuff inflated 30 mm Hg above the systolic arterial pressure. Heart rate was derived from a continuously recorded ECG.

After completion of instrumentation an intravenous saline (SAL) infusion (5.4 ml per hour) was started. Thirty minutes after start of this infusion baseline arterial (art) and venous (ven) plasma catecholamines, active plasma renin and hemodynamics were measured. Ten minutes afterwards an isometric exercise test (IET), cold pressor test (CPT), orthostatic stress test (OST) and a mental stress test (CWT) were subsequently performed, with a period of 15 minutes supine rest between each test.

After completion of the CWT the SAL-infusion was switched to an intravenous epinephrine (EPI) infusion with an infusion rate of 20 ng kg^{-1} min^{-1}. The timing and sequence of measurements and tests during the EPI-infusion were the same as during infusion of SAL. For evaluating possible sequence effects, 6 additional subjects only receiving placebo capsules (control group), were studied. In the control group, a second SAL-infusion was given instead of the EPI-infusion.

IET was performed by lifting a weight of approximately 9% of body weight over a pulley by 90° flexion of the elbow of the dominant arm, while resting supine. This test lasted three minutes. For the CPT the hand up to the wrist of the dominant arm was immersed in ice-cold water for one minute. OST was performed by 60° head-up tilting for ten minutes by means of a tilt-table. For mental stress an audio-visual version of Stroop's colour word conflict test was used. This test consists of four different colour-words written in incongruent colours. The words are randomly shown on a television monitor with a frequency of 140 words

per five minutes. The task of the subject is to ignore the written word and to mark the colour on an answer-sheet on which successive rows of randomly ordered abbreviations of the four colours are printed. The test lasts for 5 minutes.
Just before and immediately at the end of each test art and ven blood was simultaneously sampled for measurement of catecholamines. Forearm flow was measured during IET, CPT and CWT, but for technical reasons, it was not measured during OST.

Analytical Procedures and Calculations
For determination of catecholamines 10 ml of art and ven blood was collected in chilled tubes containing 12 mg gluthatione and 5 IE sodium heparin. After centrifugation at 0°C, plasma was removed and stored at —70° until assay. Plasma catecholamine concentrations were determined by high performance liquid chromatography (HPLC) with electrochemical detection (26).
The fractional extraction (FE; %) of EPI in the forearm was calculated as (EPIart-EPIven)/EPIart x100. The production of noradrenaline (NE) in the forearm was calculated as the product of forearm flow and the ven-art difference of NE plus the extraction of NE in the forearm. NE extraction was calculated as NEart x FE EPI, assuming that the FE's of NE and EPI in the forearm are similar (27).
Active plasma renin concentration was measured by RIA, as described previously (28).

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

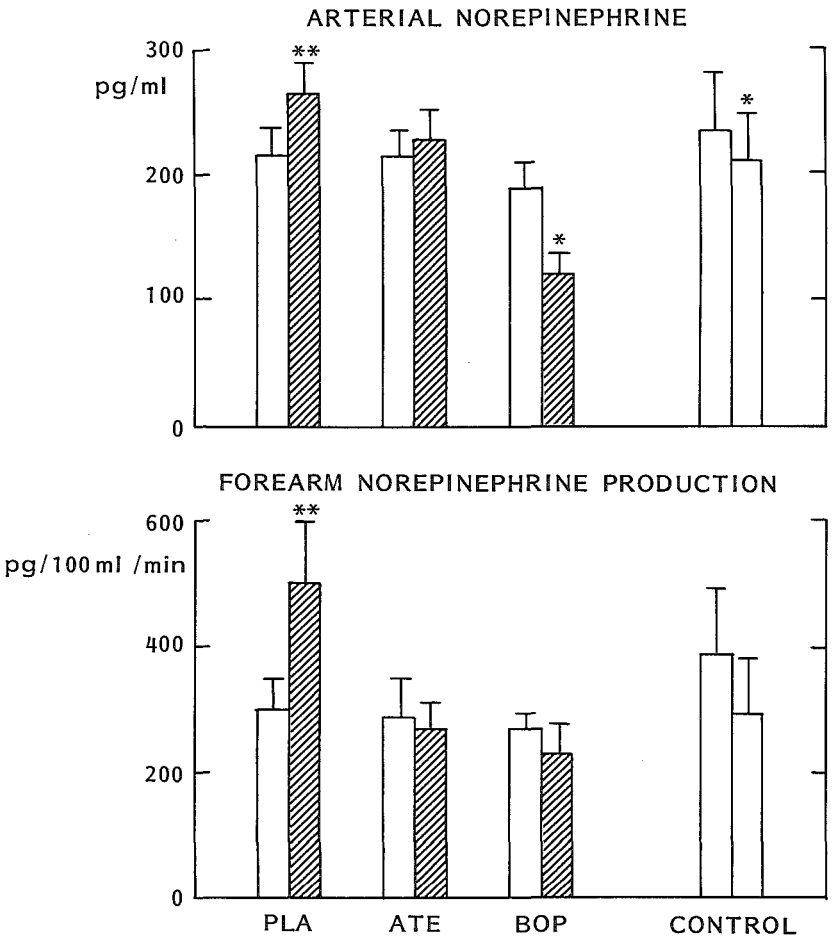

Fig. 2. Effects of epinephrine on resting concentrations of norepinephrine and forearm norepinephrine production during placebo, atenolol and bopindolol. (□ saline, (///) epinephrine infusion.
* $p < 0.05$, ** $p < 0.01$ epinephrine versus saline or saline versus saline control group.

8.4 Results

Resting Conditions

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

Table 1. *Effects of epinephrine on resting values of systolic and diastolic arterial pressure, heart rate and forearm flow during placebo, atenolol and bopindolol*

	Placebo		Atenolol		Bopindolol		Control ¶	
	SAL	EPI	SAL	EPI	SAL	EPI	SAL	SAL
SAP mm Hg	154 ± 5	151 ± 6	130 ± 5	128 ± 5	140 ± 5	143 ± 5	147 ± 4	145 ± 4
DAP mm Hg	89 ± 2	78 ± 3**	72 ± 2	67 ± 3*	79 ± 2	82 ± 3	86 ± 3	84 ± 4
HR bpm	71 ± 3	78 ± 2**	59 ± 3	58 ± 2	63 ± 3	61 ± 3	68 ± 1	65 ± 3
FAF ml/min/100ml	3.0 ± 0.3	3.5 ± 0.5	3.4 ± 0.7	2.6 ± 0.3	3.2 ± 0.3	2.9 ± 0.4	4.7 ± 1.3	3.9 ± 0.7

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

Active renin decreased to a similar degree after atenolol and bopindolol. Values were 23.1 ± 1.3 μU/ml during placebo, 11.3 ± 1.2 μU/ml ($p < 0.01$) during atenolol, and 10.9 ± 1.2 μU/

ml ($p < 0.01$) during bopindolol. Active renin did not change by infusion of EPI during any of the three treatments: the values during placebo, atenolol and bopindolol were 24.5 ± 1.2, 12.9 ± 1.2 μU/ml and 8.3 ± 1.2 μU/ml respectively.

As compared to placebo resting blood pressure and heart rate were reduced by the β-adrenoceptor antagonists (Table 1). Forearm flow was not affected by the two β-adrenoceptor antagonists. Infusion of EPI caused a decrease in diastolic arterial pressure both during placebo and during β_1-selective β-blockade with atenolol. In contrast, with bopindolol EPI-infusion led to a small increase in diastolic pressure (Table 1). Neither during placebo nor during the two β-adrenoceptor antagonists did infusion of EPI cause any change in systolic arterial pressure. Forearm flow did not change significantly by EPI during any of the three treatments. Systolic and diastolic arterial pressure, heart rate and forearm flow in the control group 30 min after the first and second SAL-infusion were not different (Table 1).

Resting values of NEart and the production of NE in the forearm 30 min after start of SAL or EPI-infusion are shown in Figure 2. Although the values of these two parameters during placebo, atenolol or bopindolol, at the time of SAL-infusion, did not differ, NEart tended to be lower with bopindolol. Infusion of EPI during placebo caused an increase in NEart ($p < 0.01$) and NE forearm production ($p < 0.05$). No EPI-induced changes in these parameters occurred with atenolol, whereas with bopindolol EPI-infusion led to a decrease in NEart ($p < 0.05$), but not to a decrease in NE forearm production (Figure 2). In the control group NEart during the second SAL-infusion was lower ($p < 0.05$) than during the first infusion, but no difference in NE forearm production was seen during the first and second SAL-infusion (Figure 2).

Stress Tests

All four tests caused an increase in NEart (Table 2). This increment was highest during orthostasis and lowest during cold provocation (Figure 3). As compared to placebo the response of NEart to IET, but not to the other three tests was higher with atenolol ($p < 0.05$) and with bopindolol ($p < 0.05$). During placebo

infusion of EPI enhanced the responses of NEart to IET ($p < 0.05$), CPT ($p < 0.05$) and CWT ($p < 0.01$), but not to OST (Figure 3). This enhancement was abolished by bopindolol as well as by atenolol. In the control group there was no difference in the responses of NEart to the respective tests during the first and second SAL-infusion (Figure 3). The responses of NE forearm production to IET, CPT and OST are summarized in Table 3. With the three different treatments (placebo, atenolol and bopindolol) the responses of NE forearm production to the stress tests showed a considerable variation, but differences were never significant. Unlike to what was observed for the responses of NEart, the NE forearm production in response to the stress tests was not enhanced by EPI-infusion during placebo. EPI had also no effect on the responses of NE forearm production during administration of atenolol or bopindolol. In the control group changes in NE forearm production in response to the first and second series of tests did not differ (Table 3).

Table 2. *Effects of epinephrine on arterial levels of norepinephrine (pg/ml) before and after isometric exercise, cold provocation, orthostatic stress and mental stress during placebo, atenolol and bopindolol*

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

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

8.5 Discussion

Effects of β-Adrenoceptor Blockade on Plasma Epinephrine

With the infusion rate of EPI currently used ven levels of EPI increased to approximately 200 pg/ml during placebo. These levels

are within the high physiological range (29). It is well known that EPI is cleared by β-adrenergic mechanisms and that β-adrenoceptor antagonists diminish the clearance of EPI (30). This explains why both art and ven levels of EPI were higher during administration of the two β-adrenoceptor antagonists. During infusion of EPI art EPI rose relatively more than ven EPI. The fractional extraction of EPI in the forearm was therefore increased. An EPI-induced increase in the fractional extraction of EPI was also observed during administration of atenolol and bopindolol, which is at variance with a report of Best and Halter (24). In their study an acute intravenous dose of propranolol during EPI-infusion undid the EPI-induced increase in forearm fractional extraction of EPI. Differences in the hemodynamic effects of β-adrenoceptor antagonists, when administered acutely or for a more prolonged period, might account for the difference between Best and Halter's findings and our study.

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

Effect of Epinephrine on Local Norepinephrine Production
In an attempt to collect also information on local sympathetic activity, predominantly in the muscles, the production of NE in the forearm (excluding the hand) was calculated. The production at rest was markedly increased by EPI but, unlike the responses of art plasma NE, the responses of NE forearm production to the stress tests were not increased by EPI. For these differential effects of EPI on art plasma NE and on NE forearm production during stress we have no easy explanation. One possibility is that changes in local muscle sympathetic activity do not run in parallel

with changes in overall sympathetic activity, a phenomenon that might be related to regional differences in sympathetic activation during different forms of stresses (31). Another possibility to consider is that during stress the changes in the fractional extraction of EPI are not representative for the changes in the fractional extraction of NE, so that under these circumstances the formula by which we calculated NE production was not valid.

Table 3. *Effects of epinephrine on forearm norepinephrine production (pg 100 ml^{-1} min^{-1}) before and after isometric exercise, cold provocation, orthostatic stress and mental stress during placebo, atenolol and bopindolol*

		Placebo		Atenolol		Bopindolol		Control ¶	
		SAL	EPI	SAL	EPI	SAL	EPI	SAL	SAL
IET	B	263 ± 55	661 ± 117	308 ± 82	310 ± 67	273 ± 92	233 ± 54	468 ± 127	362 ± 95
	A	526 ± 79	938 ± 219	747 ± 168	785 ± 131	416 ± 116	505 ± 92	919 ± 279	672 ± 343
	Δ	263 ± 81	277 ± 155	439 ± 122	474 ± 132	143 ± 69	272 ± 63	451 ± 157	309 ± 272
CPT	B	267 ± 66	558 ± 110	267 ± 43	312 ± 73	253 ± 69	251 ± 43	470 ± 166	285 ± 80
	A	352 ± 57	590 ± 135	463 ± 89	499 ± 109	334 ± 78	363 ± 73	795 ± 180	556 ± 136
	Δ	85 ± 60	32 ± 55	196 ± 79	187 ± 80	80 ± 57	112 ± 57	325 ± 154	271 ± 97
CWT	B	401 ± 67	787 ± 157	295 ± 62	395 ± 73	320 ± 76	279 ± 80	396 ± 109	441 ± 181
	A	733 ± 170	1014 ± 296	524 ± 113	502 ± 96	554 ± 114	517 ± 114	619 ± 111	701 ± 131
	Δ	332 ± 147	227 ± 239	229 ± 64	106 ± 63	223 ± 72	237 ± 73	223 ± 167	259 ± 110

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

Effects of Epinephrine on Baroreflex Activity

The hemodynamic effects of EPI might have influenced sympathetic discharge by changing afferent arterial baroreflex activity. In this regard, the influence of changes in pulse pressure appear to outweight the influence of changes in mean arterial pressure (32). It would therefore be expected that the hemodynamic effects of EPI will stimulate rather than suppress baroreceptors, and as a consequence, will diminish sympathetic activity. This is supported by a recent study of Arnold et al. (33), showing that a constant infusion of the non-selective β-adrenoceptor agonist isoproterenol, with hemodynamic effects comparable to that of EPI, caused a reflex-increase rather than a reflex-withdrawal of vagal cardiac tone. Furthermore, in the present study, although EPI-induced changes

in arterial pressure during placebo and atenolol were comparable, NE increased only during placebo, suggesting that other than hemodynamic changes underlie the alterations in NE.

Effect of Epinephrine on Norepinephrine Clearance

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

Role of Presynaptic β-Adrenoceptors

The demonstration that the EPI-induced elevations in plasma NE and NE forearm production is abolished by bopindolol as well as by atenolol, indicates the involvement of β-adrenoceptors. Similar observations were reported previously (13,14,17,18). The present study adds to this that in this regard β_1-selective blockade with atenolol is equally effective. This finding seems to contradict some pharmacological studies that suggest that presynaptic β-adrenoceptors are of a β_2-subtype (3,4). However, in other experiments it has been shown that facilitation of NE outflow by stimulation of presynaptic β-adrenoceptors can indeed be abolished by the β_1-selective β-adrenoceptor antagonist metropolol (5,7,35). Therefore, some caution is necessary, when the *in vitro* classification of the presynaptic β-adrenoceptor is extrapolated to the *in vivo* situation.

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

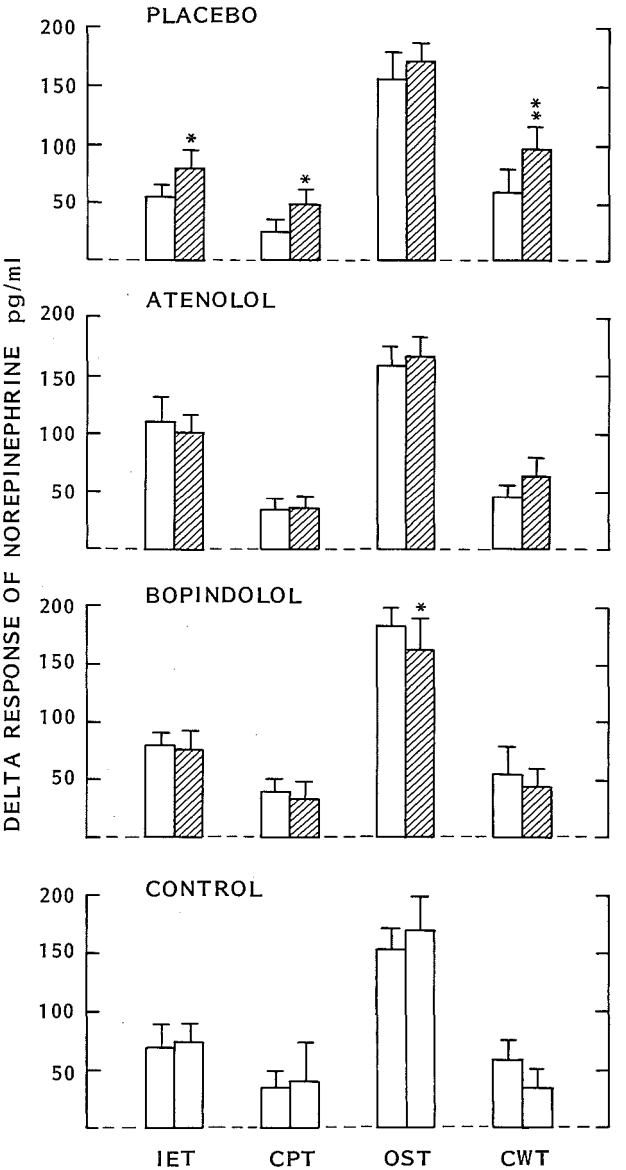

Fig. 3. Effect of epinephrine on the delta responses of arterial plasma levels of norepinephrine to isometric exercise (IET), cold provocation (CPT), orthostatic stress (OST) and mental stress (CWT) during placebo, atenolol and bopindolol. (□) saline, (///) epinephrine infusion. * $p < 0.05$, ** $p 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

In conclusion, the present results provide evidence for the hypothesis that elevated levels of EPI are capable of enhancing sympathetic activity in man. This enhancement is mediated by β-adrenoceptors and can be antagonized not only by non-selective, but apparently also by β_1-selective β-adrenoceptor blocking agents.

8.6 References

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9. Summary and conclusions

9.1 Aim of the study

Chapter 1 and 2

The aim of the studies described in this thesis was to obtain more insight in the mechanism of the antihypertensive action of β-adrenoceptor antagonists in patients with essential hypertension. For this purpose the literature was extensively reviewed. In addition a number of original studies were performed in which the acute hemodynamic and hormonal effects of β-adrenoceptor antagonists with different pharmacological properties, like partial agonist activity (PAA), β_1-selectivity and lipophilicity, were compared. The observation periods in the acute studies lasted for 24 hours. This gave us the opportunity to study in detail the time course of hemodynamic and hormonal changes between the commencement of β-adrenoceptor antagonism and the moment of the onset of the fall in blood pressure. The following five β-adrenoceptor antagonists were studied: propranolol, non-selective, highly lipohilic, no PAA; pindolol, non-selective moderately lipophilic, strong PAA: bopindolol, non-selective, moderately lipophilic, litte PAA; acebutolol, β_1-selective, moderate lipophilic, little PAA and atenolol β_1-selective, hydrophilic and devoid of PAA.

Interaction of β-adrenoceptor antagonists with β-adrenergic receptors results in a change in the density of β-receptors per cell as well as in a change of the properties of these receptors. The cellular response during adrenergic stimulation is influenced by these changes in β-adrenergic receptors. For example an increased number of β-adrenergic receptors in the heart is associated with an increased sensitivity of this organ for endogenous and exogenous catecholamines. In our studies we investigated the effects of β-adrenoceptor antagonists with different pharmacological properties on lymphocyte β-adrenergic receptor density and we have tried to correlate these effects with the chronotropic responsiveness of the heart to the non-selective β-adrenergic agonist

isoprenaline. Experiments in animals and humans have shown that changes in the number of β-adrenergic receptors on circulating lymphocytes correlate well with the changes of β-receptors in less accessible organs as the heart and the lung.
Activation of postganglionic presynaptic β-receptors, which are located on the sympathetic nerve endings, may enhance the release of noradrenaline during sympathetic stimulation by a positive feedback mechanism. Evidence indicates that adrenaline is the natural agonist of this presynaptic β-receptor, which is of the β_2-subtype. In this way adrenaline might play a role in the pathogenesis of some forms of hypertension. Otherwise it has been speculated that β-adrenoceptor antagonists interfere with this positive feedback mechanism, thereby diminishing the release of noradrenaline during sympathetic activation, which may contribute to their blood pressure lowering effect. To test this hypothesis we investigated the effects of a low intravenous dose of adrenaline on arterial plasma noradrenaline levels and on the production of noradrenaline in the forearm, both in the absence and in the presence of β_1-selective and non-selective β-adrenoceptor antagonists.

9.2 Acute effects of β-adrenoceptor antagonism

Chapter 3, 4, 5 and 6

Haemodynamics
With the exception of pindolol, a rapid fall in heart rate and cardiac output was seen after the first oral dose of all β-adrenoceptor antagonists that were studied. The fall in cardiac output was associated with an increase in vascular resistance. Since this vasoconstrictor response was proportional to the fall in cardiac output, both with the non-selective β-adrenoceptor antagonists propranolol and bopindolol as with the β_1-selective antagonists atenolol and acebutol, it is almost certain that this vasoconstriction does not reflect blockade of vasodilator vascular β_2-adrenoceptors (unopposed α-adrenergic stimulation), but represents a reflex circulatory adjustment to the decreased cardiac output.

The acute haemodynamic studies showed unequivocally that the fall in blood pressure after β-adrenoceptor antagonism at all times is due to a fall in vascular resistance. On all β-adrenoceptor antagonists, with the exception of pindolol, the fall in vascular resistance and blood pressure was delayed for several hours, whereas within 24 hours after their first administration an approximately maximal antihypertensive effect was observed, which varied from 11 to 15%. Related to its strong PAA cardiac output did not fall on pindolol and, interestingly, vascular resistance and blood pressure fell almost immediately after its first oral dose, i.e. within the absorption phase of the drug.

Analogous to direct-acting vasodilators the fall in blood pressure and vascular resistance after β-adrenoceptor antagonism was associated with a secondary increase in cardiac output. With acebutolol, atenolol, bopindolol, and propranolol cardiac output returned to pretreatment values, whereas with pindolol it rose above pretreatment values. Significant inverse correlations between the fall in blood pressure and this secondary increment in cardiac output could be demonstrated for all β-adrenoceptor antagonists. Because this increment in cardiac output occurs despite blockade of (cardiac) β-adrenoceptors withdrawal of vagal tone and afterload reduction are the factors most likely to be involved in this haemodynamic adaptation.

The acute haemodynamic studies allow us to draw some conclusions with regard to the mechanism of the blood pressure lowering effect of β-adrenoceptor antagonists. According to the theory of whole body autoregulation cardio-depression may reduce blood pressure indirectly through a readjustment of the vascular resistance to lower level in response to the decreased nutritional flow. The inverse relationships between the fall in blood pressure and the secondary rise in cardiac output after β-adrenoceptor antagonism as well as the absence of any fall in cardiac output after administration of pindolol make it very unlikely that cardio-depression plays a role in the blood pressure lowering action of β-adrenoceptor antagonists.

Active Renin and Plasma Noradrenaline

During β-adrenoceptor antagonism the secretion of active renin is suppressed. This suppression of active renin occurs simultaneously with a fall in heart rate, thus amply before there is any fall in blood pressure. In the present acute studies active renin was measured for the first time two hours after dosing. At that time a maximal suppression of renin was seen with all β-adrenoceptor antagonists, whereas on none of the drugs, with the exception of pindolol, blood pressure had been fallen. Our data further show that β_1-selective and non-selective β-adrenoceptor antagonism result in a similar degree of renin suppression, indicating that the juxta-glomerular β-adrenoceptor, involved in the secretion of renin, is of the β_1-subtype. Despite its strong PAA, the initial suppression of renin on pindolol did not differ from that of the other β-adrenoceptor antagonists. However, 24 hours after dosing of pindolol the suppression of renin was no longer significant, whereas its full antihypertensive effect was still present. With none of the β-adrenoceptor antagonists pretreatment values of active renin and the fall in blood pressure 24 hours after dosing were correlated. Considering the above mentioned findings it seems very unlikely that suppression of renin underlies the antihypertensive mechanism of β-adrenoceptor antagonists.

The plasma concentration of noradrenaline as a measure of overall sympathetic activity, did not change during the acute antihypertensive effect of the β-adrenoceptor antagonists that were studied. What is the interpretation of this finding? The plasma concentration of noradrenaline is determined both by the spillover of noradrenaline from sympathetic nerves into plasma and by its metabolic clearance rate from plasma by the lung and liver. During β-adrenoceptor antagonism the clearance of noradrenaline is impaired due to a diminished flow through liver and lung concomitant with a fall in cardiac output.

Thus unchanged plasma noradrenaline levels as was found in the present study might still be compatible with a reduced sympathetic tone. Furthermore, it should be emphasized, although the vasodilator action of the β-adrenoceptor antagonists was not associated with a decrease in plasma noradrenaline, that it was

not associated with an increase in noradrenaline either. This sharply contrasts with the effects of direct-acting vasodilators and is an indirect indication that β-adrenoceptor antagonists interfere with the sympathetic nervous system.

9.3 Chronic effects of β-adrenoceptor antagonism

Chapter 7

Haemodynamics

The chronic haemodynamic studies show that β-adrenoceptor antagonists, irrespective of their different ancillary pharmacological properties, are equally effective in lowering an elevated blood pressure when adequately dosed, but that the haemodynamic changes underlying this antihypertensive effect differ considerably. With pindolol the fall in blood pressure was entirely due to a fall in vascular resistance. Conversely, during treatment with propranolol the fall in blood pressure was predominantly associated with a decrease in cardiac output, and vascular resistance as compared to pretreatment values remained unchanged. No significant changes in vascular resistance or cardiac output were seen with atenolol, acebutolol or bopindolol. In the light of the absence of important differences of the acute and chronic haemodynamic effects of atenolol on the one hand and the effects of acebutolol and bopindolol on the other hand it can be concluded that the PAA of the latter two drugs is relatively weak.

Renal Function

As estimated from changes in serum creatinine levels β-adrenoceptor antagonists seem to have little or no adverse effects on renal function. In patients with renal failure, deterioration of renal function during β-adrenoceptor antagonism has sporadically been reported. Cardio-depression, suppression of renin and blockade of vasodilating β_2-receptors within the kidney are among the factors with may adversely effect renal function during β-adrenoceptor antagonism. In the present studies glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by means

of the clearance of ^{131}I-thalamate and ^{125}I-hippuran, respectively. No change in GFR was seen with any of the β-adrenoceptor antagonists. Changes in renal blood flow (calculated from the changes in ERPF) and changes in renal vascular resistance ran in parallel with the changes in cardiac output and systemic vascular resistance.
Thus, during pindolol renal blood flow increased and renal vascular resistance decreased, whereas these two parameters changed in the opposite direction during propranolol. Since plasma renin activity was decreased to a similar extent during chronic treatment with the β-adrenoceptor antagonists (vide infra) our findings suggest that changes in systemic haemodynamics during β-adrenoceptor antagonism are the most important determinant for the β-adrenoceptor antagonist-induced changes in renal haemodynamics.

Active Renin and Plasma Noradrenaline
The degree of renin suppression after three weeks β-adrenoceptor antagonism varied from 60 to 70%. Chronic treatment with pindolol resulted in a similar degree of renin suppression as did treatment with the other β-adrenoceptor antagonists, which have little or no PAA. This is a striking finding in the light of the effects of pindolol on the heart. Apparently the juxta-glomerular β-adrenoceptor, which is of the β_1-subtype, is not stimulated by pindolol's PAA.
For some years it is known that the human heart, besides β_1-, also contains β_2-adrenoceptors. The absence of any perceptible effect of pindolol's PAA on the juxta-glomerular β-receptor, and the clear presence of this effect on the cardiac β-receptor might be explained by assuming that the PAA of pindolol predominantly stimulates the β_2 — and not, or to a far lesser extent, the β_1-adrenoceptor. Experimental studies support this assumption. In accordance to the results of the acute study, pretreatment values of active renin and the fall in blood pressure during chronic treatment with the different β-adrenoceptor antagonists were not correlated. Plasma noradrenaline concentrations during chronic β-adrenoceptor antagonism did not change.

9.4 Lymphocyte β-adrenoceptor density and cardiac chronotropic responsiveness to isoprenaline

For the assessment of the cardiac chronotropic responsiveness the dose of isoprenaline (ng/kg/min) to increase the heart rate by 25 beats/min (CD25) was calculated for each patient. A high CD25-value indicates a low chronotropic responsiveness and vice versa. Pretreatment values of lymphocyte β-adrenoceptor density and CD25 were inversely correlated. During administration of the non-selective antagonist propranolol lymphocyte β-adrenoceptor density increased by approximately 40%, whereas during treatment with pindolol the number of lymphocyte β-adrenoceptors was decreased by a similar percentage. The changes in lymphocyte β-adrenoceptor density were apparent already 24 hours after the first dose.

Furthermore, one week after withdrawal of the two β-adrenoceptor antagonists β-adrenoceptor density was still increased with propranolol and still decreased with pindolol. As compared to pretreatment values the CD25 at that time was decreased with propranolol and increased with pindolol, indicating respectively an increased and a decreased chronotropic responsiveness of the heart.

Lymphocyte β-adrenoceptors are of a homologous β_2-subtype. It is to expected therefore that the number of these receptors do not change during administration of β_1-selective antagonists. This was indeed the case. No change in lymphocyte β-adrenoceptor density was observed during administration of atenolol and acebutolol. The CD25, as compared to pretreatment values, was decreased one week after withdrawal of atenolol, whereas the CD25 one week after withdrawal of acebutolol had not changed. The increased chronotropic responsiveness one week after withdrawal of atenolol might be explained by an increased number of β_1-receptors in the heart. Since previous studies have shown that changes in number of β-adrenoceptors on lymphocytes are representative for the changes of β-receptors in less accessible organs as the heart and the lung, our findings suggest that the so-called β-blocker-withdrawal-syndrome depends on a β-

adrenoceptor antagonist-induced increase in the number of cardiac β-adrenoceptors. This fits well with the observations that this syndrome has been described after abrupt discontinuation of treatment with propranolol and atenolol, but not after discontinuation of treatment with pindolol.
Further evidence that endogenous regulation of the number of β-adrenoceptors on lymphocyte membranes also occurs is provided by the inverse relationship between the plasma level of adrenaline and lymphocyte β-adrenoceptor density.

9.5 Adrenaline, sympathetic activity and β-adrenoceptor antagonism

Chapter 8
In accordance to previous studies of our group it was shown that a low-dose intravenous infusion of adrenaline increased arterial levels of noradrenaline as an index of overall sympathetic activity. Infusion of adrenaline also increased the production of noradrenaline in the forearm as an index of local muscle sympathetic activity. This facilitatory effect of adrenaline was abolished not only by the non-selective antagonist bopindolol, but also by the β_1-selective antagonist atenolol. This latter finding was unexpected, since most pharmacological studies have shown that the presynaptic β-adrenoceptor is of the β_2-subtype. Our finding therefore suggests that in the in-vivo situation, the presynaptic β-adrenoceptor in man is not β_2-selective, or alternatively that atenolol in the dose in which it was used has the ability to block presynaptic β-adrenoceptors.

9.6 Final conclusions

Considering the above mentioned results it can be concluded that the fall in blood pressure after β-adrenoceptor antagonism is always due to a fall in peripheral vascular resistance. The precise mechanism underlying this fall in peripheral vascular resistance cannot be directly concluded from our studies.
However, in our view it is very unlikely that a fall in cardiac

output plays a crucial role in the antihypertensive mechanism of β-adrenoceptor antagonists. Although, active renin is decreased during administration of β-adrenoceptor antagonists the differences in time courses between the fall in renin and the fall in blood pressure and the absence of a relationship between pretreatment values of renin and the fall in blood pressure make it also very unlikely that suppression of renin plays an important role in the antihypertensive effect of β-adrenoceptor antagonists. As extensively discussed circumstantial evidence indicates that β-adrenoceptor antagonism interferes with sympathetic activity, by which neurogenic α-adrenergic vasoconstriction during β-adrenoceptor antagonism may diminish. In principle blockade of β-adrenoceptors within the central nervous system as well as blockade of peripheral presynaptic β-adrenoceptors may ultimately result in a diminished sympathetic activity. The observation that hydrophilic β-adrenoceptor antagonists with a poor penetration of the blood-brain barrier are equally effective in lowering an elevated blood pressure as lipophilic β-adrenoceptors antagonists is more in favor of the second possibility. Abolishment of adrenaline-induced amplification of sympathetic activity by β-adrenoceptor antagonists adds further evidence that β-adrenoceptor antagonism interferes with sympathetic activity.

Finally our study confirms that the lymphocyte β-adrenergic receptor is a useful model for the study of cardiovascular β-adrenoceptors, since 'up' and 'down' regulation of lymphocyte β-adrenergic receptors, evoked both by endogenous adrenaline as well as by β-adrenoceptor antagonists, correlated well with the cardiac chronotropic responsiveness to isoprenaline.

10. Samenvatting en conclusies

10.1 Doel van de studie

Hoofdstuk 1 en 2

β-Adrenerge-receptor-blokkerende middelen (β-blokkers) werden oorspronkelijk ontwikkeld voor de behandeling van patiënten met angina pectoris. Het idee achter deze ontwikkeling was dat door remming van de invloed van het sympathische zenuwstelsel op het hart, met name tijdens stress-volle omstandigheden, de cardiale zuurstofkonsumptie, zou afnemen, hetgeen potentieel gunstig zou kunnen zijn voor patiënten met angina pectoris. Al spoedig nadat β-blokkers beschikbaar waren voor klinisch onderzoek, nu alweer ruim twee decennia geleden, bleek dat deze middelen een belangrijk bloeddrukverlagend effect hadden. Deze bevinding was onverwachts, daar bij dierexperimenteel onderzoek geen bloeddrukverlagend effect was vastgesteld.

Teneinde het mechanisme waardoor β-blokkers de bloeddruk verlagen te ontrafelen zijn in de loop der jaren een groot aantal studies verricht, maar tot op heden is het werkingsmechanisme van deze middelen niet zeker. Door studies met de dextro-isomeer van propranolol, een stof die geen β-blokkerende, maar wel de andere eigenschappen van propranolol bezit en die de bloeddruk bij de mens niet verlaagt, staat het vast dat het bloeddrukverlagend effect van β-blokkers inderdaad berust op blokkade van de β-adrenerge receptoren. β-adrenerge receptoren zijn in vrijwel alle weefsels aanwezig, maar voor wat betreft de bloeddrukregulatie zijn potentieel alleen die receptoren van belang die gelokaliseerd zijn in het hart, het centrale zenuwstelsel, op de uiteinden van de perifere sympathische zenuwen (postganglionaire prejunctionele β-receptoren) en op de juxta-glomerulaire cellen van de nieren. Blokkade van de receptoren in deze organen zou tot een bloeddrukdaling kunnen leiden door respectievelijk a) een afname van het hartminuutvolume, b) een vermindering van de centrale efferente sympathische activiteit en/of een nieuwe instelling van

de arteriële baroreflex, c) een vermindering van de perifere noradrenaline-vrijmaking tijdens sympathische stimulatie en d) een afname van de reninesecretie door de nier.
Door studies met β-blokkers, die een belangrijke mate van partieel agonistische activiteit (PAA) bezitten kunnen bij enkele van de bovengenoemde theorieën op zijn minst vraagtekens geplaatst worden. β-blokkers met een belangrijke mate van PAA geven in rust slechts een geringe daling van het hartminuutvolume, terwijl het bloeddrukverlagend effect van deze middelen niet minder is dan dat van β-blokkers zonder PAA. Dit suggereert dat de bloeddrukdaling tijdens β-blokkade niet zonder meer uit een daling van het hartminuutvolume verklaard kan worden. Er is ook aangetoond dat bij eenzelfde antihypertensief effect de renine-suppressie minder is op β-blokkers met PAA dan op β-blokkers zonder deze eigenschap. Deze bevinding maakt de renine-theorie als verklaring voor het bloeddrukverlagend effect van β-blokkers minder waarschijnlijk.
Een moeilijkheid bij het bestuderen van het bloeddrukverlagend mechanisme van β-blokkers is de relatief traag intredende antihypertensieve respons na toediening van deze middelen. Na acute toediening van een β-blokker daalt vrijwel onmiddellijk het hartminuutvolume. Als antwoord op deze daling van het hartminuutvolume treedt een baroreflex-gemedieerde compensatoire systemische vasoconstrictie op, waardoor de bloeddruk aanvankelijk niet verandert. Hemodynamisch wordt een reeds langer bestaande hypertensie gekarakteriseerd door een te hoge perifere vaatweerstand.
Tijdens chronische β-blokkade daalt de bloeddruk, terwijl de initiële afname van het hartminuutvolume in min of meer dezelfde mate persisteert. Door interpolatie van de acute en chronische hemodynamische effecten van β-blokkers kan geconcludeerd worden dat de bloeddrukdaling na β-blokkade tot stand komt door een afname van de initieel nog verder gestegen vaatweerstand.
Aanvankelijk werd gedacht dat tussen het starten van β-blokkade en het volledige antihypertensieve effect een periode van weken lag. Op grond van meer recent onderzoek is het waarschijnlijker dat deze periode enkele uren tot ongeveer een dag bedraagt. Het

preciese interval is evenwel niet bekend, omdat gedetailleerd onderzoek naar het tijdsverloop tussen aanvang van β-blokkade en het moment waarop de bloeddruk begint te dalen ontbreekt. De hier beschreven studies hebben primair tot doel meer inzicht te verkrijgen in de wijze waarop β-blokkers de bloeddruk verlagen bij patiënten met essentiële hypertensie. Hiertoe werden zowel op grond van een uitgebreide literatuurstudie, als op grond van eigen onderzoek de acute en chronische hemodynamische en hormonale effecten van β-blokkers met verschillende farmakologische eigenschappen, zoals PAA, β_1-selectiviteit en lipofiliteit, met elkaar vergeleken. In het eigen onderzoek was de observatieperiode tijdens de acute studies 24 uur, waardoor het tijdsverloop van de hemodynamische en hormonale veranderingen, tussen aanvang van β-blokkade en het moment waarop de bloeddrukdaling begon, in detail bestudeerd kon worden. Vijf β-blokkers werden bestudeerd te weten: propranolol, niet-selectief, sterk lipofiel, geen PAA; pindolol, niet-selectief, matig lipofiel, sterke PAA; acebutolol, β_1-selectief, matig lipofiel, geringe PAA, bopindolol, niet-selectief, matig lipofiel en gering PAA en atenolol, β_1-selectief, hydrofiel en geen PAA.

Interactie van een β-blokker met de β-adrenerge receptor kan resulteren in veranderingen van zowel de eigenschappen van de receptor als van het aantal receptoren per cel. Een toename van het aantal β-adrenerge receptoren in bijvoorbeeld het hart gaat gepaard met een verhoogde gevoeligheid van dit orgaan voor endogene of exogene catecholaminen en ligt wellicht ten grondslag aan het zogenaamde beta-blokker-ontrekkingssyndroom. Wij hebben getracht het effect van β-blokkers met verschillende farmakologische eigenschappen op de receptordichtheid van lymfocytmembranen na te gaan en deze effecten te relateren aan de chronotrope gevoeligheid van het hart voor de niet-selectieve β-agonist isoprenaline. Met name dierexperimenteel onderzoek heeft aangetoond dat veranderingen van het aantal β-adrenerge receptoren op circulerende lymphocyten goed correleren met veranderingen van deze receptoren in minder toegankelijke organen zoals hart en longen.

Door activatie van postganglionaire prejunctionele β-receptoren,

die gelokaliseerd zijn op de uiteinden van sympathische zenuwen kan via een positief terugkoppelingsmechanisme de vrijmaking van noradrenaline tijdens sympathische stimulatie gepotentieerd worden. Het "stress-hormoon" adrenaline zou de belangrijkste natuurlijke agonist zijn van deze presynaptische β-receptor, welke van het β_2-subtype is. Er is gespeculeerd dat adrenaline op deze wijze een rol zou kunnen spelen bij de pathogenese van sommige vormen van hypertensie. Anderzijds zouden β-blokkers deze positieve feedback kunnen remmen, waardoor de afgifte van noradrenaline tijdens sympathische stimulatie afneemt. Theoretisch zou via dit mechanisme de bloeddruk tijdens β-blokkade verlaagd kunnen worden. Teneinde deze hypothese te toetsen hebben wij onderzocht wat de effecten van een lage dosering adrenaline zijn op plasma noradrenaline spiegel en de productie van noradrenaline in de onderarm zowel tijdens af- en aanwezigheid van β-blokkade.

10.2 Acute effecten van β-blokkade

Hoofdstukken 3, 4, 5 en 6

Hemodynamiek

Na de eerste orale gift werd op alle onderzochte β-blokkers met uitzondering van pindolol een snelle daling van de hartfrekwentie en het hartminuutvolume gezien. De daling van het hartminuutvolume ging gepaard met een stijging van de vaatweerstand, terwijl de bloeddruk aanvankelijk niet veranderde. Omdat zowel na niet-selectieve β-blokkade (propranolol en bopindolol) als na β_1-selectieve β-blokkade (atenolol en acebutolol) de stijging van de vaatweerstand volledig kompenseerde voor de afname van het hartminuutvolume is het welhaast zeker dat deze vasoconstrictie tot stand komt door een reflectoire aanpassing van de circulatie aan het afgenomen hartminuutvolume en dat zij niet berust op blokkade van vasodilaterende vasculaire β_2-receptoren ("unopposed" α-adrenerge stimulatie).

Dat de bloeddrukdaling na β-blokkade altijd tot stand komt door een daling van de al of niet initieel gestegen perifere vaatweerstand kon in de acute hemodynamische studies op ondubbelzinnige wijze

worden aangetoond. Met uitzondering van pindolol waarop een onmiddellijke weerstands- en bloeddrukdaling werd gezien zonder cardio-depressie, trad deze weerstands- en bloeddrukdaling niet eerder dan enkele uren na aanvang van β-blokkade op, terwijl binnen 24 uur op alle β-blokkers een vrijwel maximaal en ongeveer gelijk antihypertensief effect werd bereikt, variërend van 11 tot 15%. De weerstandsdaling op de verschillende β-blokkers bleek voor dezelfde mate van bloeddrukdaling steeds hetzelfde te zijn. Hierdoor werd, rekening houdende met de basale omstandigheden waaronder onze studies werden verricht, het uiteindelijke bereikte niveau van de weerstand bepaald door de mate van initiële vasoconstrictie en dus door de mate van cardio-depressie, zoals bepaald door de mate van PAA van de toegediende β-blokker. Een opvallende en onverwachte bevinding was, dat in analogie met direct-werkende vaatverwijders, de bloeddruk- of weerstandsdaling na β-blokkade gepaard ging met een secundaire stijging van het hartminuutvolume. Het hartminuutvolume keerde terug tot uitgangsniveau tijdens acebutolol, atenolol, bopindolol en propranolol en steeg significant tot boven het uitgangsniveau na pindolol. Tussen deze stijging van het hartminuutvolume en bloeddrukdaling kon voor alle β-blokkers een significante omgekeerde relatie worden aangetoond. Afname van vagustonus en daling van de perifere vaatweerstand spelen waarschijnlijk een belangrijke rol bij deze secundaire stijging van het hartminuutvolume.

Alhoewel het op grond van de acute hemodynamische effecten niet zonder meer duidelijk is welk mechanisme ten grondslag ligt aan het bloeddruk- of weerstandsverlagend effect van β-blokkers, kunnen met betrekking tot dit mechanisme wel enige conclusies getrokken worden. Via autoregulatie zou afname van het hartminuutvolume op indirecte wijze via afname van de weefselperfusie tot vasodilatatie en bloeddrukdaling kunnen leiden. De inverse relatie tussen bloeddrukdaling en secundaire toename van het hartminuutvolume na β-blokkade en het ontbreken van een hartminuutvolumedaling op pindolol maken het erg onwaarschijnlijk dat afname van het hartminuutvolume op directe of indirecte wijze een rol speelt bij het bloeddrukverlagend mechanisme van

β-blokkers.

Actief Renine en Plasma Noradrenaline
De plasma renine activiteit daalt na β-blokkade. Het is aangetoond dat deze daling van het plasma renine snel optreedt, simultaan met de daling van de hartfrekwentie en dus ruimschoots voordat de bloeddruk gaat dalen. In de huidige acute studies werd het actief plasma renine niet eerder dan twee uur na aanvang van β-blokkade gemeten. Op dat moment was op alle onderzochte β-blokkers een maximale renine suppressie aanwezig, terwijl op geen van de middelen, met uitzondering van pindolol, de bloeddruk was gedaald. Onze gegevens toonden verder aan dat β_1-selectieve β-blokkade en niet-selectieve β-blokkade resulteerden in eenzelfde mate van reninesuppressie. Hieruit kan gekonkludeerd worden dat de juxta-glomerulaire β-receptor, die de secretie van renine medieert, van het β_1-subtype moet zijn. Ondanks de aanzienlijke mate van PAA van pindolol was de initiële suppressie van renine op dit middel niet minder dan die op de andere β-blokkers. Vierentwintig uur na toediening van pindolol was er echter geen significante daling van het actief renine meer, terwijl het bloeddruk-verlagend effect op dat moment nog volledig aanwezig was. Bij geen van de onderzochte β-blokkers waren uitgangswaarden van plasma renine activiteit en antihypertensief effect aan het eind van de 24 uurs studie gecorreleerd. Op grond van bovenstaande is het niet erg waarschijnlijk dat reninesuppressie ten grondslag ligt aan het antihypertensieve effect van β-blokkers.
De plasmaspiegel van noradrenaline, als maat voor globale sympathicus activiteit, veranderde niet tijdens het acute bloeddruk-verlagend effect van de onderzochte β-blokkers. Hoe moet dit gegeven geïnterpreteerd worden? De concentratie van noradrenaline in het plasma wordt bepaald door zowel de "spillover" van noradrenaline uit de sympathische zenuwen, als door de metabole klaring van de neurotransmitter door de lever en de longen. Door een verminderde doorbloeding van lever en longen, gekoppeld aan een gedaald hartminuutvolume, neemt de klaring van noradrenaline tijdens β-blokkade af. Bij een gelijk gebleven sympathische activiteit zal een verminderde klaring resulteren in

een verhoogde plasma noradrenaline spiegel. Een onveranderde noradrenaline concentratie, zoals werd vastgesteld in deze studie, is dus zeer wel verenigbaar met een afgenomen sympathicotonus. Ook het gegeven dat het acute vasodilaterend effect van β-blokkers, in tegenstelling tot dat van direct-werkende vaatverwijders, niet gepaard gaat met een stijging van het noradrenaline is een indirecte aanwijzing dat deze middelen interfereren met de sympathicus-activiteit.

10.3 Chronische effecten van β-blokkade

Hoofdstuk 7

Hemodynamiek
De chronische studies laten zien dat bij adequate dosering het antihypertensieve effect van β-blokkers met verschillende farmacologische eigenschappen hetzelfde is, maar dat de hemodynamische veranderingen die ten grondslag liggen aan dit bloeddrukverlagend effect sterk kunnen variëren. Zo berustte de bloeddrukdaling op pindolol vrijwel geheel op een daling van de systemische vaatweerstand en, conform de bevindingen van de acute studie, steeg het hartminuutvolume op dit middel.
Omgekeerd ging met propranolol de bloeddrukdaling vooral gepaard met een daling van het hartminuutvolume en bleef de vaatweerstand ten opzichte van de uitgangssituatie onveranderd. Geen significante veranderingen in vaatweerstand of hartminuutvolume werden gezien tijdens chronische β-blokkade met atenolol, acebutolol of bopindolol. Op grond van het ontbreken van belangrijke verschillen in de acute en chronische hemodynamische effecten van atenolol enerzijds en acebutolol en bopindolol anderzijds moet gekonkludeerd worden dat de PAA van de laatste twee middelen relatief gering is.

Nierfunctie
Het effect van β-blokkers op de nierfunctie, zoals gemeten aan het serumkreatinine gehalte, blijkt gering te zijn. Alleen bij patiënten met een reeds gestoorde nierfunctie is een verslechtering van de

nierfunctie tijdens behandeling met β-blokkers wel eens beschreven. Op theoretische gronden zouden met name door daling van hartminuutvolume en reninesuppressie de renale perfusie en glomerulaire filtratie tijdens β-blokkade kunnen afnemen. In het huidige onderzoek werd de glomerulaire filtratie snelheid (GFR) en de effectieve renale plasma flow (ERPF) gemeten door middel van de klaring van respectievelijk het I^{131}-thalamaat en het I^{125}-hippuran. Op geen van de onderzochte β-blokkers werd een significante verandering van de GFR gezien. De veranderingen in nierdoorbloeding (berekend uit de ERPF) en niervaatweerstand liepen volledig parallel aan de veranderingen in hartminuutvolume en perifere vaatweerstand. Zo steeg de nierdoorbloeding en daalde de niervaatweerstand tijdens pindolol, terwijl deze twee parameters in tegenovergestelde richting veranderden tijdens propranolol. Aangezien de plasma renineactiviteit op de verschillende β-blokkers tijdens chronische behandeling in dezelfde mate gesupprimeerd was (vide infra), is het op grond van bovenstaande aannemelijk dat de systemische hemodynamische effecten van β-blokkade de belangrijkste determinant is voor de optredende veranderingen in renale hemodynamiek.

Actief Renine en Plasma Noradrenaline

De daling van het actief renine na drie weken beta-blokkade varieerde van 60 tot 70%. Een opvallende bevinding in het licht van pindolol's cardiale effecten was dat het actief renine op dit middel even sterk daalde als op de andere β-blokkers, die geen of een veel geringere mate van PAA bezitten. Blijkbaar wordt de juxta-glomerulaire β-receptor, die van het β_1-subtype is, niet gestimuleerd door pindolol's PAA. Sinds enige tijd is het bekend dat er in hart naast $beta_1$- ook $beta_2$-receptoren gelokaliseerd zijn. De afwezigheid van enig zichtbaar effect van pindolol's PAA op juxta-glomerulaire β-receptor en de evidente aanwezigheid van dit effect op de cardiale β-receptoren zou daarom verklaard kunnen worden door aan te nemen dat de PAA van pindolol vooral de $beta_2$- en in veel mindere mate of zelfs geheel niet de β_1-receptor stimuleert. Experimenteel onderzoek lijkt deze verklaring te ondersteunen. Overeenkomstig de resultaten van de acute studies

bleek dat ook tijdens chronische behandeling met de verschillende β-blokkers de uitgangswaarden van het actief renine en het antihypertensief effect niet gecorreleerd waren.
Op geen van de β-blokkers veranderden tijdens chronische toediening de plasmaconcentraitie van noradrenaline.

10.4 β-Adrenerge receptordichtheid op lymphocytenmembranen en cardiale chronotrope gevoeligheid voor isoprenaline

De dosering van de niet-selectieve β-agonist isoprenaline waarop de hartfrekwentie met 25 slagen per minuut stijgt (CD25) werd als maat voor de chronotrope gevoeligheid van het hart gebruikt. Een hoge CD5 betekent een lage chronotrope gevoeligheid en vice versa.
Bij de 40 onderzochte patiënten bleek tussen de uitgangswaarden van de CD25 en de maximale dichtheid van β-receptoren op lymfocytmembranen een omgekeerde correlatie te bestaan. Met andere woorden hoe lager het aantal β-receptoren bij een patiënt hoe hoger de CD25 en hoe kleiner de chronotrope gevoeligheid van het hart. Tijdens behandeling met de niet-selectieve antagonist propranolol steeg het aantal receptoren met ongeveer 40%, terwijl op de niet-selectieve partiële agonist pindolol het aantal receptoren met eenzelfde percentage daalde. Op beide β-blokkers waren deze veranderingen in het aantal β-receptoren reeds 24 uur na de eerste gift aanwezig. Een week na het stoppen van de twee β-blokkers was het aantal receptoren op propranolol nog steeds verhoogd en op pindolol nog steeds verlaagd. Ten opzichte van de uitgangswaarden was op dat moment de CD25 verlaagd op propranolol en verhoogd op pindolol, wijzend op een respectievelijk toegenomen en afgenomen chronotrope gevoeligheid van het hart. Omdat de β-receptoren op lymfocyten uitsluitend van het β_2-subtype zijn, kan verwacht worden dat het aantal receptoren niet verandert na toediening van β_1-selectieve β-blokkers. Dit bleek inderdaad zo te zijn, geen veranderingen in het aantal receptoren werden gezien tijdens de behandeling met atenolol en acebutolol. Een week na het stoppen van atenolol was de CD25 ten opzichte

van de uitgangssituatie gedaald, terwijl de CD25 na het stoppen van acebutolol niet veranderd was. De toename van de cardiale chronotrope gevoeligheid na het onttrekken van atenolol kan wellicht verklaard worden uit een toename van het aantal β_1-receptoren in het hart. Op grond van studies die hebben laten zien dat veranderingen van het aantal β-adrenerge receptoren op lymfocyten representatief zijn voor de veranderingen van deze receptoren in andere organen zoals het hart, suggereren onze gegevens dat het zogenaamde β-blokker- onttrekkingssyndroom berust op een door β-blokkade geïnduceerde toename van het aantal cardiale β-receptoren. In overeenstemming hiermee is dat het β-blokker-onttrekkings syndroom wel na het stoppen van propranolol als ook na het stoppen van atenolol, maar niet na het stoppen van pindolol beschreven is. Tenslotte de inverse correlatie receptoren kunnen geïnterpreteerd worden als een aanwijzing voor een endogene regulatie van de β-receptoren.

10.5 Adrenaline, sympathische activatie en β-blokkade

Hoofdstuk 8

Verondersteld wordt, dat adrenaline door stimulatie van de perifere prejunctionele β-receptor via een positieve terugkoppeling de vrijmaking van noradrenaline uit de sympathische zenuwen faciliteert. De dosering van het intraveneuze adrenaline infuus in onze studies was zodanig dat de plasma adrenaline spiegel tot binnen het hoog fysiologische bereik steeg. De globale sympathische activiteit, gemeten aan de arteriële plasma noradrenaline spiegel tijdens basale omstandigheden en als respons op diverse stress-testen, nam toe onder invloed van adrenaline. Ook de locale skeletspier sympathische activiteit, gemeten aan de productie van noradrenaline in onderarm, steeg onder basale omstandigheden door het adrenaline infuus. Door β-blokkade kon het potentiërend effect van adrenaline op de sympathische activiteit opgeheven worden, hetgeen er op duidt dat β-adrenerge receptoren betrokken waren bij dit faciliterend effect van adrenaline. In het licht van de meeste farmakologische in-vitro studies, die suggereren dat de presynaptische β-receptor van het β_2-subtype is, was een onverwachte bevinding dat β_1-

selectieve β-blokkade met atenolol even effectief was in het antagoneren van de adrenaline gepotentieerde sympathische activiteit als de niet-selectieve β-blokker bopindolol. Deze bevinding suggereert dat de presynaptische β-receptor bij de mens in de in-vivo situatie niet selectief van het β_2-subtype is, of dat atenolol in de onze studie gebruikte dosering in staat is prejunctionele β-receptoren te blokkeren.

10.6 Eind conclusies

Bovengenoemde resultaten overziende kan geconcludeerd worden dat bloeddrukdaling na β-blokkade altijd tot stand komt door een daling van de al of niet initieel gestegen perifere vaatweerstand. Het preciese mechanisme dat aan deze weerstandsdaling ten grondslag ligt kan op grond van onze studies niet geheel opgehelderd worden. Wel staat het ons inziens vast dat daling van het hartminuutvolume geen causale rol speelt bij het bloeddrukverlagend effect van β-blokkers. Alhoewel het actief plasma renine belangrijk daalt na β-blokkade is op grond van het asynchronistisch verloop van renine- en bloeddrukdaling en op grond van de afwezigheid van een correlatie tussen uitgangswaarden van renine en uiteindelijke bloeddrukdaling het evenmin erg waarschijnlijk dat suppressie van renine een primaire causale rol speelt bij het bloeddrukverlagend effect van β-blokkers. Dat bij de relatief zeldzame vormen van hypertensie met een sterk verhoogde activiteit van het plasma renine het renine supprimerend effect van β-blokkade een bijdrage kan leveren aan de bloeddrukdaling is zeer wel mogelijk.
Zoals reeds uitvoerig besproken zijn er aanwijzingen dat β-blokkade interfereert met de sympathische activiteit, hierdoor zou de neurogene α-adrenerge vasoconstrictie tijdens β-blokkade kunnen verminderen. Interferentie met de sympathische activiteit kan in principe tot stand komen door blokkade van β-adrenerge receptoren in de hersenen of door blokkade van de postganglionaire prejunctionele β-receptoren in de perifere sympathische zenuwen. De observatie dat hydrofiele beta-blokkers met een slechte penetratie van de bloed-hersen-barrière even effectief zijn in het verlagen van de bloeddruk als lipofiele beta-blokkers wijst meer in de richting

van de tweede mogelijkheid. De bevinding, dat de door adrenaline-infusie verhoging van sympathische activiteit ongedaan gemaakt wordt door β-blokkade, is eveneens een aanwijzing dat β-blokkers kunnen interfereren met de sympathische activiteit.
Tenslotte bevestigt onze studie dat de β-receptor op de lymfocytenmembraan een goed model is voor de studie van cardiovasculaire β-receptoren, daar zowel door endogeen adrenaline veroorzaakte als door β-blokkade veroorzaakte "up" en "down" regulatie van β-adrenerge receptoren op de lymfocyt goed correleerden met de cardiale gevoeligheid voor isoprenaline.

Curriculum vitae

De schrijver van dit proefschrift werd op 13 december 1950 geboren te Breda. Na het behalen van het eindexamen Hogereburgerschool B aan het Onze-Lieve-Vrouwelyceum te Breda in 1969, begon hij in dat zelfde jaar de medische studie aan de Rijksuniversiteit Utrecht. Tijdens deze studie werkte hij twee jaar als studentassistent op de afdeling Anatomie (hoofd: Prof. dr. W. J. van Doorenmaalen). Na het artsexamen in 1976 begon hij zijn specialisatie tot internist op de afdeling Inwendige Geneeskunde (hoofd: dr. C. J. van Belle †) van het Militair Hospitaal Dr. A. Mathijsen te Utrecht. In 1979 werd de specialisatie vervolgd op de afdeling Inwendige Geneeskunde van het Academisch Ziekenhuis Utrecht (hoofden: Prof. dr. J. van der Sluys Veer en Prof. dr. A. Struyvenberg). Tijdens deze periode heeft hij onderzoek verricht op de afdeling Nierziekten en Hypertensie (hoofd Prof. dr. E. J. Dorhout Mees). Na inschrijving in het Specialisten Register in 1982 volgde een vier maanden durende waarneming in de internisten praktijk van de Nederlands Hervormde Diaconessen Inrichting te Meppel. Sinds februari 1983 tot heden is hij verbonden aan de afdeling Inwendige Geneeskunde I (hoofd: Prof. dr. M. A. D. H. Schalekamp) van het Academisch Ziekenhuis Dijkzigt.

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