IN SEARCH OF HYPERTENSIVE MECHANISMS

USE OF PHARMACOLOGICAL PROBES

(SPEURTOCHT NAAR OORZAKEN VOOR VERHOOGDE BLOEDDRUK
GENEESMIDDEL ALS ONDERZOEKSINSTRUMENT)

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"I found it so truly difficult that I almost believed that it was to be understood by God Alone".

William Harvey, De Motu Cordis (1628)

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INTRODUCTION AND AIM OF THE STUDIES

The literature on hypertension has grown at such an explosive rate that few, if any, are able to keep abreast of it. "The problem is not that we know too little, but that we don't know how much we know", these words of an anonymous session chairman cited by Folkow in 1984 seem even more appropriate to date. Hypertension has become accepted as a multifactorial disease of regulation; it results from an inbalance in various mechanisms such as hormones, enzymes, peptides, endothelial and vascular factors which all serve to control arterial pressure at a normal level. The contribution of the studies described in this thesis to this complex problem is small and sometimes allready outdated. Fortunately, the frustrated reader easily can turn to the wealth of other studies available in the field. In collecting the papers for this thesis I found solace in the words of Auguste Comte: To understand a science one must know its history.

PART 1 ACE INHIBITION

In the course of a longlasting and often passionate research of the renin-angiotensin-aldosterone system the group around professor Schalekamp has followed many tracks, but in 1979 we fell almost instantaneously in love with captopril and with the idea for which the drug stands: blocking the activity of the renin angiotensin system by angiotensin converting enzyme (ACE) inhibition. However we were not alone in our adoration and captopril appeared to be no virgin. In a rush for publication many groups opted for simplicity: captopril acted primarily by blocking the formation of circulating angiotensin II. However diversity and confusion regarding the mechanism of action of captopril soon became apparent.

CHAPTER 1

Chapter 1 of this thesis brings together studies which deal with the question of the specificity of captopril's mode of action. In studies bound to be controversial we have tried to clarify whether or not pretreatment plasma renin is an important predictor of the blood pressure response to ACE inhibition. For this purpose the effects of captopril and later ramipril on blood pressure were investigated in patients with an extremely wide range of plasma levels of renin i.e. normotensive fluid-depleted anephric patients in whom active renin in plasma was almost zero, patients with essential hypertension with low to normal renin, patients with renovascular hypertension with normal to high renin, and finally normotensive patients with heart failure with renin levels that were often extremely high.

Theoretically, the change in vascular resistance after converting enzyme inhibition might correlate more closely with pretreatment renin than does the change in blood pressure. Therefore, the goal of the last study presented in chapter 1 was to provide some insight into the relation between pretreatment plasma renin, degree of inhibition of converting enzyme

with captopril and the haemodynamic profile of action of the drug. Both short- and long-term responses of total peripheral resistance to ACE inhibition were investigated.

CHAPTER 2

We were much impressed by the beneficial effects of a single gift of captopril in hypertensive patients who appeared to be in some state of heart failure. At that time heart failure was not considered to be an indication for ACE inhibition (sic !) and the results of studies started in the United States and Switzerland were unknown to us. So we followed our own research instinct. Professor Hugenholtz and the staff of the Thoraxcentrum were very cooperative and helpful but not much impressed by the idea. However on short notice they did help us to collect a series of patients. The results of that venture are presented in the single paper of this chapter.

CHAPTER 3

In chapter 3 we go back to that black box that is of utmost importance for the regulation of blood pressure and the activity of the renin angiotensin system, the kidney. The renin story however is a very complicated one and a little history is may be helpful.

During the final decade of the last century, interest was shown in extracting various organs and measuring their effect on blood pressure, chiefly it seems because of Brown-Sequard's claim (1854) that testicular extracts had rejuvenating power. Tigerstedt and Bergman in 1898 followed the trend by making water extracts of kidneys and showing that they had pressor activity (for overview, see Page, Hypertension Mechanisms). What they showed was that the activity of extracts of rabbit kidney was destroyed by boiling, it was nondialyzable, and the active material was largely contained in the cortex. There was an initial fall in blood pressure, followed by a rise. Nephrectomy greatly prolonged its action. They theoretized that their "renin" acted on peripheral nerve centers, and not on vascular muscle. Further, they recognized a possible relationship with renal disease, but specifically disavowed proposing a new hypothesis to explain cardiorenal disease.

Not unlike many of our research associates Bergman disappeared into medical practice and was never heard from, and Tigerstedt did nothing more with his renin. This discovery of renin laid dormant for more than 30 years before its significance was recognized by Goldblatt and coworkers, who produced hypertension in the dog by clamping the renal artery. These experiments indicated that a "pressor" substance, renin, was released from the kidney with the constricted artery.

Renin ceased being a pressor substance after the discovery that it was an enzyme. This proteolytic activity of renin is responsible for the catalytic hydrolysis of the leu-10/val-11 bond of renin substrate, angiotensinogen. Because this reaction is the rate-limiting step in a series of reactions leading to the formation of the potent vasoconstrictor angiotensin-II, renin is considered to play a key role in blood pressure regulation and in sodium and water homeostasis.

In respect of its enzymatic activity, renin is comparable to other proteolytic enzymes in plasma. In contrast to precursors of polypeptide hormones, proteolytic enzymes in plasma are largely present as inactive precursor molecules that are activated outside their site of production. In case of the coagulation system, for instance, 99 percent or more of the total quantity of the individual enzymes circulates in plasma as inactive precursor. It was therefore of great interest that Lumbers and Morris and Skinner and later our group

reported on the presence of an inactive form of plasma renin that can be converted into active renin by limited proteolysis. It now appears that more than 90 percent of the renin in plasma is present in this inactive form. This inactive form of renin in plasma is identical with the biosynthetic precursor of renin in the kidney and is therefore called prorenin.

In the first part of chapter 3 we describe the development of an assay of prorenin, in which the conversion of prorenin into active renin occurred under apparently optimal condition, without any loss of prorenin, activated prorenin or naturally occurring active renin. The assay was used for measurements of prorenin and active renin in peripheral and renal vein plasma of patients with and without renal artery stenosis. ACE inhibition with captopril was used to stimulate renin release by the kidney.

The last two complimentary papers of chapter 3 focus on the renal haemodynamic influences of ACE inhibition. We studied patients with and without renal artery stenosis. This proved to be not an easy subject. Intensive investigative efforts probing the many characteristics and potential functions of the renin-angiotensin system have resulted in an exponentially expanding literature. We now know that renal artery stenosis sets into motion complex compensatory mechanisms. Because of its unique localisation and structure the juxtaglomerular apparatus, the major site of renin and angiotensin formation, plays a keyrole. Despite a lower perfusion pressure behind the stenosis renal blood flow is, at least initially, well maintained by an autoregulatory fall in preglomerular resistance. At the other side of the glomerulus locally formed angiotensin II is thought to maintain glomerular hydrostatic pressure and glomerular filtration rate by effects on postglomerular arteriolar tone. Increased renin secretion and hypertension in the systemic circulation contribute to restoration of the low perfusion pressure behind the stenosis. The non- stenotic kidney suffers from and contributes to hypertension by a reduction in cortical blood flow, glomerular filtration and excretory function in response to increased levels of circulating angiotensin II.

Thus ACE inhibitors could have a major impact on renal function, in particular of the stenotic kidney. However in unilateral renal disease such an effect may easily go unnoticed because of the functional reserve of the opposite kidney. We therefore performed split renal function studies in these patients.

CHAPTER 4

Screening for renovascular hypertension is the subject of chapter 4. It should be realized that renovascular hypertension is the most common form of secondary hypertension and that this form of hypertension is potentially curable with surgical intervention or angioplasty. It is estimated that up to 5% of the hypertensive Dutch population, or approximately 50.000, may have this form of hypertension. Current diagnostic techniques for identifying patients with renovascular hypertension are not ideal. Clinical findings, urography, and radio-isotope renography are of questionable or unproven value. Arteriography, which has been suggested as gold standard technique, is invasive, expensive, and not accurate in the sense that demonstration of renal artery stenosis in a hypertensive patient does not determine whether the stenosis is the cause of the hypertension.

Renin activity in peripheral plasma is higher on average in patients with renal artery stenosis than in patients with essential hypertension but there is a large overlap between the two groups. We hypothesized that the overlap would be reduced by administering an ACE inhibitor. There were good reasons for this assumption. First, in the kidney affected by

artery stenosis the intraglomerular pressure is critically dependent on angiotensin II. Reduction of angiotensin II by ACE inhibition will therefore activate the juxtaglomerular baroreceptor mechanism with stimulation of renin secretion as a result. Second, the juxtaglomerular apparatus is hypertrophied in kidneys with artery stenosis and may therefore expected to be hyperresponsive to stimuli for renin secretion. Finally, since the systemic blood pressure in patients with renal artery stenosis is also more dependent on angiotensin II than in patients without renal artery stenosis, the systemic and glomerular pressures were expected to fall more strongly after ACE inhibition. Evidence concerming the clinical utility of single dose captopril in the diagnosis of renovascular hypertension was evaluated in the first paper of this chapter.

The second paper of this chapter deals with the diagnostic value of renal vein renin sampling, a more sensitive method for diagnosis than measurement of peripheral plasma renin. In this study we employed a new immunoradiometric assay to measure renin. This direct assay makes use of a highly specific monoclonal antibody that recognizes active renin but not prorenin. This assay has some advantages over the indirect assay. For instance the procedure does not involve the incubation step for generating angiotensin I, thereby circumventing problems with recovery of angiotensin I and the varying levels of renin substrate. A disadvantage of the direct assay is its lower sensitivity. In the case of renal vein renin measurements we tried to solve this problem by stimulating the secretion of renin prior to sampling by giving an ACE inhibitor.

CHAPTER 5

The ACE inhibitors, captopril and enalapril, have undoubtedly proved to be effective agents for the treatment of essential hypertension. However, the two agents differ in several ways: the nature of the molecule's adherence to the active site on the converting enzyme, the form in which the agent is administered (active compound or prodrug), and the kinetics of elimination. In contrast to enalapril captopril's elimination half-life is short, 2 hours versus 11 hours. However captopril's blood pressure lowering effect has been noted to outlast its inhibition of serum ACE. Inhibition of tissue ACE or some other effect such as drug accumulation could be involved, but hard data about these possibilities are not available. Current trends favour twice-daily administration of captopril and once- daily dosing of enalapril. The results of a European multicentre trial even suggested that once-daily captopril was as effective as once-daily enalapril. A point of criticism of these studies could be the fact that blood pressure was measured, at one moment in time, 21-27 hours after dosing and that increasing doses were used. We therefore used an automatic semi-continuous ambulatory blood pressure recording device to compare, in the same patient, a twice-daily dosing regimen of captopril with an once-daily of enlapril.

PART 2 SEROTONIN ANTAGONISM

It has been known for more than a century that blood gains vasoconstrictor properties after clotting. The responsible serum factor was isolated and its structure identified in the late forties. The substance was called serotonin, and was subsequently found to be 5-hydroxytryptamine (5-HT). Its synthesis followed shortly thereafter. However the serotonin story has taken about 35 years to develop. We now know that platelets of all mammals contain serotonin and that serotonin contained in these platelets originates in the enterochromaffin cells of the gastrointestinal tract. Serotonin is taken up by platelets during

their lifetime in the circulating blood. The only blood containing measurable amounts of serotonin in plasma is that in the portal vein, problably because of release of the amine from the enterochromaffin cells of the intestinal mucosa. Under normal conditions the concentration of free serotonin is too low to produce measurable cardiovascular effects.

As the epithelial lining of the cardiovascular system, the endothelium acts as a barrier that excludes circulating cellular elements and harmful substances. However we now know that the endothelium also plays an important role in the modulation of the function of blood cells, the blood vessel wall and the tissues that the blood vessel supplies. Platelets interact both physically and biochemically with the blood vessel wall. The endothelium plays an important role in these interactions. Where the endothelium is damaged, platelets aggregate on the subendothelial collagen and smooth muscle. As an inevitable consequence of their aggregation, platelets release serotonin, nucleotides, prostaglandines, catecholamines, and various proteins, all of which may have profound effects on other platelets, the surrounding endothelium, the smooth muscle or the tissues beyond. The serotonin content of platelets is more than sufficient to exert vascular effects upon its release.

In most patients with chronic hypertension, the etiology of the increase in blood pressure remains obscure. As the cardiac output of these patients is basically normal, the chronic increase in arterial blood pressure is due primarily to an augmented peripheral vascular resistance, owing to an abnormal narrowing of the systemic arterioles. Except perhaps in patients with renovascular hypertension, primary aldosteronism or pheochromocytoma, no single derangement can be held responsible for the sustained increase in peripheral resistance. Factors such as genetic predisposition, increased activity of the sympathetic nervous system, altered function of the adrenergic neuroeffector junction, augmented activity of the renin- angiotensin-aldosterone axis, abnormal secretion of natriuretic hormone or of vasopressin, and adaptive changes in the bloodvessel wall all may contribute.

The second part of this thesis entertains the idea that the peripheral vasocontrictor effects of serotonin may contribute to the sustained increase in peripheral resistance, at least in part and in some patients with hypertension.

Serotonin however has complex and multiple actions on cardiovascular function. In the intact animal, it can cause either an increase or a decrease in blood pressure depending on the species studied and the dose used. The local effect of the monoamine on blood flow is similarly confusing: depending on the species, the vascular bed, the experimental conditions, the degree of sympathetic tone, and the route of administration, serotonin augments or reduces blood flow.

It is now realized that these bewildering cardiovascular and other effects of serotonin can be explained by activation of different receptors. According to the current classification, three main classes of serotonin receptors, designated 5-HT_1, 5-HT_2 and 5-HT_3, are distinguished. 5-HT receptors mediating contraction of vascular smooth muscle are of the 5-HT_2 type.

Our clinical interest in the cardiovascular effects of serotonin has been stimulated by the introduction of the selective 5-HT_2 serotonergic receptor antagonist ketanserin.Other serotonergic antagonists such as methylsergide, cyproheptadine, and pizotifen have inconsistent effects on arterial blood pressure. This might be related to lack of selectivity and to partial agonistic activity. Some serotonergic antagonists have marked central actions. Ketanserin is devoid of agonistic activity and animal studies have indicated that the compound is more potent as a serotonergic antagonist in the periphery than in the central nervous system. Chapters 6, 7 and 8 offer a survey of studies on the hemodynamic effects

of ketanserin in hypertensive patients and in patients with local vasoconstriction (Raynaud's phenomenon).

CHAPTER 6

The two papers of chapter 6 present data about the acute and chronic blood pressure lowering effects of ketanserin in patients with essential hypertension. In the first pilot study 10 mg ketanserin was administered intravenously to 12 patients with untreated essential hypertension and its acute effects on intra-arterial blood pressure, heart rate, right atrial pressure, pulmonary artery pressure, cardiac output, renal blood flow and glomerular filtration rate were followed for several hours. In the second study ketanserin was given orally, in a dose of 40 mg b.i.d., for six weeks to 24 subjects with mild to moderate essential hypertension. Its effects were evaluated in a placebo-controlled double-blind crossover study. In 18 subjects 24- hour ambulatory intra-arterial measurements were made,besides the usual sphygmomanometer measurements.

CHAPTER 7

During our studies it became apparent that ketanserin also interacts with other cell membrane receptors, alpha$_1$-adrenoceptors in particular. Thus, the question of alpha$_1$-adrenoceptor blockade by ketanserin has to be answered before it can be concluded that the antihypertensive and vasodilatory effect of ketanserin supports the involvement of the peripheral actions of serotonin in hypertension and other vascular disorders. In this context, one should keep in mind that conclusive evidence in this matter may only come from studies in humans, since animal models such as the spontaneously hypertensive rat may not allow better prediction of the efficacy of serotonergic blockers to lower blood pressure than they did years ago for beta-adrenergic blockers.

The first paper of chapter 7 describes the hemodynamic profiler of ketanserin's antihypertensive action in patients with essential hypertension. The possibility that the antihypertensive effect of ketanserin depends on interference with alpha$_1$-adrenoceptor-mediated vasoconstriction was tested by comparing of the pressor effects of the alpha$_1$-adrenoceptor agonist, phenylephrine, before and after ketanserin and by an assessment of the antihypertensive effect of ketanserin after administration of the alpha$_1$-adrenoceptor antagonist, prazosin.The cardiovascular effects of ketanserin were also studied in a small group of patients with autonomic insufficiency, who were unresponsive to the hypotensive action of the nonselective alpha-adrenoceptor antagonist, phentolamine. The last paper of this chapter explores these issues further.

CHAPTER 8

During its initial clinical evaluation as an antihypertensive agent, we found that ketanserin improved digital blood flow in patients with hypertension and concomitant Raynaud's phenomenon. Primary Raynaud's phenomenon is characterized by episodic reversible vasospasms of blood supply to fingers and toes, triggered by cold exposure or emotional stress. The often severe vasoconstriction has been suggested to be a consequence of overactivity of the sympathetic nervous system but therapy with alpha-adrenoceptor blocking agents has been disappointing.

In vitro, serotonin caused contraction of isolated arteries and veins from the hand of

healthy humans with an intrinsic activity approximately equal to that of noradrenaline and adrenaline. Direct infusion of serotonin into the brachial artery of humans caused a drop in the digital temperature and induced the characteristic sequential skin discoloration of Raynaud'sphenomenon.

Chapter 8 explores the mode of action of ketanserin in patients with primary Raynaud's phenomenon. Changes in digital blood flow after ketanserin were studied by venous occlusion plethysmography and laser-Doppler flowmetry. The effects on transcutaneously measured oxygen pressure were also evaluated.

CHAPTER 1

ACE INHIBITION AND PLASMA RENIN

Does captopril lower blood pressure in anephric patients?

Man in 't Veld AJ, Wenting GJ, Schalekamp MADH.
British Medical Journal 1979; **2**: 1110-1111.

Blood pressure response of nephrectomized subjects and patients with essential hypertension to ramipril (HOE 498): indirect evidence that inhibition of tissue angiotensin converting enzyme is important.

Wenting GJ, Blankestijn PJ, Poldermans D, Van Geelen JA,Derkx FHM, Man in 't Veld AJ, Schalekamp MADH.
American Journal of Cardiology 1987; **59** (suppl D): 92-97.

Hemodynamic effects of captopril in essential hypertension, renovascular hypertension and cardiac failure: correlations with plasma renin.

Wenting GJ, De Bruyn JHB, Man in 't Veld AJ, Woittiez AJJ, Derkx FHM, Schalekamp MADH.
American Journal of Cardiology 1982; **49**: 1453-1459.

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Does captopril lower blood pressure in anephric patients?

Captopril inhibits peptidyldipeptide hydrolase, the enzyme that converts angiotensin I to angiotensin II and degrades bradykinin. Captopril's antihypertensive action seems to depend on the activity of the renin-angiotensin system in relation to the state of sodium balance.[1–3] It is not clear, particularly in patients with normal or low plasma renin activity, whether captopril lowers blood pressure by eliminating the vasoconstrictor angiotensin II or by allowing the vasodilator bradykinin to accumulate. We have studied captopril's effect on supine and standing blood pressure in an anephric woman in three different states of sodium balance.

Patient, methods, and results

Captopril (25 mg by mouth) was given to a 36-year-old anephric woman on three occasions: one hour after haemodialysis when she was volume and sodium overloaded and weighed 62·2 kg; and then two and seven days after progressive ultrafiltration, when she weighed 57·9 kg and 56·7 kg respectively. Active plasma renin concentrations, measured by radioimmunoassay, ranged from 1·2 to 2·9 mU/l (normal 16-40 mU/l) in the three states and did not rise after captopril. Angiotensin-converting-enzyme activity was measured by spectrophotometric assay of the rate of production of hippuric acid from hippuryl—L—histidyl—L—leucine and expressed as a percentage of control values derived before captopril was given. Enzyme activity was inhibited by 56 ± 6% (mean $\pm$ SE of mean) 30 minutes after captopril was given and by 92 ± 7% after 120 minutes. The blood pressure was measured with a random-zero sphygmomanometer (London School of Hygiene, mk 4, No 7125) to avoid digital preference and observer bias. Heart rate was determined from a continuous electrocardiograph.

The figure shows the effect of captopril on blood pressure and heart rate in the three different states. The patient was severely hypertensive when she weighed 62·2 kg, but captopril had no effect on standing or supine blood pressure. When the patient weighed 57·9 kg her supine blood pressure was 143/90 mm Hg. She did not have postural hypotension before captopril was given, but after captopril, although her supine blood pressure rose by 10%, her blood pressure dropped from 156/101 mm Hg to 98/61 mm Hg on standing and she complained of dizziness. When her weight had fallen to 56·7 kg, captopril caused a 15% drop in supine blood pressure (144/86 mm Hg to 128/71 mm Hg), and on standing her blood pressure fell further to 79/43 mm Hg and she felt that she was going to faint. Again before captopril was given there was no postural hypotension.

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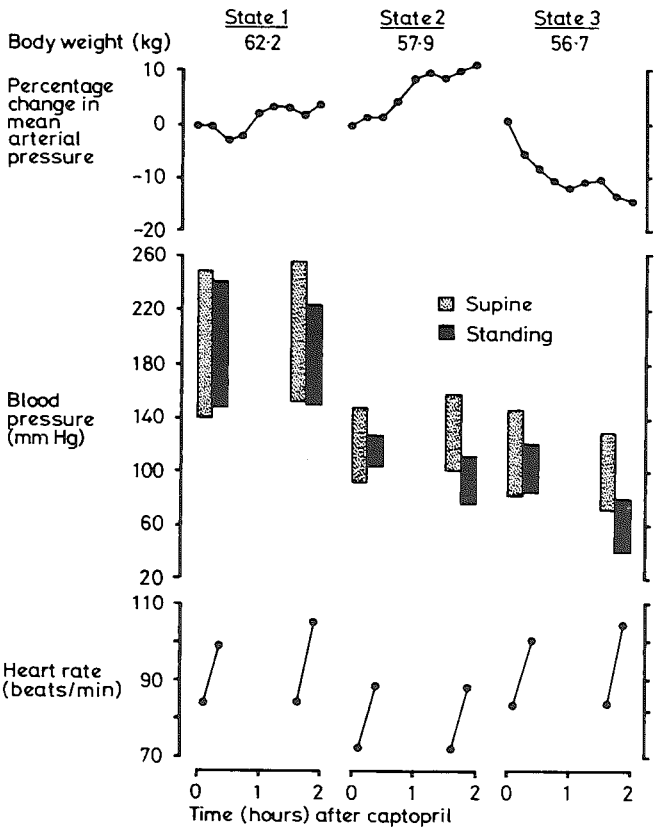

Effect of captopril (25 mg by mouth) in an anephric patient in three different states of sodium balance on supine and standing blood pressure, mean arterial pressure, and heart rate. (Mean arterial pressure was calculated from diastolic pressure and 1/3 of pulse pressure.)

Comments

Our findings show that captopril's effect on blood pressure in an anephric patient depends on the state of sodium balance. As little renin is available the effect cannot be through elimination of angiotensin II, but it may be through accumulation of bradykinin.[4] Others have not found any effect of captopril on blood pressure in anephric patients and rats,[3 4] but any hypotensive effect may have been masked by hypervolaemia. Also the blood pressure response to captopril may be partially independent of renin-sodium balance in subjects with a low plasma renin activity. If renin is inappropriately low in relation to total-body sodium then captopril may produce its effect by potentiating bradykinin action. Furthermore, our case illustrates that an

extrarenal kallikrein-kinin system may be important. The postural hypotension that developed may support a suggestion[5] that vasoactive peptides may effect venous tone. The fact that heart rate increased considerably more when the patient stood in the sodium-depleted state suggests that the baroreceptor-induced changes in sympathetic tone were appropriate. The postural hypotension may have been caused by a fall in venous return and cardiac output caused by inhibition of the angiotensin-converting enzyme increasing the capacitance of the venous system.

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[2] Case, D B, *et al*, *Progress in Cardiovascular Diseases*, 1978, **21**, 195.
[3] Case, D B, *et al*, *American Journal of Medicine*, 1976, **61**, 790.
[4] Thurston, H, and Swales, J D, *Circulation Research*, 1978, **42**, 589.
[5] Turini, G A, *et al*, *Lancet*, 1979, **1**, 1213.

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Blood Pressure Response of Nephrectomized Subjects and Patients with Essential Hypertension to Ramipril:

Indirect Evidence That Inhibition of Tissue Angiotensin Converting Enzyme Is Important

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The kinetics of blood pressure changes and plasma angiotensin converting enzyme (ACE) inhibition in response to ramipril (HOE 498), 10 mg orally, were studied in 6 nephrectomized subjects 12 hours after ultrafiltration and in 10 patients with essential hypertension. Ramipril lowered supine and standing blood pressure in both groups, but the effect was greater in essential hypertension. The maximal blood pressure response followed the effect on plasma ACE after a lag time of 3 to 4 hours in both groups. These data provide indirect evidence that ramipril lowers blood pressure, at least in part, independently of its effect on the circulating renin-angiotensin system, possibly by acting on tissue ACE.

(Am J Cardiol 1987;59:92D–97D)

Experimental and clinical evidence suggests that the antihypertensive action of angiotensin converting enzyme (ACE) inhibitors cannot be explained solely by blockade of the circulating renin-angiotensin system.[1-3] Target tissues for blood pressure regulation, such as blood vessels, kidney, adrenal gland and brain, contain renin, ACE and other components of the renin-angiotensin system, and inhibition of ACE in these tissues may contribute to the effect of ACE inhibitors. It has even been hypothesized that the primary function of the circulating renin-angiotensin system is not so much to deliver angiotensin II to the tissues but rather to deliver angiotensinogen and renin.[4]

The kidney is the main, if not the only, source of enzymatically active renin in plasma. The level of active renin is very low, if not absent, in plasma of nephrectomized subjects. In contrast, plasma angiotensinogen is high and the plasma concentration of inactive renin (prorenin) is often within the normal range.[5,6] It has been proposed that angiotensinogen and prorenin may be taken up from plasma by the tissues. Prorenin may then be activated and contribute to the local formation of angiotensin II.[7]

The novel compound HOE 498 (ramipril) is a potent inhibitor of ACE and penetrates readily into the tissues.[8] In view of the possibility that ACE inhibition on the tissue level might contribute to the antihypertensive action of ramipril, we studied the kinetics of the blood pressure response in relation to the degree of inhibition of plasma ACE by this drug both in nephrectomized subjects and in patients with essential hypertension.

Patients and Methods

Studies were undertaken in 6 nephrectomized subjects (4 women, 2 men, 29 to 54 years) and in 10 patients with essential hypertension (5 women, 5 men, 35 to 59 years). Bilateral nephrectomy had been performed 4 months to 5 years before. The reasons for nephrectomy were: intractable renal infection in 4 subjects, uncontrollable severe hypertension in 1 subject and recurrent renal bleeding (adult polycystic disease) in 1. The nephrectomized subjects were receiving maintenance

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TABLE I Baseline Clinical Data in Nephrectomized Subjects

Patient No.	Blood Pressure (mm Hg)		Heart Rate (beats/min)		Active Renin (μU/ml)		Total Renin (μU/ml)		Plasma ACE (mU/ml)	
	P	R	P	R	P	R	P	R	P	R
1	136/86	130/86	88	99	0.4	0.6	89	85	13.4	18.0
2	113/74	123/72	91	83	0.1	0.3	24	28	14.2	23.8
3	113/73	113/70	83	89	0.4	0.4	38	39	16.2	14.8
4	148/92	139/85	86	72	0.2	0.2	25	21	24.0	24.7
5	156/102	144/92	76	69	0.7	1.3	133	161	15.0	14.3
6	97/61	108/67	67	67	0.4	0.2	...	...	10.8	17.6

P = placebo; R = ramipril.

hemodialysis, twice a week, with a Fresenius A 2008 C dialyzer and a disposable polyacrylonitryl membrane kidney. None of these subjects showed clinical or radiologic evidence of congestive heart failure and none of them was receiving vasoactive drugs. In the patients with essential hypertension, antihypertensive therapy had been stopped for at least 2 weeks before the study. Sodium intake in these patients was restricted to 50 to 70 mmol daily and was checked by 24-hour urine collection.

The study was performed on an outpatient basis. The nephrectomized subjects were studied in a randomized double-blind placebo-controlled fashion. Either 10 mg ramipril or placebo was given by mouth at 9.30 A.M., 12 hours after a hemodialysis session, during which body fluid had been removed until "ideal" body weight had been reached. Three subjects began the study with active drug and 3 with placebo. Two weeks later, the subjects who had been given ramipril received placebo, and vice versa. The loss of body weight for each dialysis session was 3.1 ± 0.5 kg (mean ± standard error of the mean) for the subjects who received ramipril first, and it was 2.7 ± 0.5 kg for the subjects who received placebo first. The difference in weight loss was not statistically significant.

The patients with essential hypertension first received placebo and a week later 10 mg of ramipril orally.

Systolic, diastolic and mean blood pressures and heart rate were measured automatically (Accutorr TM 2, Datascope Corp.) every 5 minutes for 1 hour before ramipril or placebo was given and for 5 hours thereafter. Standing blood pressure and heart rate were measured each hour. Blood was obtained from an indwelling venous catheter. The concentration of enzymatically active renin and the total renin concentration of plasma were measured by radioimmunoassay as described previously.[9] Results were expressed as μU/ml, using the international human kidney renin standard (MRC 68/356) as a reference. Normal values in our laboratory are 10 to 50 μU/ml for active renin and 60 to 350 μU/ml for total renin. Plasma ACE was measured by the rate of production of hippuric acid from hippuryl-L-histidyl-L-leucine.[10] Normal values are 7 to 20 mU/ml.

Data are presented as mean ± standard error of the mean, and for statistical comparison the Student's paired *t* test was used. A p value of <0.05 was considered to indicate a statistically significant difference.

The study protocol was approved by the Hospital Ethical Review Committee, and informed consent was obtained from each patient.

Results

Effect on plasma renin, blood pressure and heart rate: Clinical data on the nephrectomized subjects before ramipril and placebo therapy were not different (Table I). As expected, active renin in plasma was hardly detectable and was unresponsive to ramipril. In contrast, the plasma concentration of prorenin ranged from low-normal to normal. Supine and standing blood pressures did not change with placebo, but decreased after ramipril therapy (Fig. 1). There was no significant difference in heart rate between the placebo and ramipril periods, and the increments of heart

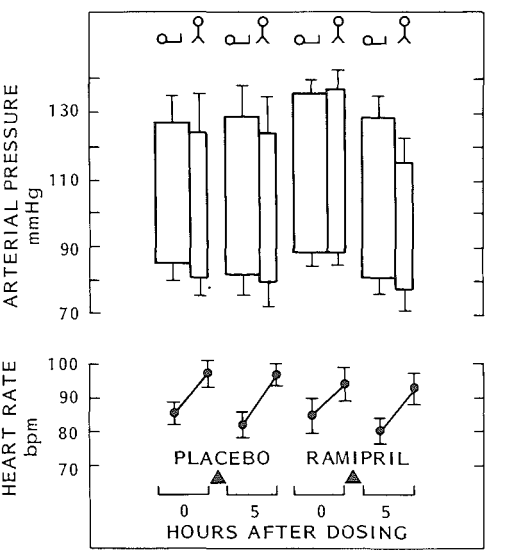

FIGURE 1. Effect of ramipril, 10 mg orally, on supine and standing blood pressure and heart rate in 6 nephrectomized subjects. Supine, systolic and diastolic blood pressures were significantly lower after ramipril ($p < 0.05$). Standing blood pressures were also lower ($p < 0.01$).

rate on standing were also not different. The decrease in blood pressure after ramipril was more pronounced in the standing position, but frank orthostatic hypotension was not observed. Figure 2 shows the changes in blood pressure in the nephrectomized subjects in more detail. The effect of ramipril on blood pressure in these subjects was maximal 4 to 5 hours after drug intake. In 3 subjects mean arterial pressure decreased by more than 10%.

Active renin in plasma was normal in the patients with essential hypertension and showed the expected increase after ramipril. Ramipril lowered blood pressure in both the supine and standing position in these patients (Fig. 3). Heart rates after ramipril and placebo were not significantly different.

Effect on blood pressure versus effect on plasma angiotensin converting enzyme: In both the nephrectomized and essential hypertension subjects, ramipril was rapidly absorbed and converted into the bioactive compound, as was shown by blockade of plasma ACE activity (Fig. 4). Inhibition of ACE was already detectable after 30 minutes and was maximal between 1 and 2 hours after administration. The effect of blood pressure became evident between 1 to 2 hours after drug intake and was maximal at 4 to 5 hours in both patient groups. Thus, the blood pressure response did not coincide with the inhibition of plasma ACE; the maximal effect on blood pressure followed the effect on plasma ACE after a lag time of 3 to 4 hours.

Discussion

Ramipril belongs to a new class of orally active, non-sulfhydryl pro-drug ACE inhibitors. Enalapril also belongs to this class. Ramipril and enalapril have a pharmacologic profile similar to that of the ACE inhibitor captopril, but they differ from captopril by their prolonged action and greater ACE inhibitory potency. Ramipril acts even longer than enalapril and may further differ from enalapril in that it has greater effect on tissue ACE. It has been reported that in spontaneously hypertensive rats the magnitude of blood pressure response to the 2 drugs was different and that this difference could not be explained by a difference in their inhibitory effect on the circulating renin-angiotensin system.[3] It was instead related to the degree of ACE inhibition in tissues. Ramipril was more potent in this respect. Differences in lipid solubility and extent of hydrolysis of the pro-drug in tissue may be important.

In the present study we gathered some data on ramipril's mode of action by relating its effect on blood pressure with its effect on plasma ACE in nephrectomized subjects with very low levels of circulating active renin, and in patients with essential hypertension

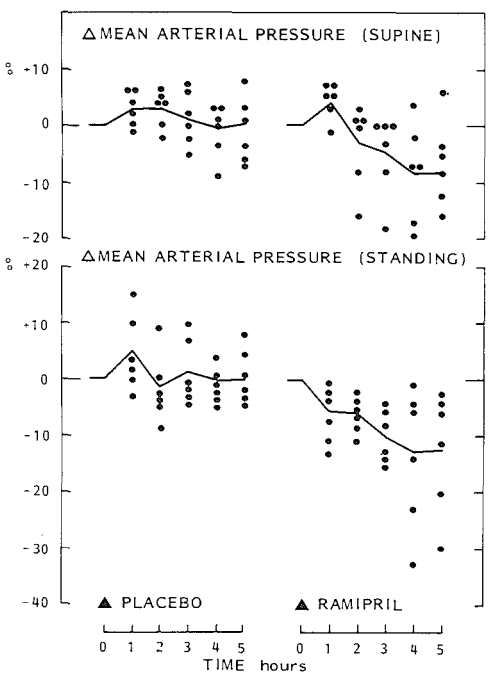

FIGURE 2. Individual blood pressure responses of nephrectomized subjects to ramipril, 10 mg orally.

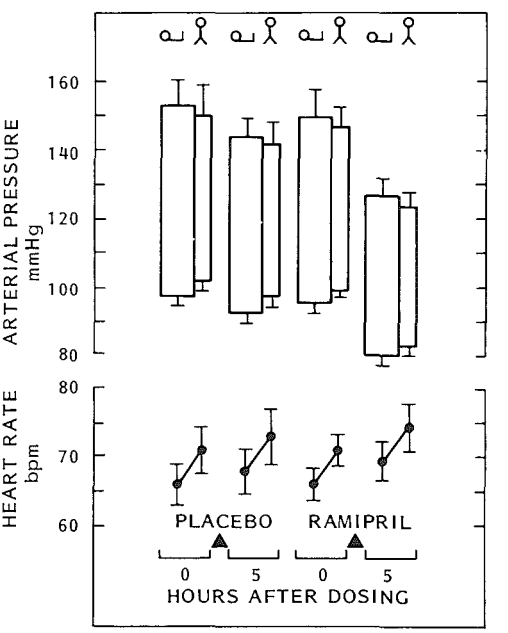

FIGURE 3. Effect of ramipril, 10 mg orally, on supine and standing blood pressure and heart rate in 10 patients with essential hypertension. Supine and standing systolic and diastolic blood pressures were significantly lower after ramipril ($p < 0.01$).

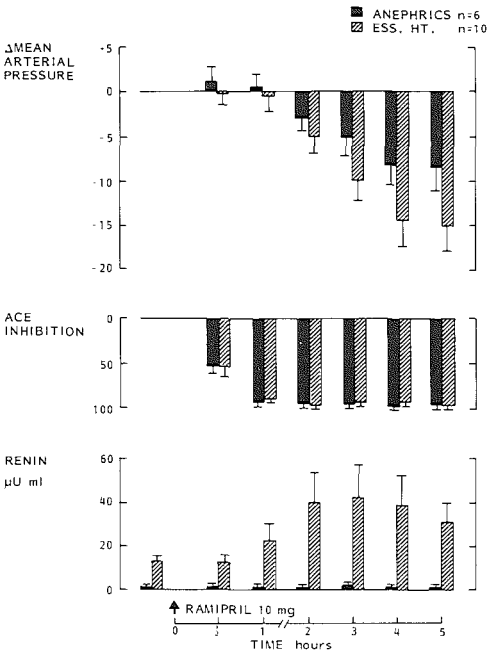

FIGURE 4. Time course of changes in blood pressure and plasma angiotensin converting enzyme (ACE) in response to ramipril, 10 mg orally, in 6 nephrectomized subjects (*black bars*) and 10 patients with essential hypertension (*hatched bars*).

with normal renin. In a previous study we showed that blood pressure was reduced in fluid-depleted nephrectomized subjects by the ACE inhibitor captopril, which was given 1 hour after completion of hemodialysis.[11] We interpreted our findings as evidence that the effect of captopril on blood pressure may not solely depend on its effect on the circulating renin-angiotensin system. However, it has been proposed that the decrease in blood pressure might have been due to a mechanism somehow related to hemodialysis.[12] Exposure of blood to the dialysis membrane might trigger the production of vasodepressor kinins. Because the kinin degrading enzyme, kininase II, is identical with ACE, reduced degradation of circulating kinins might explain our findings. Leslie et al[12] administered captopril 24 hours after dialysis, and were unable to demonstrate a hypotensive effect. However, the decrease in blood pressure we observed lasted for more then 24 hours and was progressive in time. Some patients showed symptomatic orthostatic hypotension 24 hours after captopril, and this was not observed 3 to 4 hours after completion of the dialysis procedure, even though inhibition of ACE was maximal at that time. In our view it is therefore unlikely that circulating kinins had contributed to the hypotensive effect of captopril in these patients.

To avoid possible interactions with the hemodialysis procedure, we administered ramipril 12 hours after completion of hemodialysis. The present results with ramipril confirm that ACE inhibitors are capable of lowering blood pressure in nephrectomized subjects. After ingestion of ramipril, serum ACE decreased rapidly in these subjects. It was inhibited by more than 50%, 30 minutes after dosing, and the maximum effect was at 1 to 2 hours. Blood pressure, however, decreased more gradually and the maximum response was after 4 to 5 hours. Thus, there was a considerable time lag between the 2 effects. A similar time lag was observed in the patients with essential hypertension, although the magnitude of the blood pressure response was greater in these patients than in nephrectomized subjects.

These findings, taken together, lend further support to the hypothesis that ramipril may lower blood pressure, at least in part, independently of its effect on the circulating renin-angiotensin system, possibly by acting on tissue ACE. The rapid onset of absorption and hydrolysis into the biologically effective dicarboxylic acid is somewhat surprising, but our results are supported by previous pharmacokinetic studies.[8,13]

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Hemodynamic Effects of Captopril in Essential Hypertension, Renovascular Hypertension and Cardiac Failure: Correlations With Short- and Long-Term Effects on Plasma Renin

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The hemodynamic effects of captopril were investigated in 22 patients with essential hypertension, 22 with hypertension and renal artery stenosis and 14 with refractory chronic heart failure. The effects of a first dose of captopril, 50 mg orally, were observed for 2 hours, and the effects of repeated doses, 450 mg/day in combination with mild dietary sodium restriction, for at least 4 weeks.

Short-term captopril treatment caused similar reductions in blood pressure in the three patient groups, that is, 21 ± 3 mm Hg in essential hypertension, 29 ± 6 mm Hg in renovascular hypertension and 21 ± 2 mm Hg in heart failure (mean ± standard error of the mean) despite large differences in pretreatment plasma renin. Heart rate and cardiac output did not change in hypertensive patients, and cardiac filling pressures decreased. The changes in right atrial pressure, pulmonary artery pressure and pulmonary capillary wedge pressure in essential hypertension and in renovascular hypertension did not differ. Heart rate decreased and cardiac output increased in heart failure, whereas cardiac filling pressures decreased. Blood pressure responses to long-term captopril therapy in essential and in renovascular hypertension were similar and, as with short-term treatment, changes in blood pressure were largely determined by changes in peripheral resistance. Several measurements of extracellular fluid volume showed no evidence of fluid retention by the kidneys.

Short-term but not long-term blood pressure responses were correlated with pretreatment plasma renin (percent change in mean arterial pressure, short-term, versus log renin, $r = 0.47$, $p < 0.001$, $n = 14$). Both short- and long-term responses of total peripheral resistance were correlated with plasma renin (percent change in resistance, short-term versus log renin, $r = 0.64$, $p < 0.001$, $n = 40$; percent change in resistance, long-term versus log renin, $r = 0.56$, $p < 0.001$, $n = 31$). The correlations were weak and probably not important for clinical practice. These data indicate that other factors besides circulating renin are important in captopril's hypotensive effect. The favorable hemodynamic effects of converting enzyme inhibition warrant further consideration of this principle of therapy in the clinical management of most forms of hypertension and also in the treatment of chronic heart failure.

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The efficacy of captopril in decreasing blood pressure has amply been demonstrated. Several but not all centers reported the drug to be more effective in hypertension when plasma renin is high rather than low.[1,2] Positive correlations were observed between the decrease in blood pressure shortly after a single dose of captopril and pretreatment plasma renin.[3,4] Such correlations were also recorded after long-term treatment[4,5] but again not invariably so.[6–10] Theoretically, the change in vascular resistance after captopril might correlate more closely with pretreatment renin than does the change in blood pressure. Other dif-

ferences between hemodynamic responses in patients with high-renin and low-renin hypertension may exist. Comparative studies to explore this possibility are scarce. Apart from a study by Atkinson et al.[11] indicating that total exchangeable sodium had not changed after 6 weeks of captopril therapy in eight patients with renal artery stenosis, there are few data on fluid balance during long-term captopril treatment. Such information is important because it may help to delineate more clearly the place of this drug among the antihypertensive drugs now in common use.

We have collected such data in patients with essential hypertension and in those with hypertension associated with renal artery stenosis. The results were compared with observations in so-called refractory chronic heart failure. Plasma renin was low or normal in essential hypertension, normal or high in renal artery stenosis and grossly elevated in most cases with heart failure.

Methods

Patient Groups

Forty-four hypertensive and 15 normotensive patients with chronic heart failure were studied after they had given their informed consent to the study protocol and procedures.

Essential hypertension: Twenty-two patients (7 women), aged 48 ± 2 years, were studied. Routine investigations including intravenous urography and radioisotope renography had not revealed any cause for the hypertension. In 17 patients previous antihypertensive therapy, if any, was tapered off and a placebo was given for at least 3 weeks. Blood pressure on placebo was 140 to 200 mm Hg systolic and 95 to 120 mm Hg diastolic. Five patients remained on multiple drug therapy. In these five patients blood pressure was 160/100 mm Hg or higher despite a 2 week course of standard triple therapy, that is, a combination of a diuretic (hydrochlorothiazide 100 mg/day or furosemide 80 mg/day) with a beta-adrenoceptor blocking agent (propranolol 320 mg/day) and a vasodilator (hydralazine 200 mg/day).

Hypertension associated with renal artery stenosis (renovascular hypertension): This group consisted of 22 patients (9 women), aged 42 ± 4 years. Renal arteriography and renal vein sampling was performed because of abnormalities found on routine intravenous urography or radioisotope renography, or both. In 18 patients unilateral and in 4 patients bilateral renal artery stenosis was demonstrated. The renal vein-to-artery ratio of renin on the side of stenosis ranged from 1.5 to 4.6. Of these 22 patients, 16 were treated by placebo for at least 2 weeks. Blood pressure on placebo was 150 to 200 mm Hg systolic and 95 to 120 mm Hg diastolic. Six remained on standard triple therapy for at least 2 weeks.

Chronic heart failure: Fourteen patients (3 women), aged 52 ± 2 years, were studied. Nine had ischemic heart disease and five had valvular disease. In four of the latter patients one or more prosthetic valves had been placed. All had refractory congestive heart failure and were in functional class IV (New York Heart Association) while under treatment with digoxin, a diuretic (furosemide 80 to 120 mg/day) and vasodilators (either hydralazine and isosorbide dinitrate or prazosine).

Study Design

Patients with hypertension: The study in these patients was divided into four phases: an initial outpatient precaptopril evaluation during placebo or standard triple therapy (phase I), an inpatient captopril titration period (phase II), an outpatient follow-up period of at least 4 weeks during captopril monotherapy (450 mg/day) (phase III), and in some patients a final period in which a diuretic was added to captopril (phase IV).

All measurements in phase I were made when the patients had been recumbent for at least 1 hour. Noninvasive measurements of cardiac output (vide infra) were begun when the patients were on placebo or standard triple therapy for at least 2 weeks. Dietary advice to restrict sodium intake was given but adherence to this advice was not rigorously checked. However, spot 24 hour urine collections in 30 patients during phases I and III gave values of 112 ± 8 and 108 ± 10 mmol of sodium, respectively.

For initiation of captopril treatment in phase II, patients were admitted to the hospital for a few days. The hemodynamic effects of a single first dose of captopril, 50 mg orally, were observed for several hours by invasive techniques in 14 patients with essential hypertension and in 16 with hypertension and renal artery stenosis. None of them had been on active drug for at least 3 weeks. Eleven patients on standard triple therapy were slowly titrated with increasing doses of captopril on an 8 hour schedule, while triple therapy was tapered off. In all patients a final daily dose of 450 mg was reached in a few days.

After discharge all patients were followed up in phase III in the outpatient clinic. Noninvasive measurements of cardiac output, which had been performed in phase I, were now repeated at weekly intervals. Therapy compliance was checked by pill counting.

In phase IV the hemodynamic effects of adding hydrochlorothiazide, 25 to 100 mg/day, to captopril, 450 mg/day, were studied in patients who did not become normotensive (150/90 mm Hg or less) on captopril alone.

Patients with chronic heart failure: All patients were admitted to the coronary care unit and the withdrawal of previous vasodilator therapy was covered by invasive monitoring. Twenty-four hours after the last dose of vasodilator and 12 hours after the last dose of diuretic, captopril 50 mg was given and the effects were observed for several hours.

Measurements

Hemodynamics: All hemodynamic studies were performed while the patients were in the postabsorptive state and when they had been recumbent for at least 1 hour. The short-term effects of captopril were monitored invasively and the measurements were begun 1 hour after the catheters had been placed. Systemic arterial pressure was measured through a catheter introduced into a radial or brachial artery. A Swan-Ganz flow-directed triple lumen catheter was introduced by way of an antecubital vein for recording right atrial, pulmonary artery and pulmonary capillary wedge pressures. Cardiac output was measured in triplicate by thermodilution technique.

Blood pressure: Noninvasive methods were used for repeated measurements before and during long-term captopril treatment. Blood pressure was measured with the London School of Hygiene sphygmomanometer to minimize observer bias.[12] Disappearance of sounds was taken as diastolic pressure.

Cardiac output: This was measured by an indicator dilution technique using technetium-99m–human serum albumin (100 to 200 μCi). Time-concentration curves were recorded by precordial counting of radioactivity. The counting probe, which was described previously,[13] was placed perpendicular to the chest wall without lateral rotation, over the fifth rib at

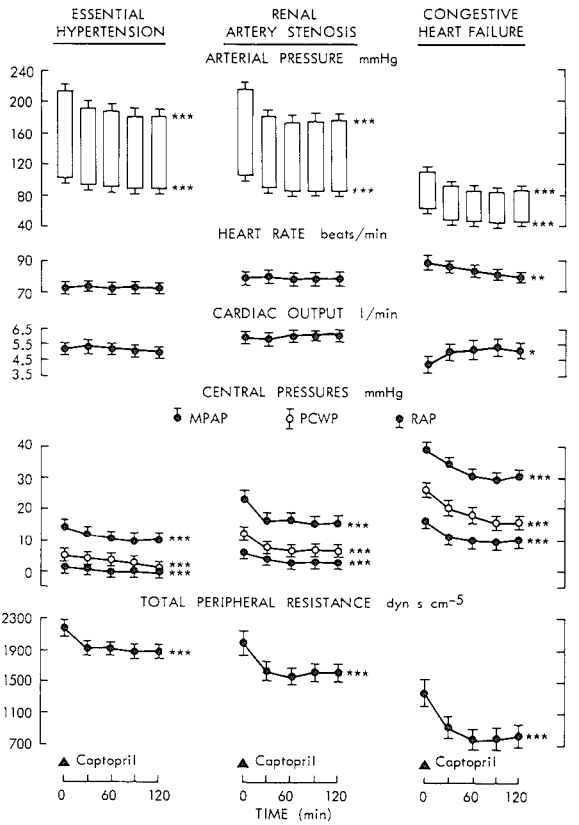

FIGURE 1. Short-term hemodynamic effects of captopril, 50 mg orally, in patients with essential hypertension (n = 14), hypertension with renal artery stenosis (n = 16) and chronic heart failure (n = 14). Data on arterial pressure, heart rate and central pressures in heart failure were obtained in 14 patients; data on cardiac output and peripheral resistance in 10. Data relevant to body size were converted to 1.73 m^2 body surface area. MPAP = mean pulmonary artery pressure; PCWP = mean pulmonary capillary wedge pressure; RAP = right atrial pressure. * p <0.05; ** p <0.01; *** p <0.001.

the midsternal line with the patient supine. Response curves were recorded for 1 to 2 minutes after rapid intravenous injection of the isotope. Additional recordings were made after 5 and 10 minutes when blood samples were taken. Cardiac output was calculated with the Stewart-Hamilton formula. From duplicate measurements in 30 subjects with cardiac output values ranging from 4 to 12 liters/min the coefficient of variation was calculated to be 6 percent. Simultaneous measurements of cardiac output by the classical indocyanine dilution method and by the isotope method showed good agreement (r = 0.92, n = 37). Heart rate was calculated from a continuously recorded electrocardiogram. Immediately after the precordial time-radioactivity curve was recorded, blood pressure was measured in triplicate. Mean blood pressure (diastolic pressure + 0.3 × pulse pressure) was used for calculating total peripheral resistance.

Extracellular fluid volume was estimated by measuring the distribution volume of intravenously injected sodium ^{35}S sulphate (50 to 60 μCi) with blood sampling at 0, 30, 60, 80, 100 and 120 minutes.[14] Blood samples were also drawn for determination of active plasma renin. The normal range of plasma renin is 15 to 40 μU/ml.[15] All values relevant to body size were converted to 1.73 m^2 body surface area.

Statistics: Data are presented as mean values ± standard error of the mean. The *t* tests for paired and unpaired data were used for comparison.

Results

Short-Term Studies

Blood pressure and hemodynamic effects (Fig. 1): The effects of 50 mg of captopril were maximal at 90 minutes. At that time mean arterial pressure had decreased from 141 ± 6 to 119 ± 7 mm Hg in patients with essential hypertension (n = 14), from 143 ± 6 to 114 ± 5 mm Hg in those with hypertension with renal artery stenosis ("renovascular") (n = 16) and from 76 ± 3 to 54 ± 4 mm Hg in those with chronic heart failure (n = 14). These responses were not significantly different among the three patient groups (p >0.05). Heart rate and cardiac output did not change in the two hypertension groups. Heart rate decreased from 90 ± 4 to 82 ± 3 beats/min (p <0.01) in the chronic heart failure group and cardiac output increased from 4.3 ± 0.5 to 5.2

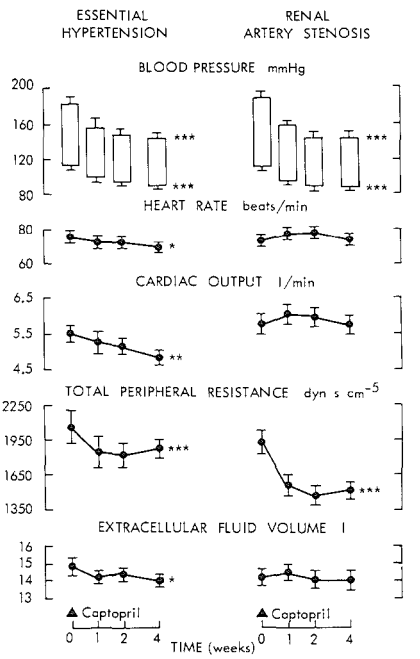

FIGURE 2. Long-term hemodynamic effects of captopril, 450 mg/day, in patients with essential hypertension (n = 17) and hypertension with renal artery stenosis (n = 14). Measurements during captopril therapy are compared with those in the last week of placebo treatment. For further details see legend to Figure 1.

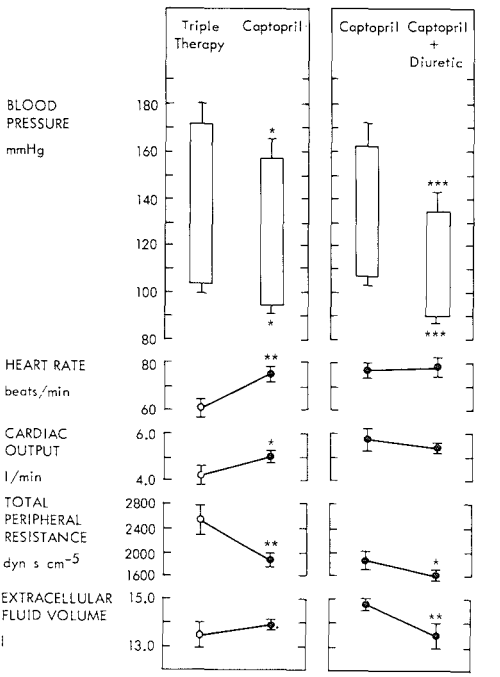

FIGURE 3. Long-term hemodynamic effects in the two hypertensive groups of captopril alone as compared with standard triple therapy (n = 11) and with captopril combined with diuretic (n = 10). For details see text and legend to Figure 1.

± 0.1 liters/min ($p < 0.05$). Right atrial pressure, pulmonary artery pressure and pulmonary capillary wedge pressures decreased in the three patient groups. The changes in these pressures were greater ($p < 0.01$) in chronic heart failure than in essential hypertension and renovascular hypertension.

Total peripheral resistance decreased from 2,170 ± 90 to 1,880 ± 90 dynes s cm^{-5} in essential hypertension, from 1,980 ± 160 to 1,600 ± 130 dynes s cm^{-5} in "renovascular" hypertension and from 1,360 ± 210 to 770 ± 130 dynes s cm^{-5} in chronic heart failure. The resistance changes were greater ($p < 0.05$) in heart failure than in the two hypertensive groups.

Plasma renin: Plasma renin increased from 27 ± 6 μU/ml before captopril to 79 ± 30 μU/ml 90 minutes after captopril in essential hypertension. It increased from 110 ± 21 to 700 ± 120 μU/ml in renovascular hypertension and from 740 ± 330 to 2,700 ± 870 μU/ml in chronic heart failure.

Long-Term Studies

Captopril monotherapy in essential and renovascular hypertension (Fig. 2): Mean blood pressure had decreased from 136 ± 4 to 108 ± 2 mm Hg after 4 weeks of captopril therapy, 450 mg/day, in the essential hypertension group (n = 17) and from 135 ± 4 to 105 ± 3 mm Hg in the renovascular hypertension group (n = 14). These responses in the two patient groups were not significantly different. Heart rate in the essential hypertension group was 76 ± 3 beats per minute before captopril and 69 ± 2 after 4 weeks of captopril therapy. Cardiac output was 5.5 ± 0.2 liters/min before the drug and 4.8 ± 0.2 after 4 weeks. These changes were statistically significant ($p < 0.05$). Stroke volume was not significantly altered in the essential hypertension group. There were no significant changes in heart rate, cardiac output and stroke volume in the renovascular hypertension group. Total peripheral resistance decreased from 2,070 ± 130 to 1,830 ± 92 dynes s cm^{-5} in the essential hypertension group and from 1,940 ± 87 to 1,470 ± 69 in the renovascular hypertension group, the change in resistance being greater ($p < 0.01$) in the latter group.

Extracellular fluid volume in the essential hypertension group was 14.8 liters before captopril and 14.0 liters after 4 weeks. In the "renovascular" hypertension

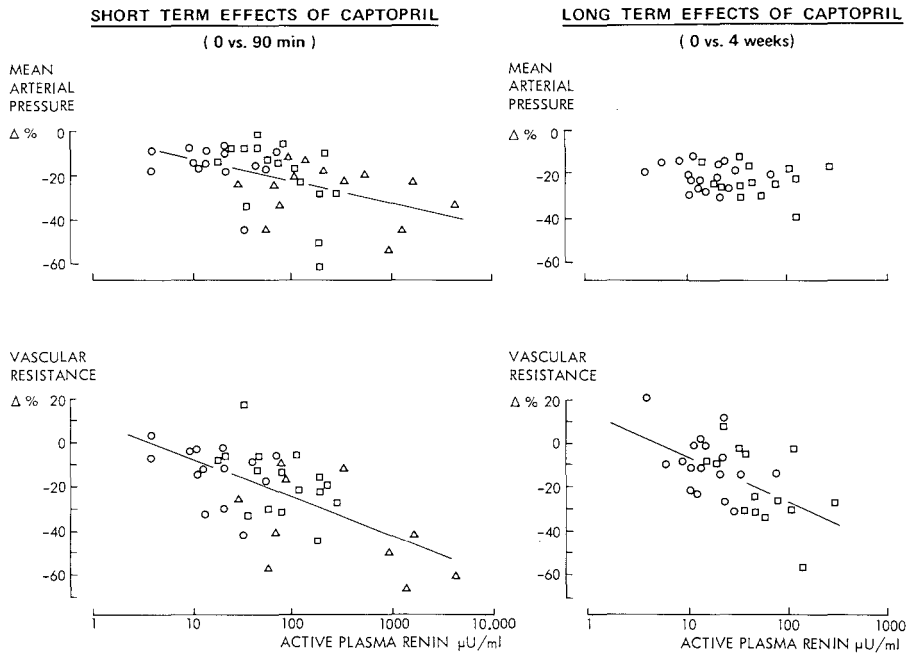

FIGURE 4. Linear regression analysis of short-term and long-term effects of captopril as related to plasma renin activity. Correlations of short-term percent changes in mean arterial pressure with log renin were significant in the group with hypertension and renal artery stenosis ($r = 0.53$, $n = 16$, $p < 0.05$), in the stenosis and essential hypertension groups together ($r = 0.43$, $n = 30$, $p < 0.01$) and in the hypertension and heart failure groups together ($r = 0.47$, $n = 44$, $p < 0.001$) but not in the essential hypertension and heart failure groups separately. Correlations of long-term percent changes in mean arterial pressure with log renin were not significant. Correlations of short-term percent changes in total peripheral resistance with log renin were significant in the hypertension groups together ($n = 0.46$, $n = 30$, $p < 0.01$) and in the hypertension and heart failure groups together ($r = 0.64$, $n = 40$, $p < 0.001$) but not in the groups separately. Correlations of long-term percent changes in total peripheral resistance with log renin were significant in the group with hypertension and renal artery stenosis ($r = 0.52$, $n = 14$, $p < 0.05$) and in the stenosis and essential hypertension groups together ($r = 0.56$, $n = 31$, $p < 0.001$). Δ% = percent change; O = essential hypertension; □ = hypertension with renal artery stenosis; Δ = chronic heart failure.

group it was 14.2 and 14.1 liters, respectively. The difference was statistically significant ($p < 0.05$) only in the essential hypertension group.

Plasma renin activity increased from 19 ± 4 μU/ml before captopril to 170 ± 90 μU/ml after 4 weeks of captopril therapy, 450 mg/day, in the essential hypertension group and from 75 ± 19 to 570 ± 130 μU/ml in the "renovascular" group.

Captopril monotherapy versus standard triple therapy (Fig. 3): The hemodynamic effects of 4 weeks of captopril monotherapy, 450 mg/day, and 4 weeks of standard triple therapy were compared in a group of 11 patients including five with essential hypertension and six with hypertension and renal artery stenosis. Systolic and diastolic arterial pressures were lower ($p < 0.05$) with captopril than with standard triple therapy (Fig. 3). Moreover, cardiac output was higher ($p < 0.05$) and total peripheral resistance was lower ($p < 0.01$) with captopril.

Captopril monotherapy versus captopril plus a diuretic (Fig. 3): The hemodynamic profiles of 4 weeks of captopril alone, 450 mg/day, and 4 weeks of captopril plus hydrochlorothiazide, 25 to 100 mg/day, were compared in 10 patients with essential hypertension who did not become normotensive (150/90 mm Hg or less) on captopril alone. The addition of hydrochlorothiazide to captopril treatment caused a sustained decrease in extracellular fluid volume with further reduction in blood pressure. Heart rate and cardiac output showed no significant changes. Thus, the decrease in blood pressure was associated with a parallel decrease in total peripheral resistance.

Correlations between hemodynamic responses and pretreatment plasma renin (Fig. 3): Short-term

blood pressure responses 90 minutes after 50 mg of captopril in the three groups of patients combined were correlated with pretreatment plasma renin (percent change in mean arterial pressure versus log renin, $p < 0.001$, $n = 40$). The correlation was rather weak ($r = 0.47$) and was not significant in the essential hypertension and heart failure groups separately. Short-term responses of total peripheral resistance were more closely correlated with pretreatment plasma renin (percent change in total peripheral resistance versus log renin, $r = 0.64$, $p < 0.001$, $n = 40$). Long-term responses of mean blood pressure were unrelated to pretreatment plasma renin, whereas long-term responses of total peripheral resistance did correlate with pretreatment renin ($r = 0.56$, $p < 0.001$, $n = 31$).

Discussion

Blood pressure effects versus plasma renin: The hypotensive effects of captopril in essential hypertension and in hypertension with renal artery stenosis ("renovascular") appeared to be similar in this study despite fourfold higher plasma renin activity in the "renovascular" hypertension group. A weak, albeit statistically significant correlation was observed between the short-term hypotensive effect of captopril and pretreatment renin in the group with renal artery stenosis and in this group and the essential hypertension group together but not in the essential hypertension group alone. No such correlations between blood pressure response and renin were observed during long-term captopril therapy (Fig. 4).

The decrease in blood pressure was mainly caused by a decrease in peripheral resistance in both hypertension groups. The changes in resistance in the two groups together were significantly correlated with pretreatment plasma renin both after short-term captopril and after long-term treatment, but again the correlations were weak and were not significant in the individual groups (Fig. 4).

Heart rate and cardiac output: Some statistically significant differences in the responses of heart rate and cardiac output to long-term captopril therapy between the two groups of hypertensive patients emerged. Heart rate and cardiac output decreased in those with essential hypertension and not in those with "renovascular" hypertension, but here again the differences were small.

Blood pressure and hemodynamic effects in heart failure: Plasma renin can be low, normal or high in chronic heart failure.[16] In our patients it ranged from 30 to 4,600 μU/ml (normal less than 40). By increasing unilateral vascular resistance the grossly elevated plasma renin in most of our patients might have contributed to the maintenance of a relatively normal blood pressure in the presence of low cardiac output. However, despite the wide range of renin values, the effect of captopril on peripheral resistance was only weakly correlated with pretreatment renin values (Fig. 4).

Blood pressure was already low in some patients with heart failure and a further decrease in pressure might have been deleterious, but captopril was well tolerated by these patients. They had no chest pain and there were no electrocardiographic changes suggestive of increased cardiac ischemia. Within 30 minutes after the intake of captopril the patients were less dyspneic. Indeed, hemodynamic measurements showed improvement: heart rate decreased and cardiac output increased in the presence of marked reduction in cardiac filling pressures. These results agree with those of previous reports on the short-term effects of captopril in heart failure.[17,18]

Mechanisms of captopril's cardiovascular effects: The most important hemodynamic effect of captopril is arteriolar dilatation. The arterioles are the major resistance to blood flow and form by far the largest contribution to total peripheral resistance. Therefore, correlations between captopril's effect on total peripheral resistance and pretreatment plasma renin support the contention that blockade of plasma renin-mediated angiotensin II formation is a component of captopril-induced arteriolar dilatation. However, the correlation coefficients in our analysis were rather small and indicate that other factors besides circulating renin are important. The observed reduction in cardiac filling pressures has been considered to reflect a dilatory effect of captopril on capacitance vessels. However, it is more likely that reduced cardiac filling pressures are an expression of improved cardiac performance. Our data do not provide definite evidence for either possibility.

Although captopril produces vasodilatation, it does not cause reflex tachycardia. From experiments in animals[19] some evidence indicates that the set-point, but not the sensitivity, of the arterial baroreflex is altered by converting enzyme inhibition. In man the baroreflex-mediated responses to upright posture seem unimpaired by captopril[20]; postural hypotension is seldom seen with this drug.

Extracellular fluid volume: role of angiotensin and aldosterone: Captopril does not cause fluid retention by the kidneys and again this contrasts with some other vasodilators. The decrease in extracellular fluid volume observed in some of our hypertensive patients and the absence of fluid retention in all can be explained, at least in part, by reduced angiotensin II formation, because this peptide is known to promote renal sodium and water retention both through its direct actions on the kidney and through aldosterone. A similar mechanism could underlie the favorable hemodynamic response to the combination of captopril with a diuretic. Our patients showed a sustained reduction of extracellular fluid volume after hydrochlorothiazide had been added to captopril, and this was associated with a decrease in total peripheral resistance. Presumably inhibition of angiotensin I conversion in these patients has prevented the compensatory increase in angiotensin II and aldosterone, which can limit the therapeutic effect of a diuretic.[21,22]

A 20 percent reduction in both systolic and diastolic blood pressures was observed in our hypertensive patients with captopril as the only drug in combination with mild dietary sodium restriction. This response is as good or even better than the blood pressure responses to thiazide diuretics and beta-receptor blocking agents.

Therapeutic implications: Captopril is an effective antihypertensive agent with a favorable hemodynamic profile. The hemodynamic responses in essential hypertension with low or normal plasma renin and in hypertension associated with renal artery stenosis with normal or high renin are very similar. The long-term hemodynamic effects of converting enzyme inhibition that have been observed in patients with hypertension warrant further consideration of captopril as a treatment of choice in most forms of clinical hypertension. In severe or refractory chronic heart failure cardiac function can improve with captopril, but further studies are required to define its place in the long-term treatment of heart failure.

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CHAPTER 2

ACE INHIBITION AND THE HEART

Effects of captopril in acute and chronic heart failure.

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Effects of captopril in acute and chronic heart failure
Correlations with plasma levels of noradrenaline, renin, and aldosterone

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SUMMARY The angiotensin-converting enzyme inhibitor, captopril, was given to 19 patients with severe heart failure. Seven patients had acute myocardial infarction and the remainder had chronic myocardial damage caused by ischaemia or valvular disease. Cardiac filling pressures were raised in all, the pulmonary capillary "wedge" pressure being 17 mmHg or more. Captopril, 50 mg orally, raised stroke volume and cardiac output, and reduced heart rate, cardiac filling pressures, systemic arterial pressure, and the plasma concentrations of aldosterone and noradrenaline. These changes were attended by clinical improvement. Decrements in cardiac filling pressures, systemic arterial pressure, and total peripheral resistance were positively correlated with pretreatment plasma renin. Long-term treatment with captopril was offered to 14 patients. Four patients with severe coronary disease died suddenly after initial clinical improvement. In nine patients haemodynamic measurements were repeated after three months. The results showed sustained effects on cardiac output and filling pressures but there was no loss of body weight. The haemodynamic effects were at least as good as with previous vasodilators. The fall in systemic arterial pressure, however, was greater with captopril. Captopril may become a valuable adjunct to the treatment of acute and chronic heart failure, but more information about its effect on coronary blood flow is required.

The acute beneficial response to vasodilators in patients with congestive heart failure is well documented.[1–4] By lowering vascular tone and left ventricular wall tension, these agents improve myocardial contraction and reduce raised filling pressures of the heart. It is now also clear that such a favourable effect is not restricted to drugs that cause vasodilatation because of their direct relaxing effect on vascular smooth muscle. Comparable haemodynamic responses are observed with drugs that reduce sympathetic vascular tone,[5,6] act by interruption of the renin-angiotensin-aldosterone cascade,[7,8] or block serotonergic vascular mechanisms.[9]

Because so many different neurohumoral factors are involved in the circulatory homeostasis of heart failure, however, it is likely that benefits from intervention in one regulatory system are lost by compensatory activation of other systems. In this regard it is significant that, as compared with the abundance of data on the acute benefits of vasodilatory treatment, little is known about its long-term efficacy.[10,11] Experiments with hydralazine[12,13] and prazosin[14–16] suggest that only part of the initial response is maintained. Weight gain, oedema, and increases in the plasma levels of renin, aldosterone, and noradrenaline have been found to accompany tolerance to these drugs. In contrast, the vasodilatory response to inhibitors of angiotensin-converting enzyme is not accompanied by increased sympathetic activity[17] and aldosterone production[18] nor by renal sodium and water retention.[19] Indeed, good results have been obtained with the use of the orally active angiotensin-converting enzyme inhibitor, captopril, even in patients with congestive heart failure refractory to conventional treatment.[20–23] The role of the renin-angiotensin system in heart failure, however, is not fully understood,[24,25] and information about the long-term cardiovascular and humoral effects of captopril in heart failure is fragmentary.

With these uncertainties in mind we have studied the acute and long-term effects of captopril in normotensive patients with heart failure and pulmonary congestion. We have tried to correlate these effects with measurements of the plasma levels of noradrenaline,

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renin, and aldosterone. These measurements were considered a rough index of the involvement of the sympathetic nervous system and the renin-angiotensin system in our patients. Two groups of patients were studied. In one group heart failure was an acute condition occurring in the course of myocardial infarction. Plasma noradrenaline was raised in these patients but plasma renin and aldosterone were normal or low in most of them. In a second group heart failure was a chronic treatment-resistant condition, and most patients had high plasma noradrenaline as well as high plasma renin and aldosterone.

Patients and methods

Nineteen patients with normal blood pressure with heart failure were studied. On the basis of onset and duration of this condition the patients were divided into two groups. Acute pump failure resulting from transmural myocardial infarction was the cause of pulmonary congestion and shortness of breath in the seven patients of group 1 (Table 1). Criteria for inclusion were: mean pulmonary capillary wedge pressure higher than 17 mmHg and systolic arterial pressure between 90 and 150 mmHg. Captopril was given to four patients within 12 hours after the onset of myocardial infarction. Digoxin, diuretics, or other vasoactive agents were not used and the interval between the last sedative or pain relieving agent and captopril was at least one hour. Intravenous lignocaine for arrhythmia was necessary in two patients and this was continued during the study. The clinical condition of three patients (Table 1, cases 5, 6, and 7) was such that initial treatment with intravenous dopamine and nitroprusside was indicated. Two to three days after the onset of myocardial infarction, these patients were weaned from intravenous therapy. Recurrence of pulmonary congestion and raised cardiac filling pressures, however, made additional treatment desirable. Captopril was then chosen as an oral substitute for the nitroprusside infusion they had received before. Treatment with dopamine and nitroprusside was stopped at least 12 hours before captopril was given. Group 2 consisted of 12 patients suffering from refractory congestive heart failure for at least two years (Table 2). The aetiology of ventricular failure was secondary to ischaemic heart disease and previous myocardial infarction in seven patients. Three of them had undergone coronary bypass surgery. Persistent poor ventricular function despite aortic or mitral valve

Table 1 *Clinical characteristics of patients with acute myocardial infarction*

Case No.	*Age (y)*	*Sex*	*Weight (kg)*	*Height (m)*	*Localisation of myocardial infarction*	*CK peak value (U/l)*	*Time between onset MI and start captopril (h)*	*Therapy (mg/d)*	
								Digoxin	*Frusemide*
1	71	M	69	1·75	Anterolateral	210	4		
2	55	M	76	1·74	Anteroseptal	870	5		
3	59	M	72	1·64	Anterior	1200	10		
4	57	M	81	1·72	Inferior	345	12		
5	45	M	60	1·78	Posterior	1600	30	0·250	240
6	62	M	90	1·76	Anteroseptal	940	52	0·250	200
7	57	F	67	1·69	Anteroseptal	1500	72	0·250	160

Note: CK, serum creatine kinase (normal value 5–30 U/l); MI, myocardial infarction.

Table 2 *Clinical characteristics and echocardiographic findings in patients with chronic heart failure*

Case No.	*Age (y)*	*Sex*	*Weight (kg)*	*Height (m)*	*Diagnosis and duration of chronic heart failure (y)*	*NYHA class*	*Echocardiographic dimensions (mm)*				*Therapy (mg/d)*						
							LA	*LA/Ao*	*LVD*	*LVS*	*Dig*	*Fru*	*Spir*	*Hyd*	*Iso*	*Pra*	*Nif*
8	64	M	72	1·68	IHD	3	IV	50	1·43	85	70	0·125	320	150	200	80		
9	49	F	72	1·63	IHD	2	IV	52	1·63	70	55	—	240	100	100	80		
10	63	M	61	1·79	IHD CB	3	IV	45	1·50	78	68	0·125	120	50	40	—		
11	62	M	76	1·82	VHD MV	2	III	72	1·89	70	60	0·250	320	—	200	80		
12	54	M	67	1·75	IHD CB	2	IV	60	2·00	75	60	0·250	120	100	—	80		40
13	67	M	66	1·68	IHD	2	IV	60	1·71	100	90	0·250	80	100	—	—	8	
14	54	M	58	1·75	VHD A+MV	3	IV	63	1·70	90	85	0·250	160	50	200	80		
15	74	F	60	1·69	VHD MV	6	IV	65	1·86	60	45	0·125	320	50	—	—		
16	59	F	65	1·68	VHD AV	2	IV	55	1·38	50	35	0·125	80	50	—	—	16	
17	46	M	78	1·80	IHD CB	3	IV	65	2·32	62	50	0·250	120	—	200	80		
18	64	F	73	1.66	VHD MV	5	IV	70	1·89	70	60	0·250	160	100	—	—	8	
19	46	M	79	1·68	IHD CB	5	III	45	1·66	73	59	0·250	80	—	—	80		40

IHD, ischaemic heart disease; VHD, valvular heart disease; CB, coronary bypass; MV, prosthetic mitral valve; AV, prosthetic aortic valve; echocardiographic dimensions: LA, left atrial dimension (normal 20–40 mm); Ao, aortic root diameter (normal 20–40 mm); LA/Ao, ratio between left atrial dimension and aortic diameter (normal 1); LVD, left ventricular end-diastolic dimension (normal 30–55 mm); LVS, left ventricular end-systolic dimension (normal value variable); Dig, digoxin; Fru, frusemide; Hyd, hydralazine; Iso, isosorbide nitrate; Pra, prazosin; Nif, nifedipine.

replacement was the cause of heart failure in five patients. All complained about shortness of breath at rest despite extensive treatment with dietary sodium restriction, digoxin, diuretics, and hydralazine-isosorbide dinitrate combinations or prazosin. Captopril was given to this treatment-resistant group 24 to 36 hours after the vasodilators or prazosin had been stopped.

HAEMODYNAMIC EVALUATION

The patients of group 1 were admitted to the coronary care unit. A Swan-Ganz thermodilution catheter was positioned in the pulmonary artery and a small Teflon cannula was placed into a radial artery. When stable baseline haemodynamic measurements had been obtained, captopril, 50 mg, was given by mouth, and its effect was followed for several hours. In the patients of group 2 invasive haemodynamic monitoring was started during the use of previous vasodilators. For this purpose the patients were transferred to the coronary care unit from the general ward where they had been admitted some days before in order to optimise medical and dietary treatment. Vasodilators or prazosin were then stopped and the withdrawal period of at least 24 hours was covered by invasive monitoring. Digoxin and diurectics were continued, but at least six hours was left between administration of these drugs and the start of captopril. With the evaluation of the first dose of captopril the invasive first part of the study was finished. After treatment lasting three months nine patients of group 2 were readmitted to the coronary care unit for invasive monitoring of the long-term effects of captopril.

MEASUREMENTS

Heart rate (HR), systemic arterial pressure, and pulmonary arterial pressure were continuously monitored. Triplicate cardiac output measurements by the thermodilution technique were performed at frequent intervals and at that time integrated mean values for systemic arterial pressure (MAP), right atrial pressure (RAP), pulmonary arterial pressure, (PAP) and pulmonary capillary "wedge" pressure (PCWP) were also taken. Pressures and cardiac output were measured with the patient in a semisupine position and the transducers were zeroed at mid-thoracic level. Cardiac output was corrected for body surface area and tabulated as cardiac index (CI). The following variables were derived: total peripheral resistance in kPa/s per l as TPR=(MAP−RAP)/CI, pulmonary vascular resistance in kPa/s per l as PVR=(PAP−PCWP)/CI, cardiac work index in J/min as CWI=(MAP−PCWP)×CI, and stroke work index in J as SWI=(MAP−PCWP)×SI, where SI is stroke volume index.

OTHER MEASUREMENTS AND STATISTICS

Estimations of cardiac chamber size were made by means of M-mode echocardiograms obtained with an ECHO-cardioVISOR SE(Organon Technica, The Netherlands) interfaced to a Honeywell LS 6 strip chart recorder. The dimensions of left atrium, aorta, and left ventricle during end-systole and end-diastole were taken with standard positions of the transducer as described previously.[26] Arterial and mixed venous oxygen saturations were measured by oximetry. The arteriovenous oxygen difference ($AV\text{-}O_2$) in mmol/l was calculated as $AV\text{-}O_2=(A_{sat}\text{-}PA_{sat}) \times Hb \times 1{\cdot}01$, where A_{sat} and PA_{sat} are the arterial and pulmonary arterial (mixed venous) oxygen saturations and Hb is the haemoglobin content in mmol/l, while 1·01 is the binding capacity in mmol 0_2 of 1 mmol Hb.

Plasma levels of active renin[27] and aldosterone[28] were measured by radioimmunoassay. The normal range for renin in our laboratory is 15 to 45 μU/ml. For aldosterone it is 100 to 500 pmol/l. A radioenzymatic technique was used for determining plasma noradrenaline.[29] The normal range is 1 to 3 nmol/l. Data are given as mean ± SEM. Plasma levels of renin, aldosterone, and noradrenaline were not distributed normally. Mean values and standard errors were therefore calculated after log transformation. Statistical analysis was performed using Student's t test for paired data. Linear regression analysis was used for calculation of correlation coefficients. Statistical significance was accepted at the 95% confidence level.

Results

PRE-CAPTOPRIL EVALUATION

Patients with myocardial infarction

Pre-captopril values of arterial pressure were somewhat higher and central pressures were lower than in the patients with chronic heart failure (Tables 3 and 4). Though plasma noradrenaline was raised in all cases, plasma renin and aldosterone were normal in the previously untreated cases (Table 5, cases 1 to 4).

Patients with chronic congestive heart failure

The severity of heart failure was not only reflected by their symptoms, as expressed according to the New York Heart Association (NYHA) classification[30] but the advanced stage of cardiac dilatation was also disclosed by echocardiography (Table 2). Both left atrium and left ventricular cavity dimensions were much increased. Moreover, filling pressures of either side of the heart were high, that is right atrial pressure 14±2 mmHg and pulmonary capillary "wedge" pressure 25±2 mmHg, while cardiac index was low. The mean value for cardiac index of 2·6±0·4 l/min is probably an overestimation because it does not include three very serious cases, in which no reliable cardiac output

Table 3 *Haemodynamic responses to captopril in patients with acute myocardial infarction*

Case No.	Heart rhythm	Heart rate (beats/min)		Mean pressure (mmHg)								Cardiac index (l/min)		Resistance (kPa/s per l)			
				Arterial		Right atrial		Pulm. artery		Pulm. cap. "wedge"				Systemic		Pulmonary	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	SR	90	86	117	107	9	6	29	19	20	11	2·9	3·4	298	238	25	19
2	SR	86	84	95	91	13	8	28	28	19	16	2·1	2·2	312	302	34	44
3	SR	91	80	113	93	12	7	31	24	26	16	2·8	2·9	289	237	14	22
4	SR	58	56	87	79	9	8	27	26	24	20	2·8	3·1	223	183	9	16
5	AF	105	78	79	62	13	8	30	23	27	18	1·7	2·4	311	180	14	17
6	SR	94	90	62	50	17	11	38	33	23	16	—	—	—	—	—	—
7	SR	109	102	98	90	5	0	27	17	17	11	2·3	2·7	324	267	35	18
Mean		90	82	93	82	11	7	30	24	22	15	2·4	2·8	293	235	22	23
±SEM		6	5	7	7	1	1	1	2	1	1	0·2	0·2	15	19	5	4
p		<0·05		<0·01		<0·001		<0·01		<0·001		<0·01		<0·01		NS	

Note: Values presented are those obtained before and 90 minutes after administration of captopril, 50 mg. SR, sinus rhythm; AF, atrial fibrillation. 1 kPa/s per l≈10 dyn s cm^{-5}.

measurements could be obtained. Systemic arterial pressure was already low before captopril, systolic 114±4 mmHg and diastolic 62±3 mmHg, and, despite extensive use of digoxin, the mean value for heart rate exceeded 80 beats/min. The plasma levels of renin, aldosterone, and noradrenaline were grossly raised (Table 5). Kidney function was moderately impaired as indicated by a serum creatinine of 133±14 μmol/l; the range was 81 to 253 μmol/l, with a value above 100 μmol/l in eight patients.

ACUTE EFFECTS OF CAPTOPRIL

Haemodynamics

Captopril improved resting haemodynamics both in the patients with heart failure caused by acute myocardial infarction (Table 3) and the patients with chronic heart failure (Table 4).

Changes in systemic arterial pressure, heart rate, cardiac output, and filling pressures were not much different for the two groups. Therefore in Fig. 1 to 3 the data of both groups have been pooled. Despite decrements in systolic arterial pressure, from 121±6 to 96±6 mmHg, and in diastolic pressure, from 66±3 to 52±4 mmHg, heart rate was also lowered by captopril, from 88±3 to 80±3 beats/min ($p<0·05$, Fig. 1). These effects became apparent after 15 minutes, while the peak effects occurred between 90 and 105 minutes. In eight patients systolic arterial pressure fell below 90 mmHg and in four below 75 mmHg. The fall in arterial pressure was well tolerated. One patient complained about blurred vision at a time when his systolic arterial pressure was 65 mmHg. No hypotensive period, however, was associated with the onset or worsening of angina or electrocardiographic abnormalities. Triple

Table 4 *Acute haemodynamic responses to captopril in patients with chronic heart failure*

Case No.	Heart rhythm	Heart rate (beats/min)		Mean pressure (mmHg)								Cardiac index (l/min)		Resistance (kPa/s per l)			
				Arterial		Right atrial		Pulm. artery		Pulm. cap. "wedge"				Systemic		Pulmonary	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
8	SR	111	89	74	34	12	3	50	24	34	10	2·3	2·4	216	103	56	47
9	SR	88	85	85	65	5	−1	38	29	23	11	1·9	2·1	337	251	63	69
10	SR	84	72	72	48	13	2	41	25	27	16	1·3	2·6	363	142	86	28
11	AF	75	76	74	65	10	8	29	26	18	13	3·3	3·5	156	130	27	30
12	SR	99	96	88	66	17	8	44	36	30	23	1·6	2·2	355	211	70	47
13	AF	104	90	83	67	16	9	38	30	27	19	—	—	—	—	—	—
14	AF	71	65	66	36	12	5	26	17	18	9	1·7	2·9	254	86	38	22
15	PM	74	74	65	30	20	8	44	31	24	14	2·8	3·1	129	57	57	44
16	AF	87	85	66	51	18	9	34	20	20	12	4·7	4·7	82	72	24	14
17	SR	82	81	93	79	27	25	45	38	33	28	—	—	—	—	—	—
18	SR	77	64	87	71	11	7	36	25	21	13	—	—	—	—	—	—
19	SR	81	80	70	51	6	−1	30	16	20	10	3·6	3·6	142	116	22	13
Mean		86	80	77	55	14	7	38	26	25	15	2·6	3·0	226	130	49	35
±SEM		4	3	3	5	2	2	2	2	2	2	0·4	0·3	36	21	8	6
p		<0·01		<0·001		<0·001		<0·001		<0·001		<0·05		<0·01		<0·05	

Note: Values presented are those obtained before and 90 minutes after administration of captopril, 50 mg. PM, pacemaker rhythm. Other abbreviations as in Table 3.

Table 5 *Hormone levels before captopril*

Case No.	*Plasma renin (μU/ml)*	*Plasma aldosterone (pmol/l)*	*Plasma noradrenaline (nmol/l)*
1	6	166	3·3
2	14	222	7·2
3	27	332	4·3
4	9	111	4·5
5	1700	9970	20·1
6	540	2270	17·5
7	—	—	—
8	990	943	18·3
9	30	665	3·7
10	4600	10500	14·6
11	88	860	2·3
12	68	2520	13·9
13	200	1270	6·5
14	1400	4700	4·5
15	58	277	2·4
16	330	1770	10·2
17	140	9690	6·8
18	110	8860	9·2
19	78	1220	2·7

Note: Plasma noradrenaline, 1 nmol/l ≈ 169 ng/l.
Plasma aldosterone, 1 pmol/l ≈ 360 pg/l.

product, that is the product of systolic arterial pressure, heart rate, and pulmonary capillary "wedge" pressure divided by 1000, fell from 253±19 to 119±13

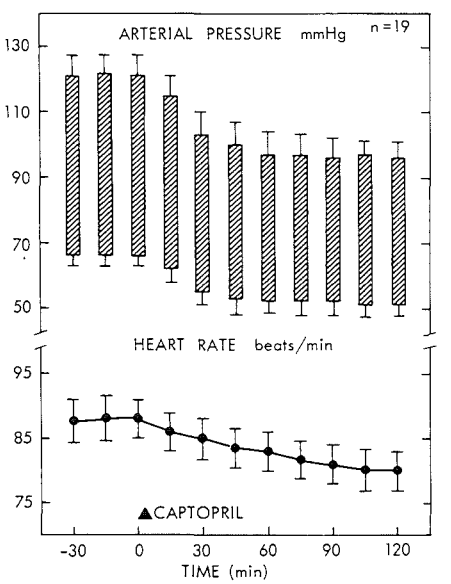

Fig. 1 *Responses of systemic arterial pressure and heart rate to a single dose of captopril (50 mg) in 19 patients with heart failure. The fall in both systolic and diastolic arterial pressure was already significant ($p<0·01$) 15 minutes after captopril. The fall in heart rate became significant ($p<0·01$) after 30 minutes.*

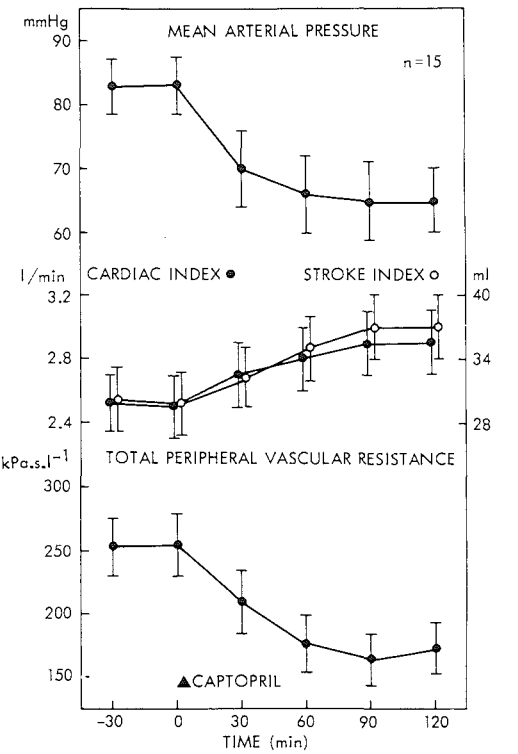

Fig. 2 *Effects of captopril (50 mg) on cardiac index, stroke index, mean arterial pressure, and total peripheral resistance. The peak effects were significant at $p<0·01$.*

($p<0·001$) presumably leading to a diminished myocardial oxygen demand.

The fall in mean arterial pressure of 25±4% was associated with a fall in total peripheral resistance of 30±5% (Fig. 2). Cardiac output rose by an increase in stroke volume. This increase in flow was also reflected in the accompanying change in arteriovenous oxygen difference. It fell significantly from 3·1±0·3 mmol/l before captopril to 2·7±0·2 mmol/l one hour after captopril ($p<0·05$) and to 2·6±0·2 mmol/l two hours after captopril ($p<0·05$). The amount of work performed by the heart did not change. Cardiac work and stroke work indices before captopril were 20·26±2·26 J/min and 0·24±0·03 J, respectively, and 90 minutes after captopril they were 19·61±2·58 J/min and 0·25±0·03 J.

Captopril caused parallel decrements in mean pulmonary arterial pressure, pulmonary capillary "wedge" pressure, and right atrial pressure (Fig. 3).

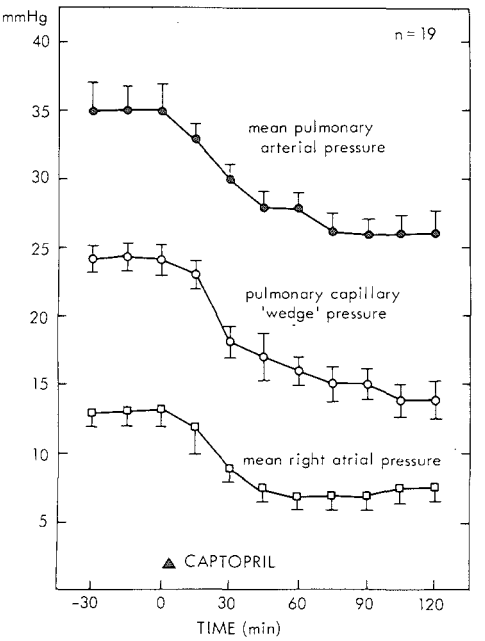

Fig. 3 *Captopril (50 mg) caused concurrent decrements in mean pulmonary arterial, pulmonary capillary "wedge", and right atrial pressures. After 30 minutes all changes were significant at p<0·01.*

Pulmonary vascular resistance fell from 38±6 to 30±4 kPa/s per l (p<0·05). These changes were most pronounced in the chronic heart failure group (Table 4).

Plasma renin rose and plasma aldosterone fell after captopril (Fig. 4) as expected. More surprising was the change in plasma noradrenaline; it fell from a mean value of 6·09 nmol/l before captopril to 5·4 nmol/l after one hour (p<0·5) and to 4·5 nmol/l after two hours (p<0·01). The higher the pretreatment value of noradrenaline, the greater was its decrement (Fig. 5).

Interrelations between humoral factors, baseline haemodynamics, and captopril-induced changes

Pretreatment plasma noradrenaline was directly correlated with pretreatment plasma renin and aldosterone (Table 6). Heart rate before captopril was directly correlated with noradrenaline, suggesting that the high plasma level of noradrenaline indeed reflected an increased sympathetic tone in these patients. Of the flow measurements, pretreatment stroke volume was inversely correlated to plasma noradrenaline but not to renin, whereas cardiac performance, expressed as cardiac work or stroke work, was inversely correlated both with noradrenaline and with renin and also with plasma aldosterone. Total peripheral resistance before captopril, however, was unrelated to the plasma levels of noradrenaline and renin. The decrease in mean and diastolic systemic arterial pressure after captopril but not the decrease in systolic pressure was correlated to pretreatment renin (Fig. 6, Table 7), which suggests that part of the pressure drop was prevented by the observed rise in stroke volume. The decrease in total peripheral resistance after captopril was directly correlated to the pretreatment plasma levels of noradrenaline, renin, and aldosterone.

CLINICAL COURSE

Patients with myocardial infarction

After the first dose of captopril all patients experienced a reduction in dyspnoea and orthopnoea and in some

Table 6 *Correlations between pretreatment haemodynamic variables and pretreatment hormone levels*

Baseline values of	*No.*	*Versus baseline values of*					
		Log plasma renin		*Log plasma aldosterone*		*Log plasma noradrenaline*	
		r	*p*	*r*	*p*	*r*	*p*
Arterial pressure systolic	18	−0·69	<0·001	−0·65	<0·01	−0·51	<0·05
diastolic	18	−0·42	NS	−0·10	NS	−0·09	NS
mean	18	−0·65	<0·01	−0·37	NS	−0·19	NS
Heart rate	18	+0·33	NS	+0·21	NS	+0·60	<0·01
Cardiac index	14	−0·31	NS	−0·37	NS	−0·40	NS
Cardiac work index	14	−0·69	<0·01	−0·64	<0·01	−0·49	NS
Stroke index	14	−0·39	NS	−0·47	NS	−0·56	<0·05
Stroke work index	14	−0·73	<0·01	−0·72	<0·01	−0·62	<0·05
Mean right atrial pressure	18	+0·24	NS	+0·34	NS	+0·30	NS
Mean pulmonary artery pressure	18	+0·31	NS	+0·29	NS	+0·40	NS
Mean pulmonary capillary "wedge" pressure	18	+0·28	NS	+0·27	NS	+0·52	<0·05
Total peripheral vascular resistance	14	+0·11	NS	+0·29	NS	+0·43	NS
Pulmonary vascular resistance	14	+0·39	NS	+0·37	NS	+0·26	NS
Log plasma renin	18	—	—	—	—	—	—
Log plasma aldosterone	18	+0·78	<0·001	—	—	—	—
Log plasma noradrenaline	18	+0·62	<0·01	+0·56	<0·05	—	—

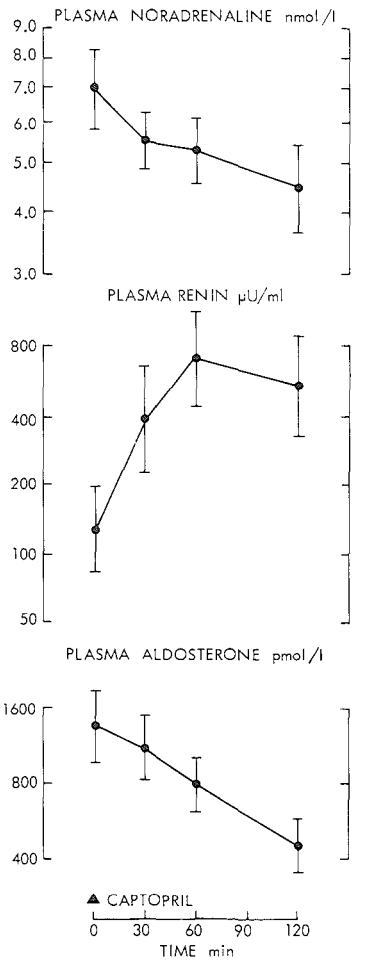

Fig. 4 *Neurohumoral responses to captopril (50 mg) in 18 patients with heart failure. Both plasma noradrenaline and aldosterone fell ($p<0·01$) after 60 minutes, whereas plasma renin rose ($p<0·01$).*

the annoying non-productive cough as a sign of imminent lung oedema disappeared. Signs of heart failure gradually faded away in four patients (cases 1 to 4) and cessation of captopril after a few doses was well tolerated. The course of their myocardial infarction was further uneventful. The three patients with more severe pump failure, who had previously been treated with dopamine and nitroprusside, remained on captopril. The dose was gradually increased to 100 mg three times a day, and digoxin and diuretics were continued. One of them, case 5, remained in a critical condition and died after a few days. The two others improved clinically and could be mobilised. Three days after discharge from the hospital, 25 days after the onset of myocardial infarction, case 6 died suddenly. Case 7 was found dead one month after myocardial infarction. At a visit to the outpatient clinic some days before her death the blood pressure was 95/75 mmHg while she was taking 300 mg captopril and 160 mg frusemide. At that time she had no complaints and there were no signs of central or peripheral congestion.

Patients with chronic heart failure

After haemodynamic assessment of the first dose of captopril, which was 50 mg, long-term treatment was instituted, initially with doses that ranged from 12·5 to 50 mg three times a day, depending on kidney function and on the blood pressure response at the time of the first dose. In case 15 captopril treatment had to be stopped after a few doses because of rapid deterioration of pre-existent mild renal insufficiency, with oliguria. Cases 8 and 14 had supine systolic arterial pressure levels that were persistently below 80 mmHg at a daily dose of 150 mg. They complained about blurred vision. In these patients the dose was reduced to 75 mg. In the remaining patients the dose of captopril was gradually increased to 300 mg per day, but in three (cases 9, 11, and 17) this led to an increase in serum creatinine, which was reversed by reducing the dose to 75 mg.

All patients showed symptomatic improvement which was mainly the result of a lessening of dyspnoea. In two patients a pleural effusion, which was refractory to treatment, disappeared in a few weeks. Captopril treatment, however, did not result in loss of weight; it was 70·4±2·4 kg before captopril and 71·5±2·5 kg after one week (n=10). Two patients (cases 8 and 10) died in the first month of treatment. Ultimately, nine patients completed a three month treatment period.

Long-term haemodynamic effects of captopril and comparison with previous vasodilatory regimens

In Fig. 7 haemodynamic measurements obtained from repeat right heart catheterisation after three months of captopril treatment were compared with those taken at the start of the study. Our study had been designed in such a way that we were able not only to compare long-term effects of captopril with its acute effects but also with the long-term effects of previous vasodilatory treatment. The significant haemodynamic improvement after the first dose of captopril was maintained in the long run. After three months of captopril, systemic arterial pressure, pulmonary arterial pressure, pulmonary capillary "wedge" pressure, cardiac index, and total peripheral resistance all still differed significantly from values measured shortly before captopril was given. The haemodynamic profile with chronic

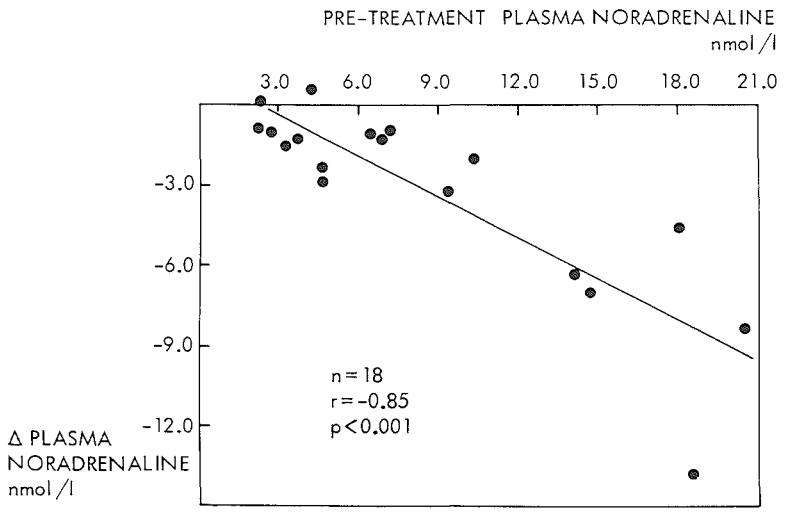

Fig. 5 *Correlation of maximal decrease in plasma noradrenaline after captopril (50 mg) with the pretreatment level of noradrenaline.*

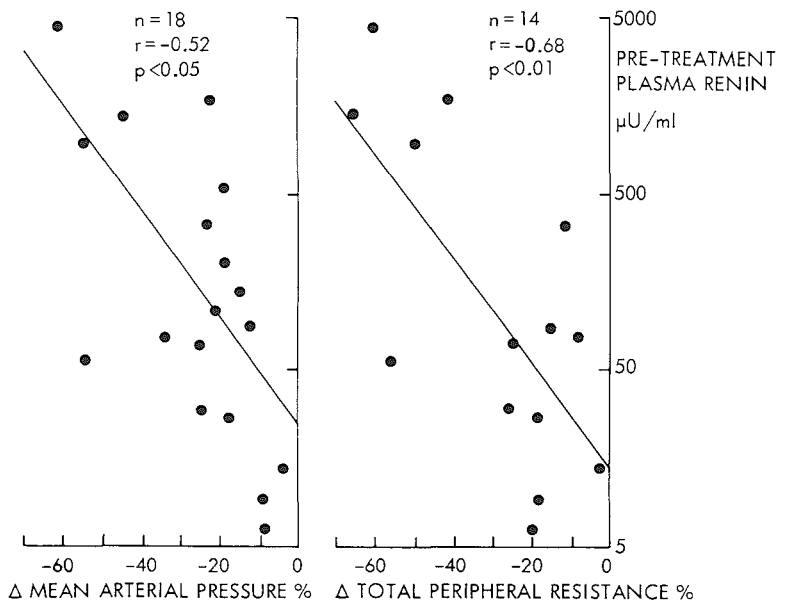

Fig. 6 *Correlations between pretreatment plasma renin and the percentage changes of mean arterial pressure and total peripheral resistance 90 minutes after captopril (50 mg).*

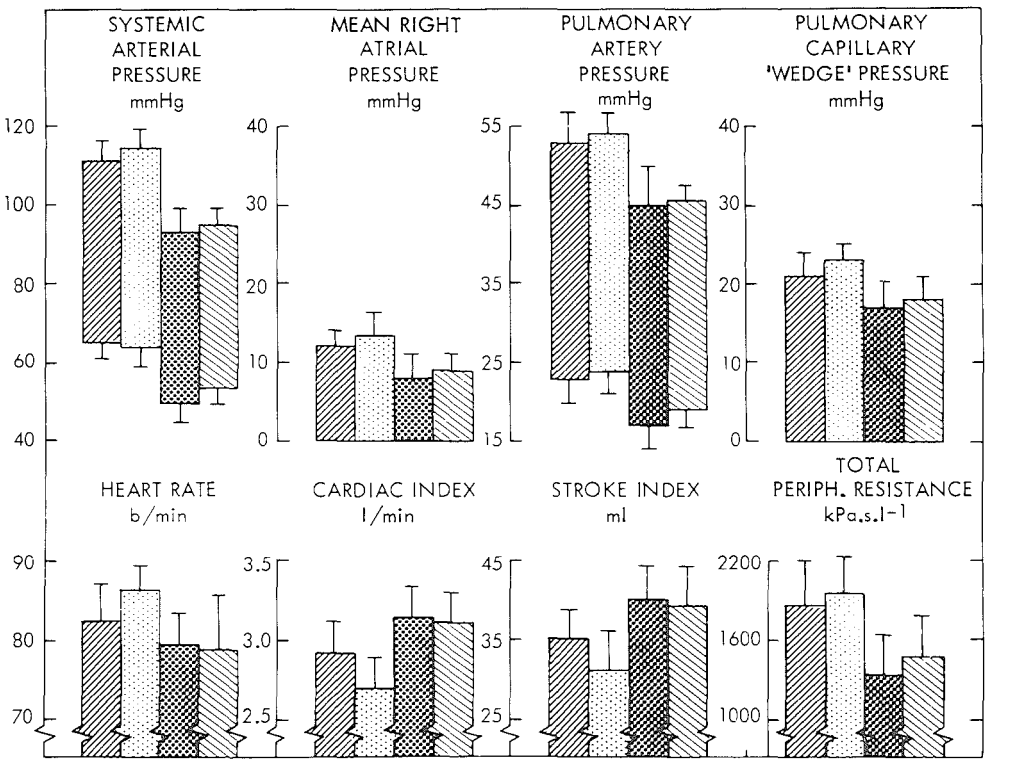

Fig. 7 *Bar graphs representing haemodynamic variables measured during the different study phases in the nine patients (cases 9, 11–14, 16–19, see Table 2), who had captopril for three months.*

measurements during the use of other vasodilatory drugs

measurements after cessation of these drugs for at least 24 hours

acute effects of captopril, 50 mg

long term effects of captopril

The acute effects of captopril in these nine patients were comparable with the responses in the whole group (Fig. 2 and 3). The acute effects of captopril were maintained during long-term treatment. When the haemodynamic effects of the previously used vasodilators were compared with long-term captopril significant differences were found for systemic arterial pressure (systolic and diastolic, $p<0.001$) and pulmonary arterial pressure (systolic and diastolic, $p<0.01$).

captopril was not much different from that with previous vasodilating treatment but systemic arterial pressure was lower with chronic captopril. Though at first we did not intend to change the dose of the diuretics, it became clear from frequent determinations of serum potassium that the dose of the aldosterone antagonist, spironolactone, could be diminished and finally stopped in all patients. The doses of frusemide and digoxin were not altered. Before captopril serum potassium was 4·0±0·2 mmol/l at a time when nearly all patients were taking spironolactone (Table 2). Evaluation at three months showed serum potassium was 4·4±0·3 mmol/l, with no patient on spironolactone or oral potassium supplements. Serum creatinine rose from 120±11 μmol/l (range 96 to 175 μmol/l) to 128±11 μmol/l (range 76 to 160 μmol/l) after three months on captopril ($p<0.01$). No significant change in body weight occurred during long-term captopril treatment; body weight was 70·2±2·9 kg after three months as compared with 71·6±2·2 kg before

Table 7 *Correlations between captopril-induced haemodynamic changes (90 min) and pretreatment hormone levels*

Changes after captopril (0–90 min)	*No.*	*Versus baseline values of*					
		Log plasma renin		*Log plasma aldosterone*		*Log plasma noradrenaline*	
		r	*p*	*r*	*p*	*r*	*p*
Arterial pressure systolic ↓	18	0·05	NS	0·27	NS	0·09	NS
diastolic ↓		0·62	<0·01	0·39		0·34	
mean ↓		0·46	<0·05	0·21		0·07	
Heart rate ↓	18	0·58	<0·05	0·41	NS	0·63	<0·01
Cardiac index ↑	14	0·57	<0·05	0·58	<0·05	0·30	NS
Cardiac work index —		0·06	NS	0·14	NS	0·24	
Stroke index ↑	14	0·65	<0·05	0·71	<0·01	0·40	NS
Stroke work index —		0·03	NS	0·16	NS	0·35	
Mean right atrial pressure ↓	18	0·48	<0·05	0·18	NS	0·26	NS
Mean pulmonary artery pressure ↓		0·47	<0·05	0·24		0·26	
Mean pulmonary capillary "wedge" pressure ↓		0·40	<0·05	0·11		0·26	
Total peripheral vascular resistance ↓	14	0·71	<0·01	0·71	<0·01	0·51	<0·05
Pulmonary vascular resistance ↓		0·61	<0·05	0·60	<0·05	0·36	NS
Plasma renin ↑	18	0·80	<0·001	0·64	<0·01	0·61	<0·01
Plasma aldosterone ↓		0·51	<0·05	0·82	<0·001	0·46	<0·05
Plasma noradrenaline ↓		0·59	<0·05	0·35	NS	0·77	<0·001

captopril. No patient developed skin rash, proteinuria, or leucopenia.

Discussion

It was remarkable how effective a single dose of captopril was in our patients whether or not they were in acute or chronic failure. As total peripheral resistance fell, the heart was relieved and stroke volume and cardiac output rose. Captopril also had a distinct effect on the preload of the heart as indicated by the decrease in cardiac filling pressures. Our study gives no detailed insight into the mechanism of this decrease. The fall in right atrial pressure has been reported to precede the fall in pulmonary arterial pressure and pulmonary capillary "wedge" pressure.[8] This suggests that an effect of captopril on venous tone rather than an improvement of forward output of the heart is responsible for the observed decrease in cardiac filling pressures. In our study, however, the changes in filling pressures of the right and left side of the heart occurred synchronously.

Plasma renin is often high in heart failure but not invariably so. In our series it was increased in most patients with chronic heart failure who were on diuretics, whereas it was normal or even low in the patients with acute myocardial infarction who were not on diuretics. Thus, stimulation of the renin angiotensin system in normotensive heart failure might be a consequence of treatment rather than a consequence of the disease. This seems to contrast with sympathetic activity as reflected by plasma noradrenaline. High concentrations of noradrenaline were observed whether or not the patients had been on diuretics. The effect of captopril on peripheral resistance was positively correlated with pretreatment plasma renin, as were the effects on stroke volume, cardiac output, and cardiac filling pressures. The correlations, however, were weak, and the changes in the patients with normal or moderately raised renin were nearly as great as in those with grossly raised renin.

A notable feature of the cardiovascular actions of captopril is its apparent interference with baroreflex function. Heart rate did not rise in our patients despite a drop in systemic arterial pressure. This has also been reported by others.[31 32] Attenuation of circulatory reflexes in congestive heart failure has been proposed as an underlying mechanism.[3] Heart rate, however, is also not increased when arterial pressure is lowered by captopril in subjects with normal heart function.[33] From experiments in dogs, Hatton *et al.*[34] concluded that captopril displaced the setpoint of the arterial baroreflex to a lower pressure without modifying the sensitivity of the reflex.

Ideally the decrease in vascular resistance after captopril should be balanced by an increase in cardiac output so that perfusion pressure of the different organs is maintained. Clearly this ideal was not met. Systemic arterial pressure fell, in some cases to an alarmingly low level with the potential danger of a deleterious effect on coronary blood flow. On the other hand, the decrease in heart rate and the concomitant reductions in cardiac filling pressures and systemic arterial pressure and thereby in left vascular wall tension suggest that myocardial oxygen demand is diminished by captopril.

Of the 14 patients on long-term captopril, four died within a month after initial clinical improvement. They had ischaemic heart disease. In congestive heart failure the cutaneous, splanchnic, and renal vascular beds are particularly prone to neurohumorally induced vasoconstriction.[35] In this way, for instance during exercise, blood flow is shunted from the renal and splanchnic circulation to that of skeletal muscle, heart,

and brain. It is therefore conceivable that in our patients who died suddenly captopril interfered with the normal redistribution of blood flow during exercise, which may have led to myocardial ischaemia.

The relatively high doses of captopril we have used may have contributed to the low systemic arterial pressure in our patients. Symptomatic hypotension, however, has also been observed with doses as low as 6·25 mg.[32] Our study does not give an indication of what the optimal dose of captopril should be, but recent experience in patients with heart failure and in patients with severe hypertension suggests that captopril in daily doses not higher than 75 mg is effective when combined with a diuretic.[32 36] Such a regimen might reduce the incidence of side effects. The side effects that occur most frequently are skin rash, loss of taste, proteinuria, and leucopenia. These were not encountered in our small series of patients.

In conclusion, oral angiotensin-converting enzyme inhibition by captopril has a profound effect on cardiocirculatory control mechanisms, which are altered during heart failure. Heart rate, total peripheral resistance, cardiac filling pressures, and the increased plasma concentrations of aldosterone and noradrenaline are lowered while stroke volume is increased. In some patients the beneficial acute effects are maintained in the long run. During treatment of heart failure with high ceiling diuretics such as frusemide and ethacrynic acid in combination with captopril, potassium supplements or aldosterone antagonists are no longer needed. In the majority of our severe cases, combined treatment with frusemide and captopril resulted in a low systemic arterial pressure. This may compromise coronary circulation, particularly during exercise. Before captopril can be recommended as a useful drug for the treatment of heart failure, particularly in coronary artery disease, more insight into its effects on coronary blood flow is required.

We thank Dr P Pigott and Dr J Hill (Squibb) for providing the captopril tablets.

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CHAPTER 3

ACE INHIBITION AND THE KIDNEY

Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis.

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Asynchronous Changes in Prorenin and Renin Secretion after Captopril in Patients with Renal Artery Stenosis

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SUMMARY **An assay of plasma prorenin was developed in which the conversion to renin occurred under apparently optimal conditions. Some characteristics of the assay were 1) prorenin was activated by Sepharose-bound trypsin at 4°C; 2) the concentration of activator was not critical provided that incubation was prolonged until renin activity had reached a plateau; and 3) this plateau was stable and had the same height as after maximal activation with acid, pepsin, plasmin or urokinase. Maximal activity with Sepharose-bound trypsin at 4°C was higher than with cryoactivation, and optimal conditions were more readily reproduced than with trypsin at 37°C or with acid-activation. The assay was used for measurements in peripheral and renal vein plasma after captopril in hypertensive patients with unilateral renal artery stenosis. Peripheral renin rose within 30 minutes after a first dose of captopril, 50 mg orally, and it remained high with chronic treatment. In contrast, peripheral prorenin fell initially and rose after 4 hours. These changes in peripheral plasma were related to changes in the secretion rates of the two forms of renin from the affected kidney. Thus chronic, but not acute, stimulation of renin release was associated with an increased secretion rate of prorenin. The late rise in prorenin is probably an indication of enhanced synthesis in the kidney, so that more prorenin is available for conversion. The data suggest that prorenin is indeed a biosynthetic precursor of renin and that, at least under certain circumstances, a major proportion of circulating prorenin originates from the kidney. (Hypertension 5: 244–256, 1983)**

KEY WORDS • **captopril** • **converting enzyme** • **prorenin** • **renal artery stenosis** • **renin**

ABOUT 80% of the renin in normal human plasma is thought to circulate in an inactive form.[1-3] Inactive renin is often called "prorenin" because it can be converted in vitro to active renin.[4] It is not certain that prorenin is a precursor of naturally occurring renin, however.

Activation of the factor XII-kallikrein pathway causes irreversible prorenin-to-renin conversion after dialysis of plasma against acid followed by restoration of pH to neutral (acid-activation)[5, 6] and possibly also in plasma that is stored at low temperature (cryoactivation).[7] Prorenin can also be activated by adding trypsin[8] or pepsin[9, 10] to plasma. These exogenous activators act independently of factor XII and kallikrein.

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Prorenin is measured by functional assays in which prorenin is converted to renin. The difference in renin activity before and after activation is taken as a measure. The implicit assumptions are that all prorenin molecules are converted and that one molecule of activated prorenin has the same enzymatic activity as one molecule of naturally occurring renin. Cryoactivation, however, often leads to incomplete prorenin-to-renin conversion.[3] Acid activation appears more complete but careful adjustment of pH is of critical importance.[11, 12] For trypsin activation, high concentrations of trypsin are required to overcome the inhibitors in plasma but such high concentrations may destroy renin.[3, 13, 14] Trypsin may also attack renin substrate and may interfere with the radioimmunoassay of angiotensin I, the final step in the assay of renin. Soybean trypsin inhibitor (SBTI) has therefore been used to prevent this. However, some commercial SBTI preparations appear to have angiotensinase activity resulting in considerable loss of angiotensin I during the assay.[15]

The present paper describes an assay of prorenin in which it is activated by trypsin that is bound to Sephar-

ose. The immobilized activator can quantitatively be removed by centrifugation. Optimal conditions were worked out for activation in plasma and the results were compared with maximal acid activation and with maximal activation by pepsin, plasmin and the plasminogen activator urokinase. The behavior of naturally occurring active renin on gel filtration and dye affinity chromatography columns was compared with that of prorenin activated by immobilized trypsin. Some enzymatic properties of the two forms of renin were also compared. The assay was then applied to a study of the effects of angiotensin converting enzyme inhibition by captopril on peripheral and renal vein plasma prorenin in patients with unilateral renal artery stenosis.

Materials and Methods

Reagents

Trypsin from bovine pancreas, 2 × crystallized, was purchased from Sigma, St. Louis, Missouri. Its specific activity was 12,000 α-N-benzoyl-L-arginine-ethyl ester (BAEE) units/mg of protein. Pepsin A, specific activity 3200 units/mg of protein, was also from Sigma. Activator-free highly purified human plasmin, specific activity 19 casein units/mg of protein, was a product of Kabi, Stockholm, Sweden. Urokinase was the urokinase reference standard of Leo, Copenhagen, Denmark. Aprotinin (Trasylol) was obtained from Bayer, Leverkusen, West Germany.

Ile^5-Angiotensin I (AI) from Serva, Heidelberg, West Germany, was dissolved in Tris/acetate buffer of pH 7.5 (vide infra) and stored at −20°C at a concentration of 40 μM. Its strength was tested by bioassay in the rat against Val^5-AII (Hypertensin) from Ciba-Geigy, Basel, Switzerland. When allowance was made for the impurities present in the Ile^5-AI preparation as stated by the manufacturer, the pressor activities of Val^5-AII and Ile^5-AI on a molar base were found to be equal.[11] Renin substrate was prepared from sheep plasma, which was taken 4–5 days after bilateral nephrectomy.[16] It was dissolved in phosphate buffer pH 7.5 and stored at −20°C at a concentration of 5 μM, expressed as the maximal quantity of Ile^5-AI equivalents that could be generated in the presence of a large excess of renin. Renin substrate prepared from human plasma[16] was also used in some experiments. A human kidney renin standard, lot MRC 68/356, was kindly supplied by the WHO International Laboratory for Biological Standards, London, England. This standard was dissolved in phosphate buffer pH 7.5 containing 0.35% bovine serum albumin. It could be stored at −20°C at a concentration of 10,000 μunits/ml for up to 3 months without loss of activity. ^{125}I-labeled Ile^5-AI and anti-Ile^5-AI rabbit antiserum were prepared as described previously.[11] Radioactive AI in 0.05 M acetic acid, containing 0.1% bovine serum albumin, 0.01% thiomersalate, 0.001% neomycin sulphate and 0.1 M NaCl, was stored in 0.25 ml portions at −20°C.

CNBr-activated Sepharose 4B, Sephadex G-100, Blue Sepharose CL-6B, Blue Dextran 2000, and the molecular weight markers for gel chromatography, ribonuclease A (Mr 13,700), chymotrypsinogen A (Mr 25,000), ovalbumin (Mr 43,000), and human serum albumin (Mr 67,000) were purchased from Pharmacia, Uppsala, Sweden. ^{14}C-ovalbumin (Mr 46,000) and ^{14}C-bovine serum albumin (Mr 69,000) were also used as molecular weight markers and were obtained from the Radiochemical Centre, Amersham, England.

Buffer Solutions

Phosphate Buffer, pH 7.5

This buffer contained 12.2 mM $Na H_2 PO_4$, 86.7 mM $Na_2 HPO_4$, 75.9 mM NaCl and 1.0 mM disodium ethylenediamine-tetraacetate (EDTA).

Glycine/HCl Buffers, pH 3.3 or pH 4.0

These buffers contained 50 mM glycine, 94.9 mM NaCl, and 5.1 mM EDTA. The pH was adjusted with concentrated HCl.

Tris/Acetate Buffer, pH 7.5

This buffer contained 0.1 M Tris, 0.35% bovine serum albumin, 0.1% lysozyme, and 0.2% neomycin sulphate. The pH was adjusted with glacial acetic acid.

Preparation of Plasma

Blood was collected in chilled plastic tubes containing EDTA in a final concentration of 5 mM. It was centrifuged at 3000 × *g* and 4°C immediately after collection. Plasma was kept frozen at −20°C before use.

Preparation of Immobilized Trypsin and Pepsin

The enzymes were covalently bound to CNBr-activated Sepharose 4B in a ratio of 30–40 mg of protein per g of dry Sepharose according to the directions of the manufacturer. In this manner more than 97% of protein was bound to Sepharose. Sepharose-bound trypsin was diluted in phosphate buffer pH 7.5. Suspensions of Sepharose-bound pepsin were stored in glycine/HCl buffer pH 3.3.

Activation of Prorenin

Activation by Immobilized Trypsin

Dilutions of Sepharose-bound trypsin (100 μl, 0.05 − 4.0 mg trypsin) were added to 1 ml plasma. The mixtures were incubated at 37° and at 4°C for various time periods as indicated in the results section. Trypsin was removed by centrifugation. The supernatants were checked for amidolytic activity with the chromogenic substrate N-benzyol-L-isoleucyl-L-glutamylglycyl-L-arginine-p-nitroanilide (S 2222, Kabi, Stockholm, Sweden). For this purpose 0.1 ml of the supernatant of the incubates was mixed with 0.2 mM substrate (about 10 times Km for trypsin) in 0.1 M Tris/HCl buffer pH 8.2 in a total volume of 1.0 ml. The linear release of p-nitroaniline was followed for 1–2 minutes at 405 nm in a 1-cm semi-microcuvette at 37°C. With this method the remaining trypsin activity in the supernatants was found to be less than 0.1% of the original activity in the incubates.

Acid Activation

Plasma samples (2 ml) were dialyzed against glycine/HCl buffer pH 3.3 for 24 h at 4°C, followed by dialysis against phosphate buffer pH 7.5, containing 6% polyvinylpyrrolidone, again for 24 hours at 4°C. Polyvinylpyrrolidone had been added to the buffer to prevent dilution of the plasma due to colloid-osmosis. The dialysis bags were emptied in calibrated plastic tubes and rinsed with phosphate buffer pH 7.5 and the volume was adjusted to 2 ml with the same buffer.

Activation by Immobilized Pepsin

Plasma samples (2 ml) were dialyzed against glycine/HCl buffer pH 3.3 for 24 h at 4°C. To 1 ml of the dialyzed plasma was added Sepharose-bound pepsin (100 μl, 0.3 mg pepsin). The mixture was incubated at 32°C for various time periods, as indicated in the Results. Pepsin was removed by centrifugation and pH was restored to 7.5 with 1 M NaOH.

Activation by Plasmin and Urokinase

Plasmin was dissolved at a concentration of 20 casein units/ml in phosphate buffer pH 7.5. Urokinase was dissolved in this buffer at a concentration of 1000 Ploug units/ml. The plasma samples were dialyzed against glycine/HCl buffer pH 4.0 for 24 hours at 4°C and pH was restored to 7.5 with 1 M NaOH. The activator solutions (100 μl) were added to 1 ml of the pH 4.0-pretreated samples and incubated at 4°C for various time periods as indicated in the Results.

Assay of Naturally Occurring Renin and Prorenin that is Activated In Vitro

For this assay, 0.10–0.25-ml samples were added to 0.5 ml of renin substrate, and the volume was adjusted to 1.0 ml with phosphate buffer pH 7.5. The final concentration of renin substrate in the mixture was 2.5 μM Ile^5-AI equivalents, which corresponds to about 10 times km (see Results). After addition of protease inhibitors, i.e., 10 μl of 0.34 M 8-hydroxyquinoline, 5 μl of 0.28 M phenylmethylsulphonyl-fluoride in ethanol, and 10 μl aprotinin (10,000 kallikrein-inhibiting units/ml), the mixtures were incubated at 37°C. The incubation time was 3 hours except when stated otherwise. The renin-containing samples had been diluted in such a way that no more than 5% of the substrate was cleaved during incubation. Parallel incubations at 4°C served as blanks. Incubations of dilutions of standard human kidney renin at 37° and 4°C were run in each assay batch. The concentration of homologous substrate in the incubation mixtures was less than 0.2 μM Ile^5-AI equivalents. Previous studies have shown that this concentration of homologous substrate did not interfere with the reaction of renin and the heterologous substrate.[11] The reaction was stopped by adding 1 ml of 0.15 M NaCl followed by heating for 10 minutes in a boiling water bath. The precipitate was removed by centrifugation. The concentration of AI in the supernatant was measured by radioimmunoassay, using ^{125}I-labeled Ile^5-AI and rabbit anti-Ile^5-AI antiserum.[11] Renin concentration is expressed as microunits of the renin standard per ml (μU/ml). Prorenin was measured as the difference between the renin concentration after activation of the test sample ("total renin") and the concentration before activation. For routine measurements in plasma, 1.0 ml plasma was incubated with 100 μl Sepharose-bound trypsin in a final concentration of 0.25 mg trypsin/ml for 24 hours at 4°C. The reasons why this procedure was selected are presented and discussed in the results and discussion sections.

Interassay variability was evaluated by weekly measurements of naturally occurring active renin and prorenin in a normal plasma pool (stored at −20°C) during a 9-month period. The mean value of naturally occurring active renin was 27 μU/ml (36 assays) with a standard deviation of 3 μU/ml (coefficient of variation 11%). The mean value of "total renin" was 254 μU/ml, with a standard deviation of 26 μU/ml (coefficient of variation 10%). The mean value of prorenin was 227 μU/ml with a standard deviation of 24 μU/ml (coefficient of variation 11%). In normal plasma the contribution of naturally occurring active renin to total renin is small but it becomes greater after stimulation of renin release. The coefficient of variation of prorenin measurements then also becomes greater since prorenin is taken as the difference between total renin and naturally occurring active renin.

In 17 healthy men (aged 24–45 years) who were recumbent for at least 1 hour before blood sampling, naturally occurring renin had a mean value of 23 μU/ml (antilog of arithmetic mean after logarithmic transformation of data) with a range of 14 to 43 μU/ml. The mean value of prorenin was 196 μU/ml with a range of 138 to 312 μU/ml; the mean value of the proportion of renin that was in the active form was 10.9%, with a range of 4.3% to 17.5%.

Gel Filtration

Untreated plasma (2 ml) or trypsin-activated plasma (2 ml) was applied to 2.6 × 90 cm columns of Sephadex G-100 equilibrated with 0.01 M Tris/HCl buffer pH 7.0 containing 0.15 M NaCl and 1 mM EDTA. The same buffer was used for elution. Flow rate was adjusted to 10 ml/hr and 1.5-ml fractions were collected. The columns were calibrated with ribonuclease A, chymotrypsinogen A, human albumin and ovalbumin. Blue dextran 2000 was used for determining void volume. ^{14}C-ovalbumin and ^{14}C-BSA were used as internal standards. Gel filtration was carried out at 4°C.

Affinity Chromatography

Untreated plasma (4 ml) or trypsin-activated plasma (4 ml) was applied to 1.6 × 25 cm columns of Blue Sepharose CL-6B equilibrated with 0.02 M phosphate buffer pH 7.1. Elution was performed with this buffer in 3 steps, i.e., without added NaCl, with 0.2 M NaCl and with 1.4 M NaCl added to the buffer.[17] Flow rate was 50 to 60 ml/hr and 2.5-ml fractions were collected. Affinity chromatography was carried out at 4°C.

Studies in Patients

Fifty-four hypertensive patients were studied after they had given their informed consent. All had unilateral renal artery stenosis as demonstrated by renal arteriography. Treatment was stopped at least 3 weeks before the studies began. The patients were recumbent for at least one hour before blood sampling.

Group 1 (n = 14)

Renal arteriography had already been performed before this study. The patients received a first dose of captopril, 50 mg orally. Peripheral venous blood was sampled from an indwelling needle for renin and prorenin measurements before and at different time intervals after captopril. Renin before captopril was 81 μU/ml (antilog of arithmetic mean after logarithmic transformation of data) with a range of 19 to 250 μU/ml, and prorenin was 190 μU/ml with a range of 77 to 410 μU/ml. The patients were then treated with captopril, 50 mg 3 times a day, and blood was taken after 1, 2, and 4 weeks 1–2 hours after the morning dose.

Group 2 (n = 15)

Blood was sampled from both renal veins and from the abdominal aorta before and 30 minutes after a first dose of captopril, 50 mg, just before renal arteriography. Because some time elapsed between sampling of the renal vein of one side and sampling at the other side, two aortic samples were taken each at exactly the same time that a renal vein sample was collected. Peripheral vein renin before captopril was 51 μU/ml (range 16–190 μU/ml) and prorenin was 170 μU/ml (range 57–320 μU/ml).

Group 3 (n = 15)

Blood was sampled from both renal veins and from the abdominal aorta 16 hours after a first dose of captopril, 50 mg, just before renal arteriography. This time interval was chosen because studies in Group 1 had shown that peripheral prorenin was significantly increased after 16 hours. Peripheral vein renin before captopril was 58 μU/ml (range 15–480 μU/ml) and prorenin was 130 μU/ml (range 20–490 μU/ml).

Group 4 (n = 10)

Blood was sampled from both renal veins and from the abdominal aorta while the patients were taking captopril, 50 mg three times a day, for 2 weeks. Sampling occurred 1–2 hours after the last 50 mg dose and just before renal arteriography. Peripheral vein renin before captopril was 66 μU/ml (range 30–360 μU/ml) and prorenin was 210 μU/ml (range 72–520 μU/ml).

Results

Activation of Plasma Prorenin by Immobilized Trypsin: Selection of Optimal Conditions

Normal plasma pool was incubated at 4° and 37°C with Sepharose-bound trypsin at concentrations ranging from 0.05 to 0.50 mg per ml of plasma at 4°C and from 0.05 to 4.0 mg/ml at 37° C. The results at 4° and 37°C were markedly different (fig. 1). First, the reaction velocity was higher at 37° C than at 4°C, but for a given trypsin concentration, the maximum level of renin activity ultimately obtained was higher at 4°C than at 37°C. Second, with trypsin concentrations ranging from 0.12 to 0.50 mg/ml, this maximum of renin activity was independent of trypsin concentration during incubation at 4°C and not at 37°C. Third, very high concentrations of trypsin were required at 37° C for approaching the maximal renin activity obtained at 4°C but these high concentrations also caused inactivation or destruction of renin.

As shown in figure 1, with the use of 0.25 mg trypsin per ml of plasma at 37°C, activation had reached its maximum after 2 hours of incubation, and at that time the renin activity was about half the maximum obtained with the same concentration of trypsin at 4°C. With this concentration of trypsin the generation of active renin at 37°C had stopped because not enough uninhibited trypsin was left. This was demonstrated by the following experiments. Plasma (1 ml) was incubated with Sepharose-bound trypsin (100 μl) in a concentration of 0.25 mg trypsin per ml of plasma for 4 hours at 37°C. After incubation the plasma was separated from the activator by centrifugation. The supernatant was transferred to a new tube and renin was measured; it was 140 μU/ml as compared to 32 μU/ml before incubation with trypsin. The supernatant was mixed with fresh Sepharose-bound trypsin, again in a concentration of 0.25 mg/ml, whereas the trypsin pellet was resuspended in fresh plasma (1 ml). The mixtures were then incubated for 24 hours at 4°C and renin was measured. Newly added trypsin caused further generation of active renin to its maximal value of 250 μU/ml in the supernatant but addition of fresh plasma to the precipitate was without effect. In contrast, at 4°C maximal activation of prorenin was obtained while active trypsin was still present. This was shown as follows: plasma (1 ml) was incubated with Sepharose-bound trypsin (100 μl) in a concentration of 0.25 mg/ml for 24 hours at 4°C. Plasma was then separated from the activator by centrifugation. The supernatant was transferred to a new test tube and renin was measured; it was 240 μU/ml as compared to 34 μU/ml before incubation with trypsin. Fresh plasma (1 ml) was added to the trypsin pellet and mixed. Renin was generated during subsequent incubation at 4°C, whereas no further activation occurred after addition of fresh trypsin to the supernatant. It is therefore very likely that at 4°C all the prorenin that could be converted by trypsin was indeed converted.

Recovery of Prorenin and Renin during Incubation with Immobilized Trypsin

The possibility that some loss of prorenin has occurred during trypsin treatment at 4°C cannot be excluded but the fact that the same maximum of renin activity was reached irrespective of the trypsin concentration in the incubate argues against such a loss of

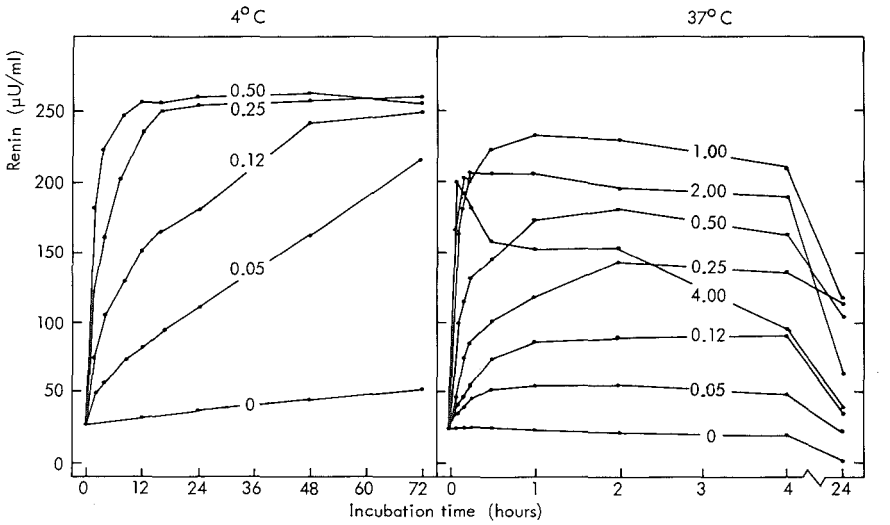

FIGURE 1. *Activation of prorenin in a pool of normal plasma by immobilized trypsin at 4°C (left) and 37°C (right). The concentrations of trypsin, in mg/ml of plasma, are indicated. Results are the means of three experiments.*

prorenin. Renin activity decreased with prolonged incubation with trypsin at 37°C but not at 4°C, thereby indicating that active renin was inactivated or destroyed at 37°C. This was substantiated by the following experiment (fig. 2). Standard human kidney renin (1000 μU/ml) in phosphate buffer pH 7.5 was incubated at 4° and 37°C with Sepharose-bound trypsin in a concentration of 0.25 mg/ml. After various time intervals trypsin was removed by centrifugation, and renin activity in the supernatant was determined. Incubation for 24 hours at 4°C had practically no effect on renin activity but at 37°C renin was rapidly inactivated or destroyed. It should be noted that these incubates are free of trypsin inhibitors, so that the concentration of uninhibited trypsin is higher under these circumstances than when the same quantity of trypsin is added to plasma.

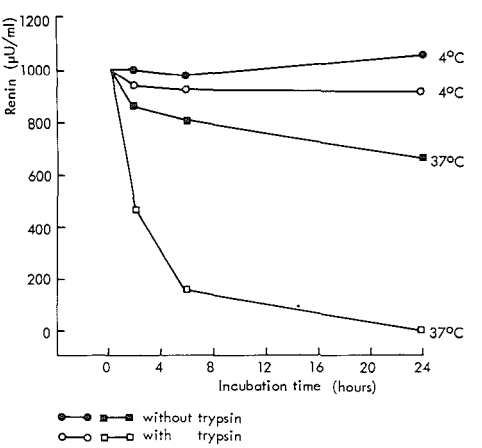

FIGURE 2. *Inactivation or destruction of human kidney renin (MRC standard) by immobilized trypsin (0.25 mg/ml of incubate) at 4°C (closed squares) and at 37°C (open circles). Controls without trypsin are shown as closed symbols.*

As shown in figure 2, at 37°C some destruction of renin occurred in the absence of trypsin. This may be due to a change in pH; in some examples it rose during 24 hours of incubation at 37°C from 7.4 to maximally 8.1. Renin has been reported to be destroyed at this temperature at alkaline pH.[3]

Recovery of Angiotensin I in Trypsin-Treated Plasma

Aliquots of normal plasma (1 ml), to which Ile^5-AI had been added in a final concentration of 0.04 μM, were incubated for 24 h at 4°C with immobilized trypsin (100 μl) in a concentration of 0.25 mg/ml or with phosphate buffer pH 7.5 (100 μl). After incubation the immobilized trypsin was removed by centrifugation and 0.5 ml of the plasma samples was incubated with 0.5 ml of sheep renin substrate in the presence of protease inhibitors as described in the methods section. The recovery of added Ile^5-AI was 98 ± 10.1% in trypsin-treated plasma and 98 ± 9.3% in untreated plasma (mean ± SEM, n = 5).

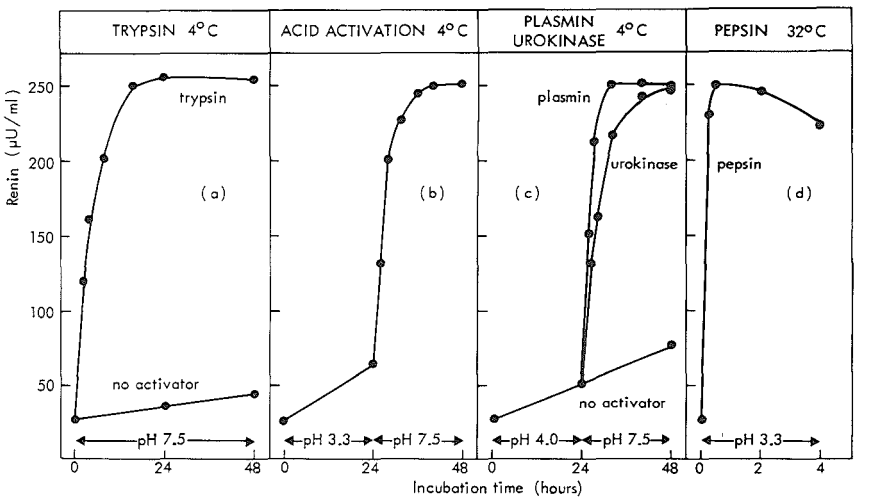

FIGURE 3. *Comparison between various activation procedures: (a) incubation of a pool of normal plasma with immobilized trypsin (0.25 mg/ml of plasma) at 4°C; (b) dialysis at pH 3.3 followed by dialysis at pH 7.5 both at 4°C; (c) incubation with plasmin (2 casein units/ml) or urokinase (100 Ploug units/ml) at 4°C following dialysis at pH 4.0 and restoration of pH by 1 M NaOH; and (d) incubation with pepsin (0.3 mg/ml) at pH 3.3 and 32°C followed by restoration of pH by 1 M NaOH. Results are the mean of three experiments.*

Comparison between Trypsin Activation of Prorenin and other Activation Procedures

The following procedures were compared (fig. 3): 1) incubation of normal plasma pool at 4°C with immobilized trypsin (0.25 mg/ml); 2) dialysis for 24 hours at pH 3.3 and 4°C followed by dialysis at pH 7.5 again at 4°C; 3) dialysis for 24 hours at pH 4.0 and 4°C followed by restoration of pH to 7.5 with 1 M NaOH and incubation at 4°C with plasmin (2 casein units/ml) or with urokinase (100 Ploug units/ml); and 4) dialysis for 24 hours at pH 3.3 and 4°C followed by incubation at 32°C with immobilized pepsin (0.3 mg/ml) and subsequent restoration of pH to 7.5 with 1 M NaOH. The maximum levels of renin activity obtained with each of these procedures appeared not different. This is an indication that all the prorenin that could be converted to active renin by proteolytic attack was indeed converted.

Comparison between Trypsin-Activated Prorenin and Naturally Occurring Renin

Gel Filtration

The eluates were treated with trypsin for measuring prorenin. The conditions were the same as for plasma, i.e., incubation with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C. We have not rigorously checked whether the conditions chosen for plasma were also appropriate for measuring prorenin in the column eluates. They probably are because, when normal plasma was treated with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C *before* it was subjected to chromatography, the quantity of renin in the eluate was the same as when native plasma was applied to the column and the eluate was *subsequently* treated with trypsin. This quantity was 15 times higher than the quantity of renin that was recovered from the column, when trypsin-treatment had been omitted both *before* and *after* chromatography. The factor of 15, as found in the column eluates, was the same as the ratio between total renin and naturally occurring active renin in the plasma itself.

When plasma was activated prior to chromatography, only active renin was recovered from the column, with its peak appearing at the same elution volume as the peak of naturally occurring active renin (fig. 4). Mr of naturally occurring active renin was 49,000 and Mr of prorenin was 56,000 (mean value of three experiments).

Dye-Ligand Affinity Chromatography

The eluates were treated with immobilized trypsin in the same way as after gel filtration. When native plasma was subjected to affinity chromatography, the bulk of active renin passed through the column, while prorenin was eluted with 0.2 M NaCl. The quantity of renin that was recovered after trypsin-treatment of the eluate was about 15 times higher than before trypsin-treatment, which agrees with the ratio between total renin and naturally occurring active renin in the plasma itself. This suggests that the method of trypsin-treat-

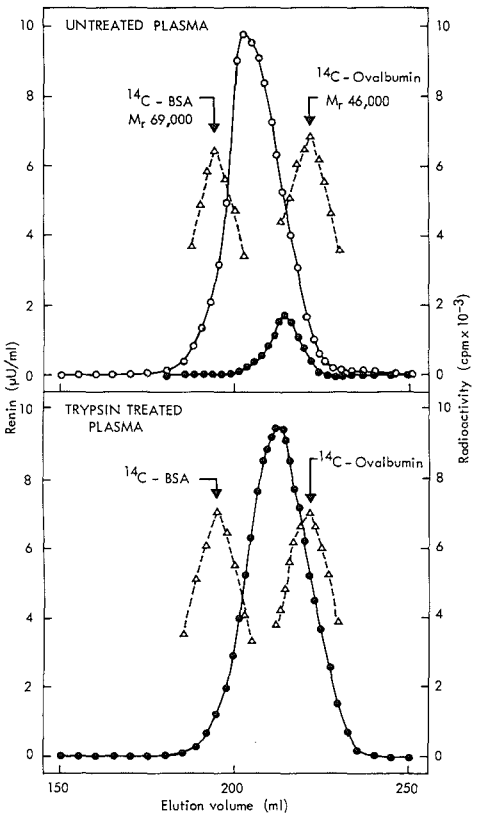

FIGURE 4. *Gel filtration on Sephadex G-100 of untreated normal plasma and of plasma treated with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C. The molecular weight markers ^{14}C bovine serum albumin (Mr 69,000) and ^{14}C-ovalbumin (Mr 46,000) were used as internal standards. Renin was determined in the eluate before (closed circles) and after (open circles) incubation with immobilized trypsin. Calculated Mr-values: plasma prorenin 56,000, plasma renin 49,000 and human kidney renin (MRC standard) 42,000.*

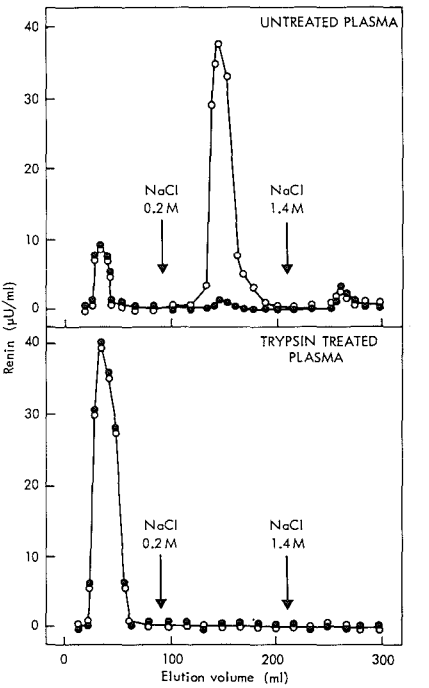

FIGURE 5. *Affinity chromatography on Blue Sepharose of untreated normal plasma and of plasma treated with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C. Renin was determined in the eluate before (closed circles) and after incubation with immobilized trypsin (open circles).*

ment was appropriate for measuring prorenin in the column eluates.

When plasma had been activated by trypsin prior to chromatography, all the renin appeared to pass through the column, since no renin was detected both before and after trypsin treatment of the eluates at 0.2 M NaCl and at 1.4 M NaCl (fig. 5).

Enzyme Kinetics of Naturally Occurring Renin and Prorenin that is Activated by Trypsin

Under the conditions of the renin assay the generation of AI from sheep renin substrate by both forms of renin proceeded linearly with time and was proportional to the concentration of active renin. The activity of human kidney renin (MRC standard) was not influenced by the addition of untreated or trypsin-treated normal plasma, indicating that substances interfering with the reaction between renin and its substrate were absent.

Active renin was isolated from plasma of a patient with hypertension and renal artery stenosis. The concentration of naturally occurring renin in this plasma was very high, 1200 μU/ml. Renin was isolated by Sephadex G-100 gel filtration and further purified by DEAE-Sepharose ion exchange chromatography[10, 18] followed by affinity chromatography on Blue-Sepharose.[17] The preparation was free of renin substrate and prorenin. Prorenin was isolated from normal plasma by Sephadex-G-100 gel filtration followed by chromatography on Blue Sepharose. The preparation was free of renin substrate and renin. Prorenin was then activated with immobilized trypsin as described above. The renin preparations were incubated with sheep renin substrate for 1 hour at 37°C and AI that had been

formed was determined for constructing Lineweaver-Burk plots (fig. 6). Km-values for naturally occurring plasma renin and standard human kidney renin and for plasma prorenin that was activated by trypsin appeared similar; they ranged from 0.21 to 0.28 μM.

pH-Optimum Curves of Naturally Occurring Renin and Prorenin that is Activated by Trypsin

The sources of naturally occurring renin and of prorenin that was activated by trypsin were the same as in the previous experiment. The renin preparations and sheep renin substrate were dialyzed for 24 hours at 4°C against phosphate buffers with pH values ranging from 4.5 to 9.0. The renin preparations were then incubated with sheep renin substrate or with human renin substrate at these various pH-values for 1 hour at 37°C. The concentrations of sheep substrate in these incubates was 2.5 μM AI equivalents/ml, and the concentration of human substrate was 0.4 μM AI equivalents/ml. The pH optimum curves of naturally occurring renin and of prorenin that was activated by trypsin appeared not different (fig. 7). The pH-optimum was 7.5 for the reaction with sheep substrate and 5.8 with human substrate.

Measurements of Plasma Renin and Prorenin in Patients

Group 1

Results are shown in figure 8. Renin in peripheral plasma rose within 30 minutes after the first dose of captopril and reached a peak value after 1–2 hours. It remained high with chronic treatment. Prorenin fell initially ($p < 0.05$; paired t test) but with long-term treatment it reached a value as high or higher than renin.

Groups 2, 3, and 4

Basal values of peripheral vein renin and prorenin were comparable in the three groups (see Methods section). Blood pressure fell from 208 ± 6/112 ± 5 mm Hg to 164 ± 10/94 ± 6 mm Hg 30 minutes after captopril in Group 2. Blood pressure was 211 ± 7/110 ± 6 mm Hg before captopril and 198 ± 8/104 ± 7 mm Hg 16 hours after captopril in Group 3. In Group 4 blood pressure was 202 ± 9/109 ± 4 mm Hg before captopril and 143 ± 6/91 ± 5 mm Hg 1–2 hours after the last dose of captopril. Data collected at the time of renal vein sampling are shown in figure 9 and in table 1. Renin in the renal vein on the affected side, but not contralaterally, was higher than in the aorta before captopril and 30 minutes after a first dose of captopril ($p < 0.001$, Group 2), and at 16 hours after a first dose of captopril ($p < 0.001$, Group 3), and also with chronic captopril treatment ($p < 0.001$, Group 4). Prorenin in the renal vein on the affected side was significantly higher than in the aorta before and 16 hours after a first dose of captopril ($p < 0.05$ and $p < 0.01$ in Groups 2 and 3 respectively), and also with chronic captopril treatment ($p < 0.01$, Group 4). Contralaterally the prorenin levels in the renal vein and aorta were not different neither before captopril nor

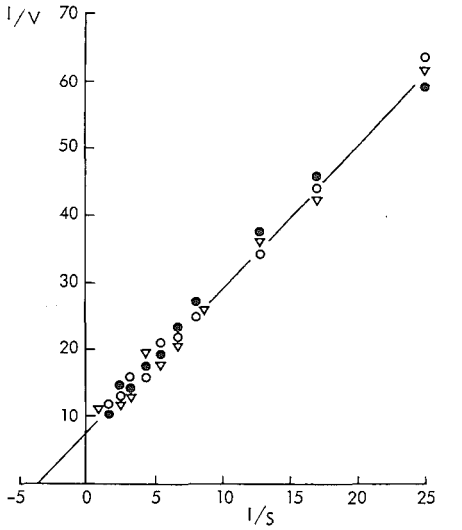

FIGURE 6. *Enzyme kinetics of naturally occurring active plasma renin (closed circles), trypsin-activated plasma prorenin (open circles) and human kidney renin (MRC standard, triangles). The renins were incubated at 37°C with sheep renin substrate for 5, 10, 20, 30, and 60 minutes. v = initial velocity in μM angiotensin I per hour. s = substrate concentration in μM angiotensin I-equivalents.*

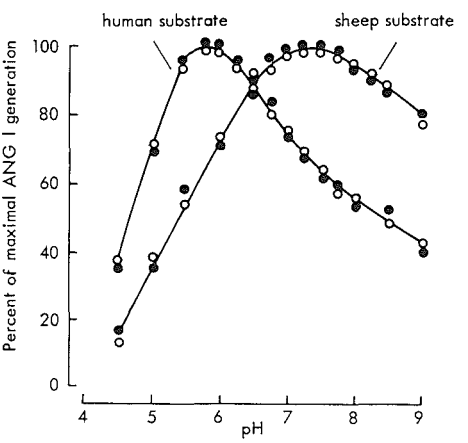

FIGURE 7. *Optimum pH curves for naturally occurring renin (closed circles) and trypsin-activated prorenin (open circles) with human renin substrate and with sheep renin substrate. Results are the means of two experiments.*

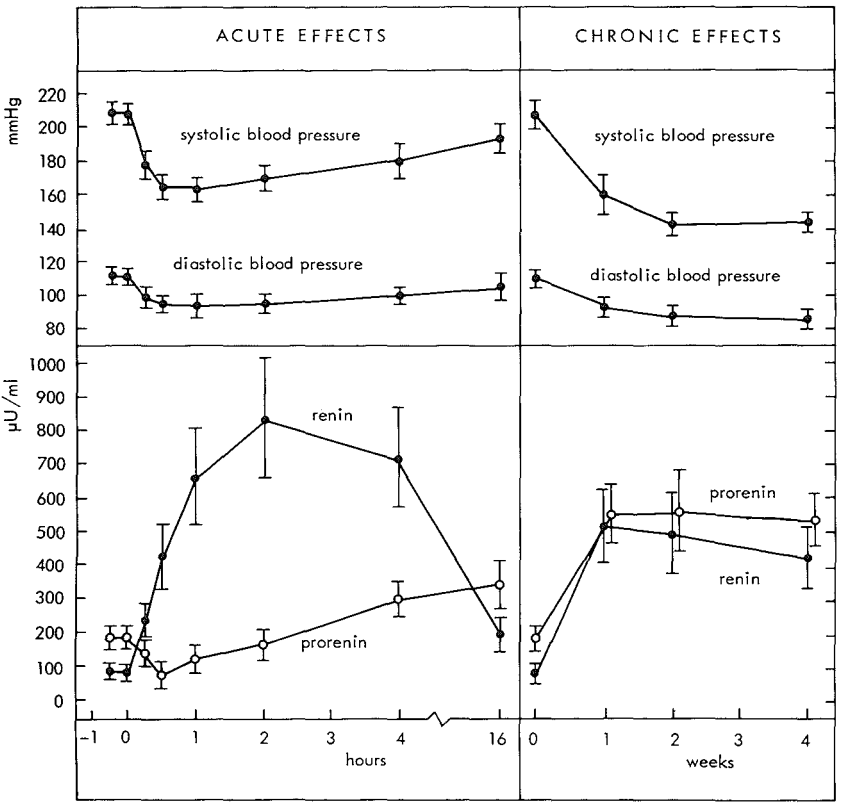

FIGURE 8. *Effects of a first dose of captopril, 50 mg orally, and of chronic captopril treatment, 50 mg three times a day, on blood pressure and on renin and prorenin in peripheral plasma. Prorenin at 30 and 60 minutes after the first dose of captopril was significantly below the control value ($p < 0.05$, paired t test). At 4 and 16 hours it was above control ($p < 0.001$). During chronic treatment blood was sampled 1 to 2 hours after the morning dose of captopril. Prorenin in these samples was 3 times higher than control ($p < 0.001$). Values in normal plasma ($n = 17$) are 23 μU/ml (range 14–43 μU/ml) for renin and 196 μU/ml (range 138–312 μU/ml) for prorenin.*

TABLE 1. *Renin and Prorenin in Plasma of Renal Vein and Aorta in Patients with Renal Artery Stenosis*

Patient group*	Study design	Blood sampling time	^{10}Log renin (μU/ml) Affected side Aorta	Affected side Renal vein	Contralateral Aorta	Contralateral Renal vein
Group 2 (n = 15)	Captopril 50 mg single dose	Before first dose of captopril	1.73 0.10	2.09 0.10	1.72 0.09	1.79 0.09
		30 min after first dose of captopril	2.66 0.11	3.12 0.13	2.70 0.11	2.71 0.10
Group 3 (n = 15)	Captopril 50 mg single dose	16 hrs after first dose of captopril	2.02 0.15	2.37 0.19	2.01 0.15	2.01 0.15
Group 4 (n = 10)	Captopril 50 mg t.i.d. for 2 wks	1–2 hrs after last dose of captopril	2.91 0.13	3.42 0.12	2.89 0.12	2.89 0.13

*For details see Methods section. †$p < 0.05$. ‡$p < 0.01$. §$p < 0.001$ for difference from 1.00.

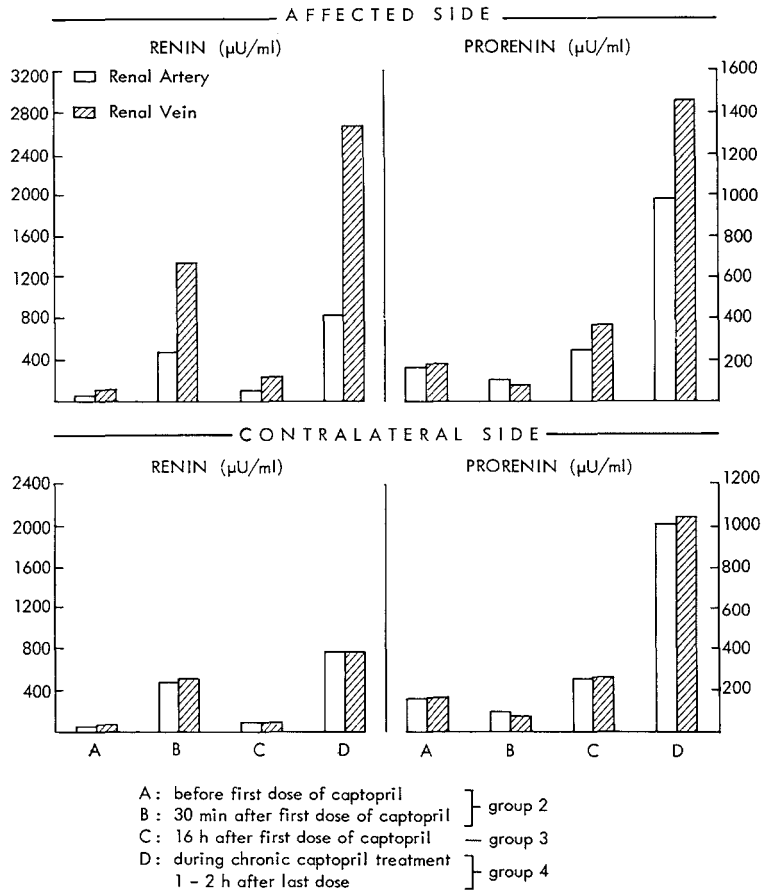

FIGURE 9. *Effects of a first dose of captopril, 50 mg orally, and of chronic captopril treatment, 50 mg three times a day, on plasma renin and prorenin in the renal vein and the abdominal aorta. Statistics of these data and details on study design are presented in table 1.*

TABLE 1. *(Continued)*

^{10}Log prorenin (μU/ml)				Renal vein-to-aorta ratio of renin		Renal vein-to-aorta ratio of prorenin		
Affected side		Contralateral						
Aorta	Renal vein	Aorta	Renal vein	Affected side	Contra-lateral	Affected side	Contra-lateral	
2.23	2.26	2.21	2.21	2.38§	1.16	1.19†	1.04	Mean
0.04	0.06	0.06	0.06	0.21	0.09	0.08	0.08	SEM
2.02	1.93	2.01	1.89	3.25§	1.05	1.14	1.04	Mean
0.09	0.20	0.14	0.19	0.50	0.05	0.26	0.13	SEM
2.41	2.57	2.41	2.42	2.72§	1.00	1.53‡	1.03	Mean
0.13	0.13	0.12	0.13	0.50	0.02	0.16	0.03	SEM
2.99	3.16	3.01	3.02	3.39§	1.00	1.50‡	1.03	Mean
0.06	0.06	0.05	0.05	0.48	0.03	0.11	0.02	SEM

after captopril. High levels of renin were measured in the aorta 30 min after a single dose of captopril and with chronic treatment. High levels of prorenin were measured in the aorta with chronic captopril. This is in agreement with the measurement of peripheral vein renin in Group 1.

Discussion

Methodological Aspects

For the results of functional assays of prorenin to be valid, conversion of the prorenin in the test sample should be complete and the enzymatic activities of equimolar quantities of activated prorenin and naturally occurring renin should not differ. These criteria are likely to be met when immobilized trypsin is used for prorenin activation in human plasma under the assay conditions we have worked out. Several lines of evidence support this conclusion. First, identical results were obtained with acid-activation, with activation by pepsin, plasmin, and urokinase and with immobilized trypsin, provided that optimal conditions were selected. A plateau of maximal renin activity was obtained, and the level of this plateau had the same height with each of these procedures.

Second, Km-values and pH-optimum curves for the reactions of trypsin-activated prorenin and naturally occurring renin with sheep renin substrate did not appear different. Third, the behavior of trypsin-activated prorenin on Sephadex-G100 and Blue Sepharose chromatography columns was similar to that of naturally occurring renin. The Mr-values of naturally occurring active renin (49,000) and prorenin (56,000) as estimated by gel filtration, are in agreement with those reported by others[19, 20] and confirm that the Mr of these plasma renins is greater than the Mr of human kidney renin (42,000).

Maximal activation of prorenin was observed, when plasma was incubated with trypsin at 4°C. Other workers have incubated at 37°C.[14] Our results indicate that it is difficult, if not impossible, to obtain complete activation of prorenin at this temperature. Moreover, the results at 37°C are strongly dependent on the concentration of trypsin and on the incubation time. At 4°C these variables are less critical, provided that incubation is prolonged until renin activity has reached a plateau. At 37°C a larger proportion of added trypsin is inhibited by plasma than at 4°C, and at 37°C the remaining uninhibited trypsin causes progressive inactivation or destruction of renin. Both the inhibition of trypsin, which leads to incomplete activation of prorenin, and the inactivation of renin by trypsin are the cause of measuring falsely low prorenin values. Low values have also been obtained after cryoactivation. It is therefore not surprising that the results of prorenin measurements reported in the literature are widely different. A literature search showed that the measured percentage of renin in normal plasma is highest with cryoactivation (35%–53%),[8, 21–23] whereas it is intermediate with acid-activation (20%–50%),[1, 2, 10, 11, 13, 22, 24–32] and lowest with trypsin (12%–34%).[3, 12, 14, 33] In our study, 11% of the renin in normal plasma was in the active form.

The methodological difficulties are amplified when prorenin is measured after maneuvers that are known to increase circulating renin. The accuracy of renin assays expressed as absolute values is inversely correlated to the height of the measured value. Thus, the higher the renin, the less accurate is its measurement. This causes problems particularly when the difference in renin before and after activation of plasma is small. It explains why there is confusion on whether, after certain stimuli, increments in circulating renin are associated with decrements in prorenin.[1, 2, 25, 27, 30, 31, 34, 35]

Changes in Prorenin after Captopril

As shown by the present study on patients with renal artery stenosis, stimulation of renin release by angiotensin converting enzyme inhibition with captopril causes a precipitous rise in circulating renin with initially no change or even a fall in prorenin. Later also prorenin begins to rise. A transient fall in plasma prorenin after captopril has recently been reported,[36, 37] whereas earlier studies had failed to demonstrate such a fall.[38–40]

Before stimulation with captopril we found a renal vein-to-aorta prorenin ratio of 1.19 ± 0.08 (mean ± SEM) on the affected side and of 1.04 ± 0.08 contralaterally; the value on the affected side was just significantly different from 1.00 ($p < 0.05$). Also in earlier studies renal secretion of prorenin was difficult to demonstrate under basal conditions.[2, 11, 25, 27, 41] It has therefore been postulated that prorenin is formed by extrarenal inactivation of intrarenally produced renin. However, since prorenin appears to have a longer plasma half-life than renin,[11] a relatively low secretion rate of prorenin may suffice to maintain a relatively high plasma level. It is possible therefore that the venoarterial difference in prorenin across the kidney is often too small to detect with an assay that has an accuracy that is not better than 10%.

After acute stimulation with captopril the secretion of prorenin by the affected kidney was not significantly increased, despite a tenfold rise in the secretion of renin. With chronic stimulation however, the venoarterial difference in prorenin became large enough to be easily detectable. Thus it appears that the kidney is indeed capable of secreting prorenin. This finding is probably not an artifact since secretion could be demonstrated on the affected side but not contralaterally. In fact, our data indicate that the changes in peripheral prorenin concentration after captopril are a consequence, at least in part, of corresponding changes in the rate of prorenin secretion from the affected kidney. Both the concentration of prorenin in peripheral plasma and its secretion are increased with prolonged stimulation of renin release but not with acute stimulation. An extrarenal source of plasma prorenin, however, cannot be entirely ignored since low to normal concentrations of prorenin are present in the plasma of nephrectomized subjects.[11, 25, 42]

This pattern of changes in renin and prorenin after captopril is similar to that of insulin and proinsulin following an oral glucose load; insulin rises within a few minutes but proinsulin begins to rise not earlier than after 1–2 hours.[43] The late rise in proinsulin is a manifestation of an increased rate of synthesis in the pancreas. More prohormone is then available for conversion to the active hormone before it is released into the circulation. These points of resemblance between the two hormonal systems should not distract from the fact that under normal basal conditions the concentration of insulin in peripheral plasma is 8–9 times higher than that of proinsulin, whereas the reverse is true for renin and prorenin.

Our results do not answer the question whether or not prorenin is a storage form of renin but they do suggest that prorenin enters the circulation either by leakage from the juxtaglomerular cells or by corelease before it is converted to renin. In conclusion, these data indicate that prorenin is indeed a biosynthetic precursor of renin and that under some circumstances, if not mostly, a major proportion of prorenin in the circulation originates from the kidney.

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Split renal function after captopril in unilateral renal artery stenosis

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Abstract

The renal extraction ratios of ^{131}I-sodium iodohippurate (^{131}I-Hippuran) and ^{125}I-thalamate were greatly reduced on the affected side by 50 mg captopril in seven out of 14 patients with unilateral renal artery stenosis. With long term captopril 150 mg daily the uptake of ^{99m}Tc-diethylenetriaminepenta-acetic acid by the affected kidney, which was determined by scintillation camera renography, became almost zero in these seven patients, indicating severe reduction of the glomerular filtration rate. Function of the affected kidney returned on discontinuing treatment. The reduced extraction of sodium iodohippurate probably reflected a shortened plasma transit time through the kidney due to intrarenal vasodilatation. The reduced extraction of thalamate reflected a low filtration fraction, suggesting that the vasodilatation was, at least in part, at the level of the postglomerular arterioles. Captopril had little effect on the contralateral kidney and on the kidneys of 17 patients with essential hypertension, and serum creatinine concentrations showed minor changes.

Radioisotope renography should be performed after beginning captopril treatment in patients with renal artery stenosis. This is also recommended for patients given captopril as a third line drug when renal artery stenosis has not been excluded. Hypertension in these patients is often severe and difficult to control. Renal artery disease is not rare in this difficult group and finding seriously impaired renal function on one side during captopril treatment may be diagnostic.

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Introduction

Captopril is now widely used for severe hypertension, including that associated with renal artery stenosis.[1-4] Renal failure, however, may occur in patients receiving captopril who have bilateral renal artery stenosis or a stenosis affecting a solitary functioning kidney.[5-10] Increase in systemic arterial pressure, dilatation of preglomerular arterioles, postglomerular vasoconstriction, and possibly other mechanisms may help to maintain glomerular filtration when renal perfusion is compromised by artery stenosis.[11 12] Some of these mechanisms depend, at least in part, on an intact renin-angiotensin system. Converting enzyme inhibition, by interfering with angiotensin II formation, has therefore the potential to disturb the fine balance between pressure and flow required for optimal regulation of glomerular filtration in renal artery disease. In unilateral disease such an effect may easily go unnoticed because of the functional reserve of the opposite kidney.

We report on the effects of captopril on split renal function in these patients.

Patients and methods

Thirty one hypertensive patients were selected from a larger series of consecutive patients because they were shown to have unilateral renal artery stenosis on renal arteriography (n = 14; table I) or because their renal arteries were found to be normal on both sides (n = 17). The patients were admitted to this hospital for a diagnostic work up because their hypertension was difficult to control; they remained hypertensive despite combined treatment with high doses of diuretics, β blockers, hydralazine, and in some cases also methyldopa. Results of urine analysis, serum electrolyte,

urea, and creatinine concentrations, and urinary excretion of vanillylmandelic acid were normal. Medication had been stopped for at least two weeks before renal function studies and renal vein catheterisation. The renal arteriogram was made after renal vein sampling in the same session.

^{131}I-Sodium iodohippurate (^{131}I-Hippuran) and ^{125}I-thalamate were administered by constant infusion into an arm vein. After reaching the steady state blood samples were taken simultaneously from the abdominal aorta and the renal vein. Samples from the same sites were used for renin measurements. The extraction ratio of ^{131}I-sodium iodohippurate (E_H) and of ^{125}I-thalamate (E_T) and the aortic and renal vein plasma renin values were measured 10-15 minutes before captopril and 30-45 minutes after 50 mg of this drug. Blood samples were also taken at 15 minute intervals from a peripheral vein for estimating total renal clearance of sodium iodohippurate and thalamate.[13 14] All blood samples were centrifuged immediately and radioactivity measured in plasma. Single-kidney extraction ratio (extraction efficiency) was calculated as $(A-V)/A \times 100\%$, where A = activity in abdominal aorta and V = activity in renal vein. The clearance of sodium iodohippurate was taken as a measure of total effective renal plasma flow, and the clearance of thalamate was taken as a measure of total glomerular filtration rate.

The single kidney uptake of ^{99m}Tc-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA) was determined by scintillation camera renography.[15] Approximately 5-10 mCi ^{99m}Tc-DTPA was injected intravenously. Lightpen "regions of interest" corresponding to the left and right kidneys were traced on the display screen using the three minute summation image. Time activity curves of each kidney region were displayed. Counting rates from the kidney areas were corrected for background activity using a region of interest between the kidneys. Single kidney function was estimated from the radioactivity over the kidney regions 60-120 seconds after injection and expressed as activity ratio—that is, right/(right + left). This ratio is a measure of the single kidney's contribution to total glomerular filtration rate.[16] The kidney scans were made before treatment and after three to five weeks of captopril 150 mg daily.

The concentration of active renin in plasma was measured by radioimmunoassay.[17] Blood pressure was measured intra-arterially in the acute study during renal vein catheterisation and indirectly with the London School of Hygiene sphygmomanometer in the long term study.

Grouped data are presented as means (SEM in parentheses), and differences were analysed for statistical significance by Student's *t* tests for paired and unpaired data.

Results

Values of E_H and E_T were significantly decreased after captopril on both sides both in patients with unilateral renal artery stenosis and in essential hypertension (table II; fig 1). The effects of captopril on kidneys with a stenotic artery were much greater than on kidneys with normal arteries. The renal extraction ratio of a substance equals its renal clearance divided by the renal plasma flow. Thus E_T = clearance of thalamate/true renal plasma flow, or glomerular filtration rate/true renal plasma flow—that is, filtration fraction. Our results therefore indicate that the single kidney filtration fraction was lowered by captopril, particularly when the kidney was affected by renal artery stenosis.

Since the clearance of sodium iodohippurate did not change after captopril (table II; fig 2) and E_H = clearance of sodium iodohippurate/true renal plasma flow, the observed reduction of E_H after captopril implies that true renal plasma flow and therefore the total renal blood flow was increased.

As shown in figure 3, ^{99m}Tc-DTPA uptake by the affected kidney became almost zero after captopril in seven patients with unilateral renal artery stenosis (group 1) and was essentially unchanged in the remaining seven patients with renal artery stenosis (group 2). It was also unchanged in the patients with essential hypertension. Reductions in E_H and E_T after the first dose of 50 mg captopril were greater in group 1 than in group 2 (table II). Serum creatinine concentration rose significantly during long term captopril in group 1 but not in group 2 (table III). Neither the changes in blood pressure nor the pressure levels that were reached after captopril were, however, different in the two groups. None of the patients developed troublesome proteinuria.

The loss of renal function after captopril in group 1 appeared not to be due to irreversible parenchymal damage. In four patients DTPA uptake was restored one to two weeks after captopril had been stopped (figure 4 gives an example). By that time the plasma creatinine concentration had also returned to its original value. The

TABLE I—*Clinical data on patients with unilateral renal artery stenosis*

Case No	Age (years)	Sex	Cause of renal artery stenosis	Plasma renin mU/l†	Renal vein to artery renin ratio	
					Affected kidney	Contralateral kidney
			Group 1			
1	47	M	Atherosclerosis	233	5·97	0·92
2	57	M	Atherosclerosis	61	3·29	1·02
3	56	M	Atherosclerosis	37	2·42	0·94
4	64	M	Atherosclerosis	38	1·58	1·12
5	47	M	Atherosclerosis	37	1·71	1·17
6	31	F	Fibromuscular hyperplasia	23	2·65	0·88
7	57	M	Atherosclerosis	480	1·39	0·91
			Group 2			
8	68	F	Atherosclerosis	178	5·81	0·89
9	57	M	Atherosclerosis	353	2·09	0·81
10	58	M	Atherosclerosis	75	3·08	1·03
11	65	M	Atherosclerosis	208	2·20	1·40
12	25	F	Fibromuscular hyperplasia	34	1·53	1·04
13	68	M	Atherosclerosis	40	1·36	1·55
14	50	F	Atherosclerosis	42	2·17	0·96

Patients separated into two groups based on effect of captopril on uptake of ^{99m}Tc-diethylenetriaminepenta-acetic acid by affected kidney (see text).
†Normal range 5-45 mU/l.

TABLE II—*Acute effects of captopril in patients with unilateral renal artery stenosis (group 1 v group 2). Values are means (SEM in parentheses)*

		Group 1 (cases 1-7)		Group 2 (cases 8-14)		p Values for differences between groups 1 and 2	
		Before captopril	After captopril	Before captopril	After captopril	Before captopril	After captopril
Mean arterial pressure (mm Hg)		139 (5)	114 (4)***	137 (10)	110 (10)**	NS	NS
Total effective renal plasma flow (ml/min)		333 (35)	343 (42)	320 (46)	328 (48)	NS	NS
Total glomerular filtration rate (ml/min)		95 (6)	82 (7)**	87 (8)	81 (7)*	NS	NS
Single-kidney extraction ratio of sodium iodohippurate (%)	Affected kidney	60 (7)	29 (7)***	64 (5)	53 (9)*	NS	<0·001
	Contralateral kidney	75 (3)	71 (3)	74 (3)	70 (5)	NS	NS
Single-kidney extraction ratio of thalamate (%)	Affected kidney	18 (1)	6 (1)***	17 (2)	13 (3)**	NS	<0·001
	Contralateral kidney	24 (2)	22 (3)	22 (2)	19 (3)	NS	NS

NS = Not significant.
*p < 0·05. **p < 0·01. ***p < 0·001.

TABLE III—*Long term effects of captopril in patients with unilateral renal artery stenosis (group 1 v group 2). Values are means (SEM in parentheses)*

	Group 1 (cases 1-7)		Group 2 (cases 8-14)		p Values for differences between groups 1 and 2	
	Before captopril	After captopril	Before captopril	After captopril	Before captopril	After captopril
Mean arterial pressure (mm Hg)	143 (7)	111 (6)***	140 (8)	114 (5)***	NS	NS
Serum creatinine (μmol/l)	100 (6)	122 (9)**	113 (12)	116 (12)	NS	NS
Uptake of ^{99m}Tc-DTPA by affected kidney (% of total uptake)	34 (3)	<10	31 (4)	30 (4)		

NS = Not significant.
**p<0·01, ***p<0·001.
Conversion: SI to traditional units—Creatinine: 1 μmol/l ≈ 0·01 mg/100 ml.

remaining three patients were not restudied after discontinuation of captopril treatment, but DTPA kidney scans after reconstructive vascular surgery showed improved uptake on the affected side.

Discussion

This study shows that in a substantial number of patients with unilateral renal artery stenosis the renal extraction ratio of both ^{131}I-sodium iodohippurate (E_H) and ^{125}I-thalamate (E_T) is greatly reduced on the affected side when captopril is given as the only drug. The fall in E_H may be explained by a shortened plasma transit time through the kidney due to intrarenal vasodilatation. This has also been observed with vasodilatation induced by other agents.[18] E_T equals filtration fraction, and the fall in E_T after captopril may reflect the dilatation of postglomerular arterioles.[10] Captopril also lowered E_H and E_T of kidneys with a normal artery but the changes were not as great as for kidneys with artery stenosis. In our patients the fact that the decrease in E_H on both sides was not associated with a decrease in total clearance of sodium iodohippurate is further support for vasodilatation in the kidney, probably on the affected as well as the non-affected side. Increase in total renal blood flow and decrease in total filtration fraction after captopril have been reported in patients with essential hypertension.[19] In those studies the clearance of para-aminohippurate was used as an estimate of renal plasma flow with the implicit assumption that the renal extraction efficiency was high and remained constant. This, however, may be misleading, as shown by our results; the effects of captopril on renal blood flow and filtration fraction would be grossly underestimated in some patients.

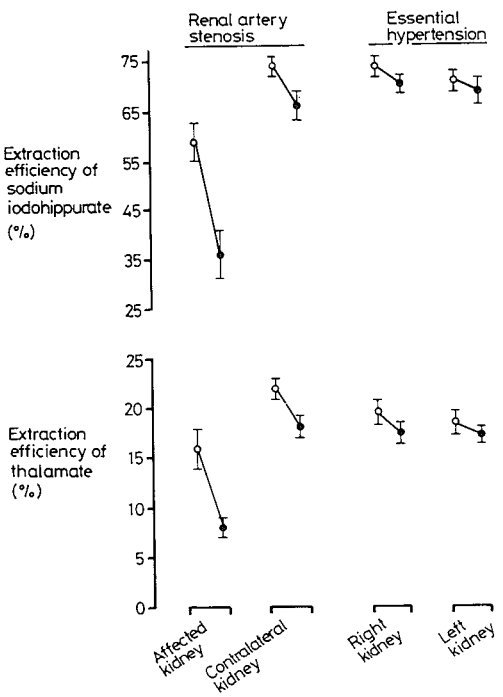

FIG 1—Effect of 50 mg captopril on renal extraction efficiencies of ^{131}I-sodium iodohippurate (E_H) and ^{125}I-thalamate (E_T) in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. In patients with renal artery stenosis changes in E_H and E_T were significant on both sides (p<0·01). Changes in essential hypertension were also significant (p<0·05).

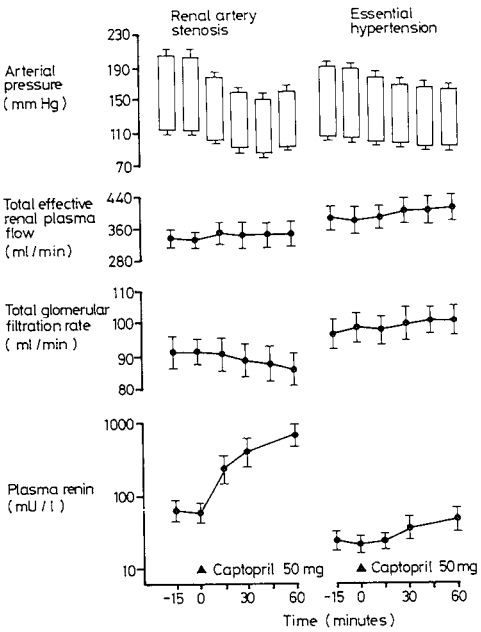

FIG 2—Effect of 50 mg captopril on total clearances of ^{131}I-sodium iodohippurate (effective renal plasma flow) and ^{125}I-thalamate (glomerular filtration rate) in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. Effect of captopril after 60 minutes was significant for systolic and diastolic intra-arterial pressure (p<0·001) and for renin (p<0·01).

Other significant findings were the changes in the ^{99m}Tc-DTPA kidney scans showing a decrease in glomerular filtration rate during long term captopril treatment. This was seen only with kidneys affected by artery stenosis. It also appeared to be an all or none phenomenon—that is, the uptake of DTPA by the affected kidney became either almost zero or showed little change. The deterioration in renal function was observed in half of our patients, but this high incidence may have been related to selection; all had been referred to us because of severe hypertension that was difficult to control.

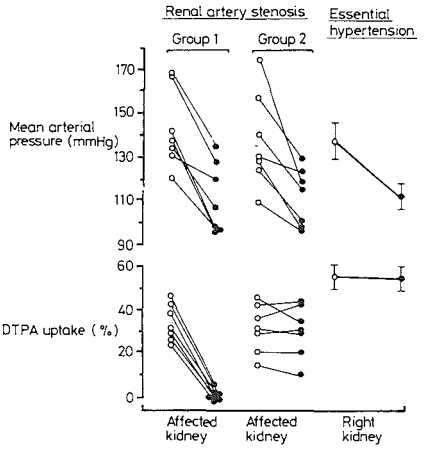

FIG 3—Effect of long term captopril 150 mg daily on blood pressure and single kidney uptake of ^{99m}Tc-DTPA in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. Patients with renal artery stenosis divided into two groups according to change in DTPA uptake (see table III for statistics). Values in patients with essential hypertension presented as means and SEM. Mean arterial pressure calculated as diastolic pressure + 0·3 × pulse pressure. London School of Hygiene sphygmomanometer used. Three consecutive readings with patient in recumbent position were averaged. Effect of captopril on mean arterial pressure in patients with essential hypertension was not different from effect in two groups of patients with renal artery stenosis.

Deterioration of renal function does not occur only with captopril,[20] but conceivably converting enzyme inhibitors may be especially likely to cause this complication. Acute converting enzyme inhibition with captopril or angiotensin II blockade with saralasin caused renal failure in rats with chronic two kidney, two clip hypertension pretreated with frusemide.[21] By contrast, the direct smooth muscle relaxants minoxidil and dihydralazine did not have this effect, despite a similar fall in systemic arterial pressure. Such findings have also been reported in a few patients with bilateral renal artery stenosis or with a stenotic artery to a solitary functioning kidney.[7-9] Most of these patients had been treated with captopril in combination with other drugs, particularly diuretics. More work is needed to establish whether captopril either alone or combined with a diuretic is more harmful for the kidney affected by artery stenosis than other antihypertensive drugs.

The effect of captopril on systemic arterial pressure in our patients who responded with loss of filtration on the affected side was not greater than in those who maintained filtration. Thus the degree of reduction in blood pressure is probably not the only factor determining whether or not renal function can be maintained during captopril. Experimental constriction of a renal artery is known to be followed by vasoconstriction within the affected kidney, and there is good evidence that the post-glomerular vascular resistance is increased so that filtration pressure is restored and glomerular filtration rate is maintained. This mechanism is impaired by converting enzyme inhibition, and filtration pressure may fall, particularly when systemic arterial pressure also falls.[11 12] Increased glomerular blood flow after intrarenal vasodilatation may partly compensate for this.[22] When filtration pressure falls below a critical level, however, the kidney stops filtering. It is tempting to assume that this occurred in some of our patients. It was the patients with the greatest reductions in E_H and E_T after captopril who responded with loss of filtration. Presumably these were the patients with the most severe artery stenosis. An alternative or additional mechanism contributing to the fall in glomerular filtration rate might be that a critically severe stenosis of a large artery becomes more severe after dilatation of the distal vascular bed.[23 24] This has been reported in renal artery stenosis induced by cuff constrictors in intact instrumented dogs.[25]

Fortunately, in none of our patients were the effects of captopril on renal function associated with irreversible damage to the renal parenchyma. DTPA uptake was restored by discontinuing captopril or after reconstructive vascular surgery. Radioisotope renography should be performed in any patient with renal artery stenosis who is taking captopril. Perhaps we should go even further. Until now captopril has been used in hypertension mainly as a third line drug when other drugs have failed. Renovascular hypertension is not uncommon in this difficult group. Hence radioisotope renography should probably be performed in every patient who has been given captopril because of poor response to other drugs when the possibility of renal artery stenosis has not been excluded. We believe that finding severely impaired renal function on one side during captopril treatment calls for withdrawing the drug or perhaps lowering the dose. In such cases renal artery stenosis is likely to be the underlying disease.

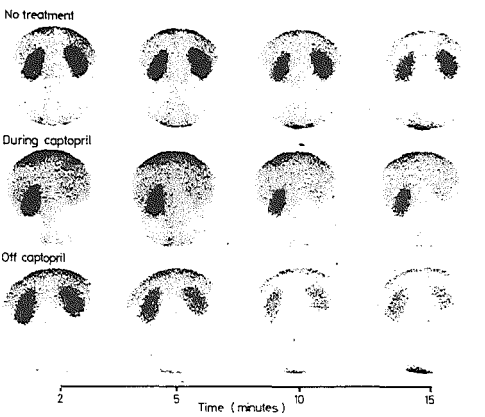

FIG 4—Sequential ^{99m}Tc-DTPA kidney scans in patient with unilateral renal artery stenosis (case 3; table I) before captopril, after four weeks of captopril 150 mg daily, and one week after stopping captopril. Time after radioisotope injection indicated.

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Risks of angiotensin converting enzyme inhibition in renal artery stenosis

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ACE inhibitors are now widely used in the treatment of severe hypertension, including hypertension associated with renal artery stenosis. Several authors have reported on deterioration of renal function upon institution of angiotensin converting enzyme (ACE) inhibition in patients characterized by having marked degrees of bilateral renal artery stenosis or a stenosis affecting a solitary functioning kidney. These effects have been considered to be associated with a profound fall in arterial pressure by some authors [1–4] but not by others [5–7].

To date, the exact mechanism behind this loss of renal function has not been clarified, but the current explanation is that autoregulation of glomerular filtration depends on an intact renin–angiotensin system [8]. ACE inhibition, by interfering with angiotensin 11 formation, has therefore the potential to disturb the balance between the pressure and flow required for optimal regulation of glomerular filtration in renal artery disease. In unilateral disease, adverse effects of ACE inhibition may easily go unnoticed because of the functional reserve of the opposite nonstenotic kidney. We therefore studied split renal function in 25 patients with hypertension and unilateral renal artery disease. In one patient with a stenosis of a saphenous-vein graft to a solitary functioning kidney, we further tried to elucidate the mechanism involved in the shutdown of glomerular filtration during ACE inhibition.

Patients and methods

Twenty-five patients (10 male, 15 female; aged 16–66 years) with unilateral renal artery stenosis on renal arteriography and near-normal overall renal function were studied. The cause of the renal artery stenosis was classified, according to the arteriographic appearance, as due to either atheroma (21 patients) or fibromuscular dysplasia (4 patients). One patient (a heavy smoker, male, aged 62 years) had had an aorto-iliac graft and a saphenous-vein graft had been anastomosed to the prosthesis and end to end to the right renal artery beyond the occluded portion. A small contracted nonfunctioning kidney was removed in the same session. Blood pressure was initially well controlled without hypotensive drugs but the recurrence of severe hypertension suggested re-stenosis. Aortography showed a tight stenosis of the saphenous-vein graft. In all patients, previous antihypertensive treatment was withdrawn for at least one week. The patients were admitted to hospital for detailed measurements of blood pressure, and for renal function studies and renal vein catherization. Renal arteriography was performed after renal vein sampling.

^{131}I-sodium iodohippurate (^{131}I-hippuran) and ^{125}I-thalamate were administered by constant infusion into an arm vein. After reaching the steady state, blood samples were taken simultaneously from the abdominal aorta and the renal vein. Samples from the same sites were used for renin measurements. The extraction ratio of ^{131}I-hippuran (E_H) and of ^{125}I-thalamate (E_T), and the aortic and renal vein plasma renins, were measured 10–15 min before, and 30–45 min after, administration of 50 mg of captopril.

Blood samples were also taken at 15-min intervals from a peripheral vein for estimating total renal clearance of sodium iodohippurate and thalamate [9, 10]. All blood samples were centrifuged immediately and radioactivity was measured in plasma. Single-kidney extraction ratio (extraction efficiency) was calculated as $[(A - V)/A \times 100\%)]$, where A is the activity in abdominal aorta and V is the activity in renal vein. The clearance of sodium iodohippurate was taken as a measure of total effective renal plasma flow, and the clearance of thalamate was taken as a measure of total glomerular filtration rate (GFR).

The single kidney uptake of ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) was determined by scintillation camera renography [11]. Approximately 5–10 mCi ^{99m}Tc-DTPA was injected intravenously. Light-pen "regions of interest" corresponding to the left and right kidneys were traced on the display screen using the three-minute summation image. Time activity curves of each kidney region were displayed. Counting rates from the kidney areas were corrected for background activity using a region of interest between the kidneys. Single kidney function was estimated from the radioactivity over the kidney regions 60–120 seconds after injection and expressed as activity ratio—that is, right/ (right + left). This ratio is a measure of the single kidney's contribution to total GFR [12]. The kidney scans were made in 14 patients before treatment and after 3–5 weeks of captopril 150 mg daily. The concentration of active renin in plasma was measured by radioimmunoassay [13]. Blood pressure was measured intraarterially in the acute study during

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Table 1. Acute effects of 50 mg captopril in 25 patients with hypertension and unilateral renal artery stenosis

		Before captopril	After captopril	*P* values
Arterial pressure, *mm Hg*		205 ± 6/114 ± 3	161 ± 6/84 ± 5	<0.001/<0.001
Total effective renal plasma flow, *ml/min*		328 ± 24	340 ± 32	NS
Total glomerular filtration rate, *ml/min*		91 ± 4	85 ± 5	<0.01
Single-kidney extraction ratio of sodium iodohippurate, %	Affected kidney	54 ± 5	34 ± 4	<0.01
	Contralateral kidney	74 ± 2	66 ± 3	<0.01
Single-kidney extraction ratio of thalamate, %	Affected kidney	15 ± 2	8 ± 1	<0.01
	Contralateral kidney	22 ± 1	18 ± 1	<0.01

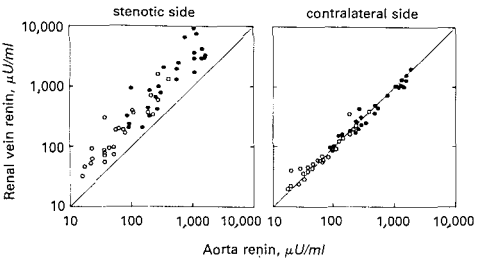

Fig. 1. *Acute effect of 50 mg captopril on the plasma concentration of active renin in blood from the renal veins and aorta.* Symbols are: (○) before captopril, (●) after captopril.

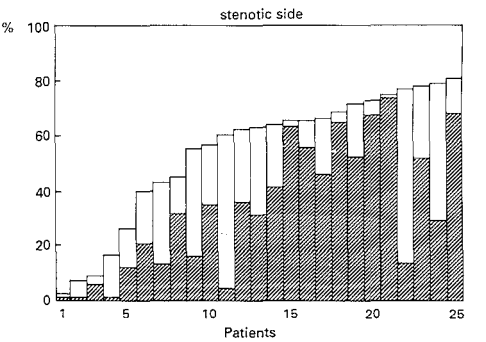

Fig. 2. *Acute effect of 50 mg captopril on the extraction efficiency of ^{131}I-sodium iodohippurate of the stenotic kidney.* Open bars before captopril, hatched bars during captopril treatment.

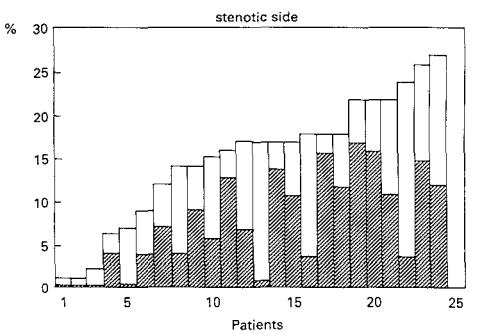

Fig. 3. *Acute effect of 50 mg captopril on the extraction efficiency of ^{125}I-thalamate of the stenotic kidney.* Open bars before captopril, hatched bars during captopril treatment.

renal vein catherization and indirectly with the London School of Hygiene sphygmomanometer in the long-term study. Grouped data are presented as means ± SEM and differences were analysed for statistical signficance by Student's *t* tests for paired data.

Results

Acute effects of captopril

Renal vein renins. Before captopril treatment, the plasma concentration of active renin was higher in blood from the renal vein of the stenotic kidney than from the aorta (Fig. 1), the renal vein-to-aorta renin ratio (*V/A*) being 2.86 ± 0.28. Veno-arterial differences for active renin across the contralateral kidney were small, *V/A* being 1.09 ± 0.06. After the first dose of 50 mg captopril, *V/A* of the stenotic side rose to 3.70 ± 0.46 ($P < 0.01$).

No change of *V/A* was found for the contralateral side, with *V/A* remaining 1.03 ± 0.03. Renin in peripheral plasma rose from 100 ± 20 to 607 ± 98 μU/ml.

Blood pressure and renal function. Despite a fall in blood pressure, the overall clearance of sodium iodohippurate and thalamate was not much altered (Table 1). However, captopril had a pronounced, and sometimes dramatic, effect on the extraction efficiency of the stenotic kidney for these radiolabels (Figs. 2 and 3). E_H fell by more than 20% in 9 patients. The renal extraction ratio of a substance equals its renal clearance divided by the renal plasma flow. Thus E_T = clearance of thalamate/true renal plasma flow, or GFR/true renal plasma flow, that is, filtration fraction. Our results therefore demonstrate that the single kidney filtration fraction was lowered by captopril, particularly when the kidney was affected by renal artery stenosis. Since the overall clearance of sodium iodohippurate did not change after captopril treatment and E_H = clearance of sodium iodohippurate/true renal plasma flow, the observed reduction of E_H after captopril implies that true renal plasma flow and therefore the total renal blood flow was increased. It is, however, possible that in some cases the blood flow of the nonaffected kidney is increased, whereas the flow of the affected kidney is decreased.

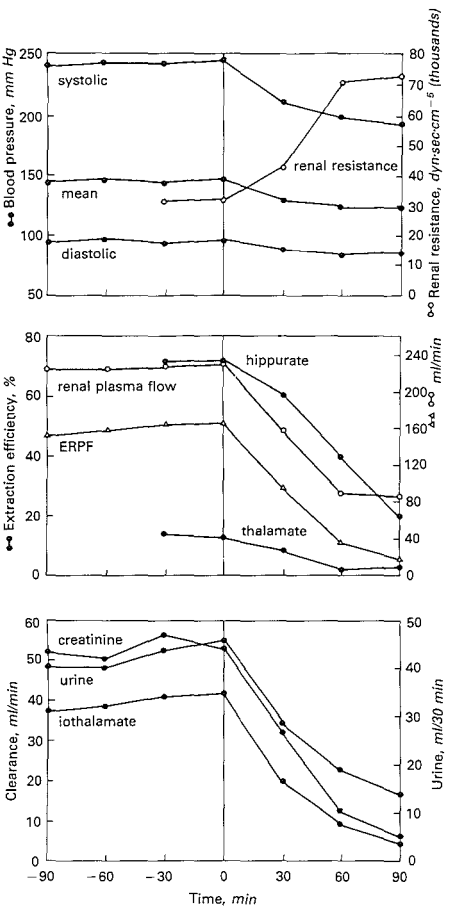

Fig. 4. *Acute effect of 50 mg captopril in a patient with a tight stenosis of a saphenous-vein bypass graft to the right artery of a solitary functioning kidney.*

Function of a solitary kidney. Data on blood pressure, urine production, GFR, and renal haemodynamics after a single dose of 50 mg captopril in the patient with a tight stenosis of the saphenous-vein bypass are shown in Fig. 4. The fall in blood pressure after captopril was modest, but renal perfusion and glomerular filtration almost ceased. Although captopril probably had a vasodilatory effect within the kidney, vascular resistance over the total circuit, including the bypass, rose.

Long-term effects of captopril

As shown in Fig. 5, ^{99m}Tc-DTPA uptake by the affected kidney almost ceased after captopril treatment in seven patients (group 1), but was essentially unchanged in the remaining group

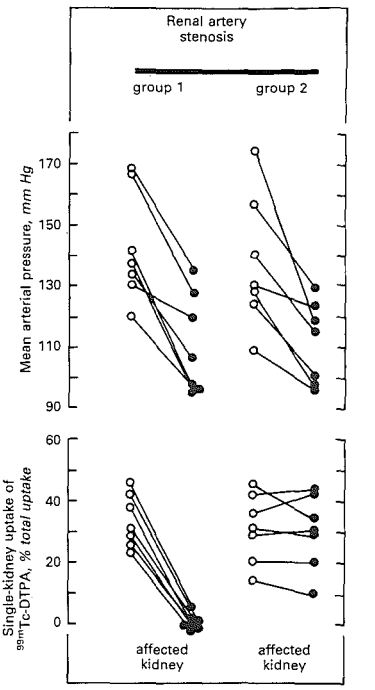

Fig. 5. *Effect of long-term captopril, 150 mg daily, on blood pressure and single kidney uptake of ^{99m}Tc-DTPA in 14 patients with unilateral renal artery stenosis.* Patients with renal artery stenosis divided into two groups according to change in DTPA uptake, Mean arterial pressure calculated as diastolic pressure + 0.3 × pulse pressure.

(group 2). Neither the changes in blood pressure nor the pressure levels that were reached after captopril treatment were, however, different in the two groups (Fig. 5). Reduction in E_H and E_T after the first dose of 50 mg captopril were correlated to the changes in ^{99m}Tc-DTPA uptake ($r = 0.78$, $P < 0.01$, and $r = 0.72$, $P < 0.01$, respectively). Serum creatinine concentration rose significantly from 100 ± 6 to 122 ± 9 μmol/l ($P < 0.01$) in group 1 but not in group 2 (113 ± 12 μmol/l before captopril, 116 ± 12 μmol/l during captopril). The loss of renal function after captopril treatment in group 1 appeared not associated with irreversible parenchymal damage. In 4 patients, DTPA uptake was restored after captopril had been stopped and serum creatinine had also returned to its original value. The remaining three patients were not restudied after discontinuation of captopril treatment but DTPA kidney scans after reconstructive vascular surgery showed improved uptake on the affected side.

Discussion

The present study extends earlier observations [14] that, in a substantial number of patients with unilateral renal artery stenosis, the renal extraction ratio of both ^{131}I-sodium iodohip-

purate (E_H) and ^{125}I-thalamate (E_T) is greatly reduced on the affected side when captopril is given as the only drug. The fall in E_H may be explained by a shortened plasma transit time through the kidney due to intrarenal vasodilatation. This has also been observed with vasodilatation induced by other agents [15]. E_T reflects filtration fraction, and the fall in E_T after captopril treatment may result from the dilatation of postglomerular arterioles [16]. Captopril also lowered E_H and E_T of the contralateral kidney, but the changes were small and comparable to those found in patients with essential hypertension [13]. Other significant findings were the changes in ^{99m}Tc-DTPA kidney scans showing a decrease in GFR during long-term captopril treatment. This was seen only with kidneys affected by artery stenosis. It also appeared to be an all or none phenomenon—that is, the uptake of DTPA by the affected kidney either became almost zero or showed little change. The effect of captopril on systemic arterial pressure in our patients who responded with loss of filtration on the affected side was not greater in those who maintained filtration. Thus the degree of reduction in blood pressure is probably not a decisive factor determining whether or not renal function can be maintained during captopril treatment. Experimental constriction of a renal artery is known to be followed by vasoconstriction within the affected kidney, and there is good evidence that the postglomerular vascular resistance is increased (by local formation of angiotensin 11) so that filtration pressure is restored and glomerular filtration rate is maintained. This mechanism is impaired by ACE inhibition, and filtration pressure may fall. Increased glomerular blood flow, after intrarenal vasodilatation, may partly compensate for this [17]. When filtration pressure falls below a critical level, however, the kidney stops filtering. It is tempting to assume that this occurred in some of our patients. An alternative or additional mechanism contributing to the fall in GFR might be that a critically severe stenosis of a large artery becomes more severe after dilatation of the distal vascular bed [18, 19]. This has been reported in renal artery stenosis induced by cuff constrictors in intact, instrumented dogs [20]. The rise in vascular resistance over the total renal circuit in our patient with the tight stenosis of the saphenous-vein graft suggests that this rheological phenomenon also occurs in humans. It is clear that, in exceptional cases, E_H will also fall as a result of severe reduction of renal perfusion. Fortunately, in none of our patients were the effects of captopril on renal function associated with irreversible damage to the renal parenchyma. However, the consequences of a long-term, captopril-mediated reduction in GFR are unknown. When renal blood flow is gravely compromised, renal artery thrombosis may occur [21]. We recommend that radioisotope renography should be performed after beginning ACE inhibition in patients with renal artery stenosis. This is also recommended for patients given captopril as a third-line drug when renal artery stenosis has not been excluded. Hypertension in these patients is often severe and difficult to control. Renal artery disease is not rare in this difficult group, and finding seriously impaired renal function on one side during captopril treatment may be diagnostic.

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CHAPTER 4

ACE INHIBITION AS DIAGNOSTIC TOOL

Captopril test for diagnosis of renovascular hypertension.

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CAPTOPRIL TEST FOR DIAGNOSIS OF RENAL ARTERY STENOSIS

Summary

One hundred seventy-nine patients were selected for a diagnostic work-up of renovascular hypertension from a consecutive series of hypertensive subjects referred to a single outpatient clinic between January 1978 and January 1985. Selection criteria were: 1. diastolic blood pressure 120 mm Hg or above in patients of 40 yr or younger, irrespective of whether or not they were on treatment; and/or 2. diastolic blood pressure of 100 mm Hg or above despite a combination therapy with at least three drugs. Diagnosis of renal artery stenosis was made by renal angiography. The response of blood pressure and the plasma concentration of naturally occurring active renin, one hour after 50 mg captopril orally, were assessed. Before the test, the patients had been off drug treatment for at least two weeks. The predictive value of the test was 0.92 using a renin concentration of 200 μU/ml or above as criterion for a positive test. The predictive value of a negative test was 0.81. Using the blood pressure response as a criterion, the predictive values were lower. Predictive values were not improved by combining the renin level with the blood pressure response. The prevalence of renal artery stenosis in our patients with severe or therapy-resistant hypertension was 50% (unilateral stenosis 34%). Plasma renin one hour after 50 mg captopril (captopril test) is a useful criterion for selecting cases for renal angiography, at least in a hypertension clinic where a patient population is seen with a similarly high prevalence of renal artery stenosis.

Introduction

There are no clinical characteristics that can be used to differentiate unequivocally between patients with essential hypertension and patients with hypertension due to renal artery stenosis (3). Renal angiography is the only effective technique for demonstrating the presence of a stenotic lesion in the renal artery. Obviously, this technique cannot be used for screening the hypertensive population for renal artery disease. Several procedures, such as intravenous urography and radioisotope renography have been used for selecting patients for renal angiography. However, the values of these diagnostic tests has been disputed (3).

Plasma renin activity and the plasma concentration of active renin is higher on average in patients with renal artery stenosis than in patients with essential hypertension but there is a large overlap between the two groups. This might be related, at least in part, to the fact that renin measurements were not always performed under strictly standardized conditions (sodium intake, posture, antihypertensive treatment).

Until recently, the saralasin infusion test has been advocated as a diagnostic tool in patients with suspected renal artery stenosis. Blood pressure generally falls after infusion of this competitive angiotensin II antagonist when plasma renin is high, but not when it is normal or low. The discriminatory value of this test as a diagnostic indicator of renin-

dependent hypertension however, is also disputed (7, 8).

Inhibition of angiotensin-converting enzyme is another possibility to inferfere with the renin-angiotensin system. In this paper we have tried to answer the following question: Are measurements of plasma active renin under strictly standardized conditions before and after a single oral dose of the angiotensin-converting enzyme inhibitor captopril (50 mg) of any use for diagnosing renal artery stenosis ?

Patients and methods

Patients

The 179 patients in this study were selected from a larger consecutive series of caucasian hypertensive subjects referred to our outpatient clinic between January 1978 and January 1985. Inclusion criteria for this study were:

1. diastolic blood pressure repeatedly 120 mm Hg or above in patients of 40 yrs or younger, irrespective of whether or not they were under antihypertensive therapy; and/or
2. diastolic blood pressure of 100 mm Hg or above, despite a combination therapy with at least three different antihypertensive drugs.

The patients were hospitalized during the time of the study and had a sodium intake between 50 and 70 mmoles per day. Antihypertensive therapy was stopped at least two weeks before the study. All patients underwent renal angiography at the end of the study. The distinction between fibromuscular dysplasia and atherosclerotic disease was made by angiography criteria (2). The patients had given their informed consent.

Sixty-two patients had unilateral renal artery stenosis and 28 had bilateral renal artery stenosis. In 10 of these patients, renal artery stenosis was due to fibromuscular dysplasia and in the remainder to atherosclerotic lesions.No abnormalities were found in 89 patients, and this group was considered to have essential hypertension.

Captopril test

The test was performed while the patients were recumbent after one hour bed rest. Captopril, 50 mg was given by mouth. Blood pressure was measured every 5 min with an automatic oscillometric device (Accutor, Datascope, Paramus, NJ, USA) from 30 min before to 4 hours after captopril administration. Blood samples for renin measurements were taken from an indwelling catheter in a forearm vein at -30 min, 0 min, 30 min, 1 hr, 2 hrs and 4 hrs after captopril. The first blood sample was taken between 11 and 12 a.m.

Renin measurements

Blood was collected in chilled plastic tubes containing EDTA in a final concentration of 5 mM. It was centrifuged at 3000 g for 10 min at 4°C immediately after collection. Plasma was stored at -20°C. Naturally occurring active renin was measured as described before (4, 5). For this assay 0.1-0.25 ml plasma was added to 0.5 ml purified sheep renin-substrate, to which the appropriate enzyme inhibitors were added. The volume was adjusted to 1.0 ml with 0.1 M sodium phosphate buffer, pH 7.5, containing 0.05 M NaCl. The final concentration of sheep renin-substrate in the incubation mixture was 1.5 μM, which corresponds with approximately 6xK_m. The samples were incubated at 37°C for periods up to one hour. The reaction was stopped by pipetting 50 μl aliquots into prechilled plastic tubes placed on melting ice. The quantity of angiotensin I that was generated during incubation was measured by radioimmunoassay. The zero-incubation time was taken as the blank. The concentration of enzymatically active renin is expressed as microunits per ml of

plasma (μU/ml), using as a reference the human kidney renin standard 68/356 of the Medical Research Council (MRC) National Institute for Biological Standards and Control (London, UK) (1)

Statistics

The best trade-off between sensitivity and specificity for the various test criteria was determined from a Receiver Operator Characteristic Curve (6).

Results

Peripheral vein renin under basal conditions

The peripheral vein renin levels of enzymatically active renin in untreated patients with unilateral renal artery stenosis, bilateral renal artery stenosis and essential hypertension, who were off antihypertensive therapy for at least two weeks are shown in Figure 1.

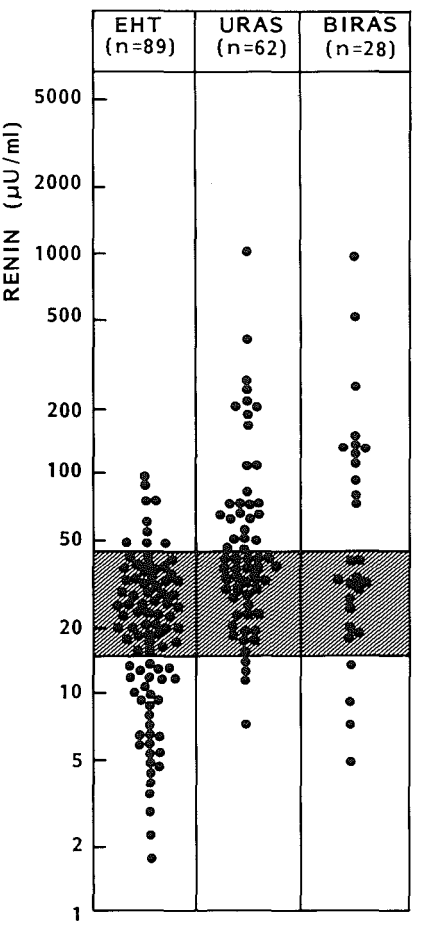

Figure 1: Active renin in the peripheral vein of patients who were off antihypertensive therapy for at least 2 weeks. EHT = essential hypertension. URAS = unilateral renal artery stenosis. BIRAS =bilateral renal artery stenosis.

The normal renin level in our laboratory is between 15-45 μU/ml. Basal renin did not discriminate for renal artery stenosis. Half of the patients with renal artery stenosis had renin values within the normal range. The predictive values of a positive test (renin ≥ 45 μU/ml) and a negative test are given in Table 1.

TABLE 1. Diagnostic value of different tests

Test criterion	Specificity	Sensitivity	P(D+T+)	P(D-T-)
Renin before captopril (≥ 45 μU/ml)	0.43	0.90	0.81	0.61
Decrease in systolic BP one hour after captopril (≥ 30 mm Hg)	0.66	0.77	0.74	0.68
Renin one hour after captopril (≥ 200 μU/ml)	0.84	0.93	0.92	0.81
Decrease in systolic BB x renin after captopril (≥ 5000 mm Hg.μU/ml)	0.85	0.92	0.94	0.81

P(D+T+) = predictive value of positive test = chance for someone with positive test (T+) to have renal artery stenosis (D+).
P(D-T-) = predictive value of negative test = chance for someone with negative test (T-) to have essential hypertension (D-).

Captopril test

The decrease in blood pressure in response to captopril cannot be used as a diagnostic test (Figure 2 and Table 1).

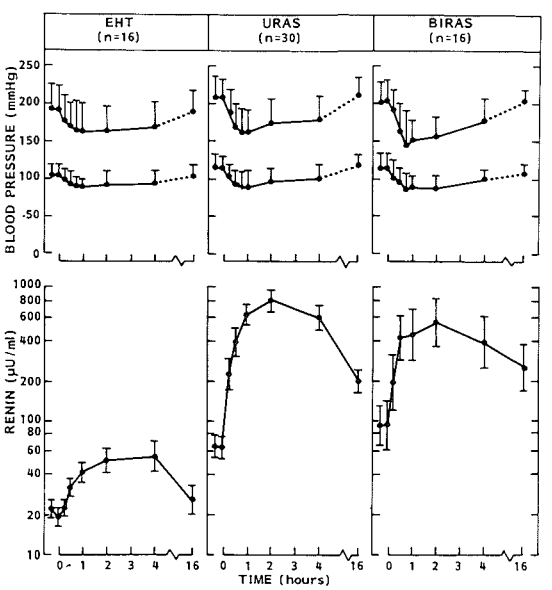

Figure 2: Effects (mean±SEM) of a single dose of oral captopril, 50 mg,on blood pressure and peripheral vein active renin. EHT = essential hypertension. URAS = unilateral renal artery stenosis.BIRAS = bilateral renal artery stenosis.

The best criterion for predicting renal artery stenosis was a plasma renin level of more than 200 μU/ml one hour after captopril (Figure 3 and Table 1). The predicted value of a positive test was 0.92, and the predictive value of a negative test was 0.81 (Table 1). Combining the renin levels 1 hour after captopril with the blood pressure response caused no improvement in predictive values (Table 1).

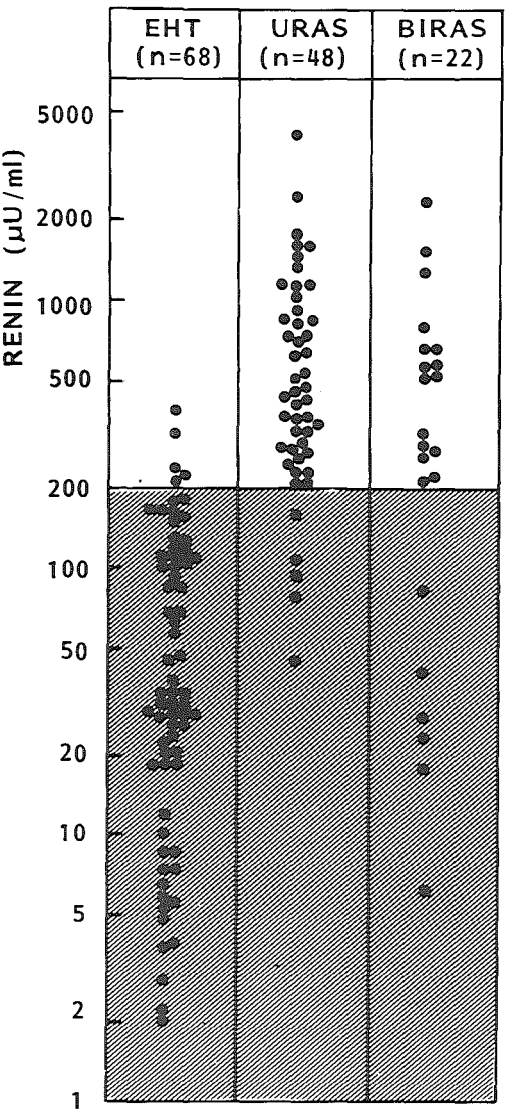

Figure 3: Active renin in the peripheral vein 1 hour after 50 mg captopril.EHT = essential hypertension. URAS = unilateral renal arterystenosis. BIRAS = bilateral renal artery stenosis.

Discussion

The diagnostic specificity of measurements of the peripheral vein renin under basal conditions is low, even when these conditions are carefully standardized as in this study. Also the blood pressure response to captopril is not of much diagnostic value. Both the specificity and the sensitivity of this effect were low. The discriminative power of peripheral vein renin measurements are improved when the blood samples are taken after stimulation of renin release by captopril. Renin values one hour after this drug showed little overlap between patients with renal artery stenosis and those with essential hypertension.

In our population of patients with severe or therapy-resistant hypertension the prevalence of renal artery stenosis was 50%.

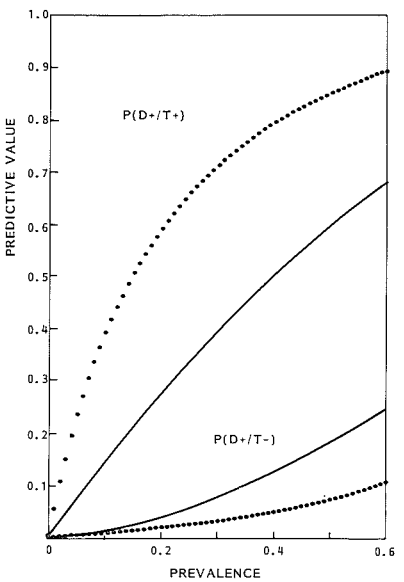

Figure 4: Relationship between prevalence of renal artery stenosis and predictive values of renin (closed lines) and captopril renin (dotted lines). P(D+/T+) = change for someone with positive test(renin ≥ 45 μU/ml; captopril renin ≥ 200 μU/ml) to have renal artery stenosis. P(D+/T-) = chance for someone with negative test to have renal artery stenosis. Note that the predictive values of plasma renin greatly improved after stimulation of renin by captopril. Calculations were made on the basis of a specificity of 0.84 and a sensitivity of 0.93. Prevalence of renal artery stenosis in our patient population however was high, approximately 50% (see also Table 1). D+ = Disease (renal artery stenosis) present; D- = Disease (renal artery stenosis) absent; T+ = Test positive; T- = Test negative.

Figure 4 shows a computer-calculated relationship (6) between the prevalence of renal artery stenosis and the predictive value of a positive captopril test (≥ 200 μU/ml). It can been seen that in a population with a prevalence of 30%, the predictive value of a positive test would be 0.83. In the same case the predictive value of a negative test would be 0.72. These are still acceptable values.

Our results indicate that the captopril test is useful for selecting patients for renal angiography.

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CAPTOPRIL TREATMENT DOES NOT IMPROVE RENAL VEIN RENIN LATERALIZATION. MEASUREMENT OF RENIN WITH MONOCLONAL RENIN ANTIBODIES.

ABSTRACT

The renal vein-to-artery (V/A) renin ratios were determined in 160 patients with essential hypertension (EHT) and 116 with unilateral renal artery stenosis (URAS). Drug treatment was stopped for two weeks in 76 EHT and 75 URAS. The remaining patients were on captopril 50 mg tid for 14 days. Renin was measured by direct asay using monoclonal renin antibodies as well as indirect assay. The renal V/A renin ratios obtained with the two assays were not different. In untreated EHT V/A was 1.285 ± 0.029 (right kidney, mean $\pm$ sem) and 1.296 ± 0.028 (left). In untreated URAS it was 3.008 ± 0.211 on the affected side and 1.049 ± 0.028 contralaterally. With captopril treatment peripheral plasma renin rose by a factor of 3 in EHT and by a factor of 7 in URAS. The renal V/A renin ratios on both sides were not altered by captopril treatment, both in URAS and EHT. The renal V/A difference rather than V/A ratio is a measure of renal secretion of renin. Provided the clearance rate of renin is not altered by captopril the V/A ratio will only increase after this drug when renal plasma flow is decreased. Our findings are in accordance with the experience that generally renal perfusion is well maintained during converting enzyme inhibitor treatment.

INTRODUCTION

An elevated renal vein-to-artery (V/A) ratio of renin on the affected side, together with contralateral suppression, is considered to be a useful index of the functional importance of unilateral renal artery stenosis in patients with renovascular hypertension (1-3). The renal V/A ratio of renin is also used for predicting the outcome of surgical correction (4-8). A variety of stimuli of the renal secretion of renin, such as low sodium diet (9,10), head-up tilting (10,11) and treatment with a diuretic (9), vasodilator (12), saralasin (13) or angiotensin-converting enzyme inhibitors (14-16), have been advocated to improve the diagnostic value of V/A renin measurements.

However, the renal V-A difference rather than the V/A ratio is a measure of renin secretion, and there is no a priori reason for expecting V/A to increase after stimulation of renin secretion. The V-A difference cannot be used in clinical practice, because of the large variability of this difference. This variability is partly due to the fact that the standard deviation of repeated renin measurements is large as compared to the V-A difference, which is often small. Whereas the renal V/A will not increase necessarily with an increase in renin secretion, V/A , at a given rate of secretion, will increase with a decrease in renal plasma flow (2). Although renal plasma flow is generally well maintained during treatment

with angiotensin-converting enzyme inhibitors, lowering of the systemic blood pressure by these drugs may impair renal perfusion, particularly when the renal artery is affected by stenotic disease (17).

We therefore decided to perform a prospective study on the usefulness of captopril as a diagnostic tool for increasing the renal V/A ratio of renin in patients with renal artery stenosis. A second issue we wanted to address was the method of renin assay. In most studies the PRA method is used. In this method angiotensin I (ANG I) generated from endogenous renin substrate is measured by RIA. The rate of ANG I generation in this method depends not only on the concentration of renin but also on that of renin substrate. The latter may be decreased due to increased consumption when large amounts of ANG I are produced, as may occur during converting enzyme inhibitor treatment (18). In patients with renal artery stenosis this may lead to an erroneously low V/A ratio on the affected side, particularly, when the renal vein renin level on that side is very high.

We therefore measured ANG I generation in the presence of saturating amounts of exogeneous substrate and compared this *indirect* RIA with a novel *direct* RIA using a monoclonal renin antibody that recognizes renin and does not bind to its precursor, prorenin.

PATIENTS AND METHODS

Patients.

Patients who were referred for the diagnostic work-up of renal artery stenosis were studied. These include:

1) patients, 40 yr or less, with a diastolic blood pressure of at least 105 mm Hg in the absence of any drug treatment, and 2) patients, regardless of age, with a diastolic blood pressure of at least 105 mm Hg despite treatment with at least 3 different classes of antihypertensive drugs.

Patients with a small contracted kidney not caused by renal artery stenosis and patients whose kidneys were affected by pyelonephritis, tuberculosis or radiation were not included. Patients with bilateral renal artery stenosis were also not included. This report deals with data on 276 consecutive cases.

Renal vein blood sampling.

In 151 patients (75 with unilateral renal artery stenosis) antihypertensive drugs were stopped for at least 14 days before the patients were taken into hospital. Captopril was given to 125 patients (41 with unilateral renal artery stenosis) for 14 days in a dose of 50 mg three times daily. After the patients were admitted to the hospital they were put on a diet containing 60-90 mmol sodium per day. After 3 days the renal veins were sequentially catheterized which a single catheter via the Seldinger technique. Arterial blood samples were obtained via a catheter in the abdominal aorta. In patients receiving captopril, renal vein sampling was performed 5-6 hours after dosing. Since plasma renin may change during the catheterization procedure and since it takes some time to move the catheter from one renal vein to the other, the arterial samples were collected at exactly the same time as the corresponding renal vein samples. The position of the catheters was checked by measuring O_2 saturation of blood. Renal angiography was performed immediately after the sampling procedure had been finished. Blood for renin measurements was collected in tubes containing 0.1 volume of 0.13 M trisodium citrate to which soybean trypsin inhibitor (Sigma, St Louis, Mo,USA), 10 mg/ml, had been added. The blood samples were

immediately centrifuged at 3000 g for 10 min at room temperature, and plasma was stored at -20°C. Renal angiography showed that renal artery stenosis (more than 70%) was caused by atherosclerosis in 106 patients and by fibromuscular dysplasia in 10. The remainder had essential hypertension and no abnormality of the renal arteries. The patient data are summarized in Table 1.

Table 1. Patient groups

Diagnosis	Female/Male	Age yr	Duration of hypertension yr
Unilateral renal artery stenosis			
fibromuscular dysplasia	10/0	31.8 ± 3.	2.9 ± 1.0
atherosclerosis	30/76	50.0 ± 1.3	4.0 ± 0.6
Essential hypertension	65/95	46.2 ± 1.1	7.9 ± 0.6

Results are expressed as mean ± sem

Renin assays.

Renin was measured by an immunoradiometric assay using the monoclonal antibodies 3E8 and 4G1 (Diagnostic Pasteur, Marnes la Coquette, France). The characteristics of these antibodies have been described elsewhere (19). Antibody 3E8 recognized both renin and prorenin and was covalently bound to a magnetic solid phase (Magnogel, Diagnostic Pasteur). Antibody 4G1 specifically recognized the catalytic site of active renin and did not bind to prorenin. This antibody was labelled with iodine 125 using the chloramine-T method. Details of this *direct* renin assay have been described elsewhere (20-21). Results are expressed as pg/ml, using highly purified human kidney renin (Ciba Geigy, Basle, Switzerland) as a standard. The MRC human kidney renin standard 68/356 (Medical Research Council, National Institute for Biological Standards and Control, London, UK) was also run in each assay batch. One pg of the Ciba Geigy standard corresponded with 0.7 μU of the MRC standard.

In the enzyme-kinetic assay of renin (*indirect* renin assay) the velocity of ANG I generation, in the presence of saturating amounts of sheep renin substrate, was measured by RIA of ANG I (22). Results are expressed as μU of the MRC standard per ml. The renal vein and artery plasma samples from each patient were always run in the same assay batch.

RESULTS

Comparison between direct and indirect renin assays.

The direct assay of renin is less sensitive than the indirect assay, the lower limit of detection being 7 μU/ml (10 pg/ml) and 0.4 μU/ml respectively. For a plasma pool with a renin level of 25 pg/ml, the within- and between-assay variability coefficient of variation (CV) of the direct assay was 11 and 16% respectively, and for a plasma pool with high renin content, 90 pg/ml, it was 6 and 8%, which is similar to the CV of the indirect assay.

The results of the direct and indirect renin assays in the patients with renal artery stenosis and essential hypertension are presented in Fig.1.

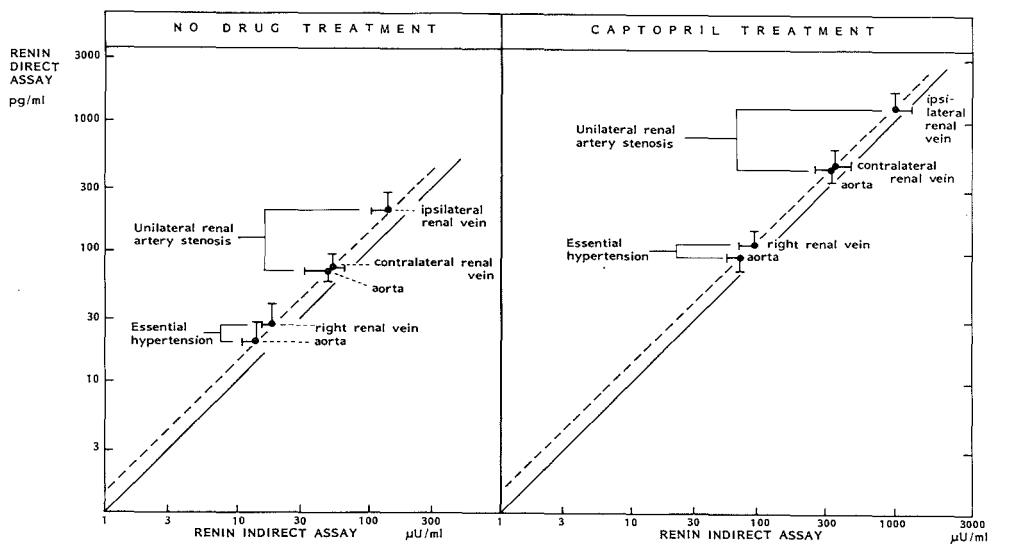

Fig.1. Correlation between the results of direct and indirect renin measurements in patients with unilateral renal artery stenosis or essential hypertension. In the direct assay renin was measured by an immunoradiometric technique using highly specific monoclonal renin antibodies. In the indirect assay renin was measured by the enzyme-kinetic method and the generated angiotensin I was quantitated by RIA. The renal artery stenosis group consisted of 16 patients on captopril, 50 mg tid for two weeks, and 16 on no drugs. The essential hypertension group consisted of 12 patients on captopril and 12 on no drugs. Continuous line is the line of identity. Results are expressed as mean and sem.

By measuring renin both with the direct and the indirect assay, the enzymatic activity of renin can be expressed as μU per pg. From Fig.1 it appears that, in patients with unilateral renal artery stenosis, renin, expressed in this way, in the ipsilateral renal vein was not different from that in the contralateral vein and in the aorta, and this was also true for patients with essential hypertension. Thus, there was no evidence for the existence of substances in plasma interfering with the action of renin on its substrate. The V/A renin ratios calculated from the results of the direct assay were not different from those of the indirect assay (Fig.2).

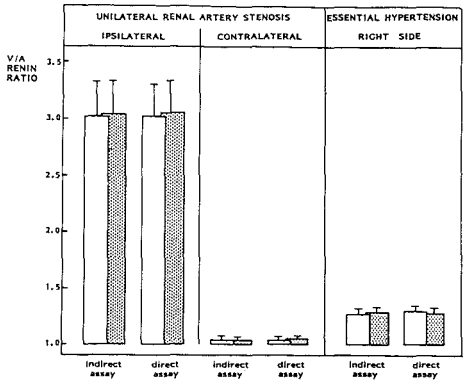

Fig.2. The renal vein-to-artery (V/A) renin ratio in patients with unilateral renal artery stenosis or essential hypertension. The V/A ratios were calculated from the results of both the direct and indirect renin assays. Closed bars: patients on captopril, 50 mg tid for two weeks. Open bars: patients on no drugs. For numbers of patients see legend of Fig.1. Results are expressed as mean ± sem.

Effect of captopril on the V/A ratio of renin.

The effects of two-week captopril treatment on peripheral vein renin and on serum creatinine, creatinine clearance and blood pressure in patients with renal artery stenosis or essential hypertension are summarized in Table 2.

Table 2. Effects of captopril

Diagnosis	Capto-pril	Number of patients	Serum creatinine µmol/l	Creatinine clearance ml/min	Blood pressure systolic mm Hg	Blood pressure diastolic mm Hg	Peripheral plasma renin µU/ml
Renal artery stenosis	no	75	112 ± 4	76 ± 3	192 ± 2	114 ± 2	53.2(21.0-135)
	yes	41	134 ± 8*	63 ± 4*	163 ± 3**	99 ± 2**	359(124-1040)**
Essential hypertension	no	76	102 ± 5	83 ± 4	191 ± 6	116 ± 4	17.6(7.1-43.8)
	yes	84	122 ± 9*	70 ± 5*	162 ± 5*	102 ± 4**	50.6(13.1-195)**

Data on creatinine and blood pressure are expressed as mean ± sem. Statistical analysis for the renin measurements was performed after logarithmic transformation of the results in order to obtain a Gaussian distribution. Results are expressed as geometric mean (between brackets geometric mean minus SD and geometric mean plus SD).
* P<0.01, ** P<0.001 from untreated patients (unpaired t-test)

Captopril caused a decrease in creatinine clearance, a fall in blood pressure and a rise in renin. The effects on creatinine and blood pressure were similar in the two groups.
In contrast, the increase in renin was much greater in the patients with renal artery stenosis.

The best trade-off between sensitivity and specificity of measurements of peripheral plasma renin for predicting renal artery stenosis was determined from a Receiver Operator Characteristic Curve. The best trade-off was obtained with a renin level of 45 μU/ml or higher in the untreated patients and 200 μU/ml or higher in the captopril group.
The sensitivity of measurements of unstimulated peripheral plasma renin as a test for predicting renal artery stenosis was 0.49 with a specificity of 0.89. The sensitivity of measurements of captopril-stimulated renin to predict renal artery stenosis was 0.70 and the specificity 0.84. Thus, when using peripheral renin as a criterion, it is the sensitivity of this test that is markedly improved by captopril treatment.

In essential hypertension the V/A renin ratio of the right kidney was 1.285 ± 0.029 (mean ± sem) in the untreated patients and 1.298 ± 0.030 in the group treated with captopril (Fig.2).The V/A renin ratio on the left side was not different from that on the right. The V/A renin ratio was 3.008 ± 0.211 on the affected side and 1.049 ± 0.028 contralaterally in the untreated patients with unilateral renal artery stenosis (Fig.2)

In the captopril-treated patients the V/A renin ratio was 2.991 ± 0.215 and 1.035 ± 0.017 respectively, which again was not different from the V/A ratios in the untreated patients. A V/A renin ratio on the affected side of 1.50 or more is considered to indicate renal artery stenosis on that side (1-3). Figure 3 shows that 93% of the patients with renal artery stenosis satisfied this criterion on the affected side, as compared to only two patients (2%) on the unaffected side. In contrast, 17% of the patients with essential hypertension had a V/A ratio of 1.50 or more on the right side and 16% had this increased ratio on the left side. If one defines contralateral suppression as a V/A ratio of 1.10 or less, 77% of the renal artery stenosis patients had contralateral suppression, whereas 18% of the patients with essential hypertension had a suppressed ratio on the right side and 20% on the left.

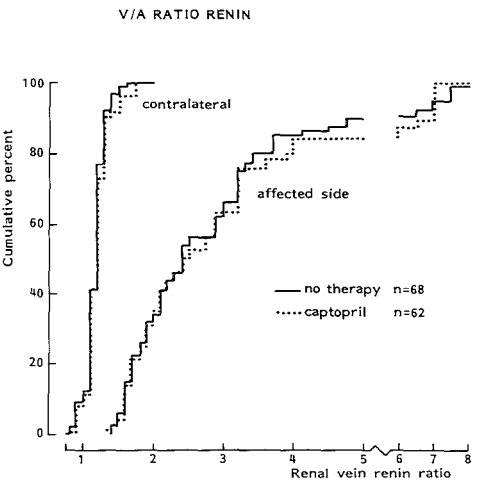

Fig.3. The cumulative distribution curve of the V/A renin ratio in patients with unilateral renal artery stenosis. Broken line: patients (n=41) on captopril, 50 mg tid. Continuous line: patients (n=75) on no drugs.

DISCUSSION

In this study we employed a new immunoradiometric assay to measure renin. This direct assay of renin makes use of a highly specific monoclonal antibody that recognizes active renin but not prorenin (19). This assay has some advantages over the indirect assay: 1) The procedure does not involve an incubation step for generating ANG I; the problems of incomplete recovery of ANG I and non-optimal pH during incubation are therefore circumvented, 2) results are not influenced by the concentration of renin substrate (23), and 3) one technician can easily handle up to 100 samples in one day; the indirect renin assay takes at least two days.

A disadvantage of the direct renin assay is its lower sensitivity, which makes it difficult to measure low-normal levels. In the case of renal vein measurements this problem can be solved by stimulating the secretion of renin, for instance with captopril, prior to sampling. With such stimulation both types of assay produce comparable results.

With captopril treatment we found a 6- to 7-fold increase in peripheral vein renin in patients with unilateral renal artery stenosis. In these patients nearly all renin is secreted by the affected kidney and, because the increase in peripheral renin is caused by a parallel increase in renin secretion, the V/A renin ratio of this kidney will not be altered by captopril as long as renal plasma flow remains constant. Several authors , including ourselves (15, 16, 22), found an increase of the V/A ratio 30-60 min after captopril administration. One of the reasons might be that a steady state had not been reached in these acute studies. Another reason for an increase of the renal V/A renin ratio in the first 60 minutes is the precipitous fall in blood pressure that occurs in some patients. This may cause a reduction in renal plasma flow and thereby an increase in the V/A renin ratio.

Some investigators used the renal vein renin ratio between the affected and non-affected side instead of the V/A renin ratios (15). There is however a steep rise of renin during the first 60 minutes after captopril administration (22), and it takes some time to maneuver the catheter from one renal vein to the other. Therefore it is extremely important that the renal vein and artery plasma samples are drawn at exactly the same time. Instead of the renal artery one can use the forearm vein.

Our study demonstrates that the V/A renin ratio of the contralateral kidney remains suppressed during chronic angiotensin-converting enzyme inhibition. The suppression is therefore unlikely to be solely caused by feed-back suppression of renin secretion by angiotensin II. It is also unlikely that the unaffected kidney produces a renin inhibitor because we found the enzymatic activity of renin, expressed as μU per pg of renin, in the renal vein of that kidney not to be different from that of pure kidney renin.

In our group of patients with renal artery stenosis more than 90% had a renal V/A renin ratio on the affected side of 1.50 or more. Thus, renal vein renin measurements are of limited use as an adjunct to renal angiography for detecting renal artery stenosis, at least in patients with unilateral disease. It is however possible that these measurements have some predictive value with respect to the response to surgery or percutaneous transluminal angioplasty, in the sense that a higher V/A ratio may predict a higher probability of a successful outcome.

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CHAPTER 5

ACE INHIBITION AND TWENTY-FOUR BLOOD PRESSURE PROFILES

Twenty-four blood pressure profiles during chronic ACE inhibition. A comparative study of twice-daily captopril versus once-daily enalapril in patients with essential hypertension.

Blankestijn PJ, Wenting GJ, Brouwer RML, Van den Meiracker AH, Schalekamp MADH.
Journal of Hypertension 1989; in press.

Twenty-four hour blood pressure profiles during chronic ACE inhibition. A comparative study of twice daily captopril versus once daily enalapril in patients with essential hypertension

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INTRODUCTION

The angiotensin converting enzyme (ACE) inhibitors, captopril and enalapril, have undoubtedly proved to be effective agents for the treatment of essential hypertension. However, the two agents differ in several ways: the nature of the molecule's adherence to the active site on the converting enzyme, the form in which the agent is administered (active compound or prodrug), and the kinetics of elimination. The binding of captopril to the converting enzyme by means of its sulphydryl group is rather loose, its absorption after oral administration is fast but influenced by food, and its elimination half-life from plasma is short (for review, see Williams 1988). Enalapril scores better on all these points but, nevertheless, there is little evidence that these differences are of any clinical significance.

Current trends favour twice-daily administration of captopril and once-daily dosing of enalapril. The results of a European multicentre trial (Garanin 1986) even suggested that once-daily captopril was as effective as once-daily enalapril. A criticism of this and other studies could be the fact that blood pressures were only measured 21–27 hours after dosing and that increasing doses were used. In the present study, an automatic ambulatory blood pressure recording device was used to compare, in the same patients, a twice-daily dosing regimen of captopril with a once-daily dosing regimen of enalapril. Treatment order was randomised and a fixed dosage ratio of 4:1 was used to compare captopril and enalapril.

PATIENTS AND METHODS

PATIENTS AND STUDY PROTOCOL

Ten patients with essential hypertension (aged 39–64 years, eight men) controlled on captopril (50–200 mg/day) or enalapril (10–20 mg/day) monotherapy participated in this single-blind, crossover study, which was conducted in the Hypertension Outpatient Clinic. After they had given their informed consent to the study protocol, they were randomised (five patients in either group) to receive twice-daily captopril (morning dose 37.5 mg, evening dose 37.5 mg) or once-daily enalapril (morning dose 20 mg, evening dose matching placebo). Each patient received his/her drug regimen for eight weeks before the first 24-hour ambulatory blood pressure monitoring was undertaken. Following this procedure the patients were switched to the other ACE inhibitor, and eight weeks after the crossover they were re-studied over a 24-hour period to assess blood pressure control. Drug administration was standardised to between 07.00–08.00 hours (morning dose) and 18.00–19.00 hours (evening dose). Within these time frames, the patients were requested to take the medication with a constant relationship to breakfast or evening meal. Tablet counts were performed at two-week intervals to verify compliance. No other antihypertensive drugs were added during the treatment period.

BLOOD PRESSURE MEASUREMENTS

After the eight-week treatment periods, blood pressure was measured with a London School of Hygiene sphygmomanometer. Patients rested supine for 10 minutes before measurements were made, and an average was obtained from three readings. These measurements were performed around 08.00 hours, 12 hours after the last drug dose. After the patients had taken their morning dose, the ambulatory blood pressure monitoring equipment (Spacelabs 5300 Monitor, Spacelabs Inc., Hillsboro, Oregon, USA) was fixed to the patients. At the start and end of these measurements, blood pressure was measured simul-

taneously with a standard sphygmomanometer connected to the ambulatory equipment via a T-tube, to verify proper function of the equipment and to ensure that no gross alteration had occurred in the ability of the device to read blood pressure during its use. The Spacelabs 5300 Monitor makes use of an occlusion cuff and a microphone or oscillometer to detect systolic and diastolic blood pressure. The microphone was taped over the brachial artery and, whenever the microphone failed to identify Korotkoff sounds the oscillometer intervened. The cuff is automatically inflated by a minipump fastened at the subject's waist, and blood pressure data are stored in digit format on a RAM package by a built-in computer. The data from this memory were fed into a computer which produced a printout of all measurements made. The data were averaged over periods of one hour. The device was programmed to measure blood pressure and heart rate every 15 minutes from 06.00 to 24.00 hours and every 30 minutes from 24.00 to 06.00 hours. At the time of each measurement, the patients were instructed to 'freeze' their activities and keep their arm motionless. Each patient was studied twice (captopril, enalapril) on the same day of the week (eight weeks apart) and the timing of the study was always the same.

STATISTICAL ANALYSIS

Results are expressed as the mean and standard error of the mean. Resting office measurements were analysed by a Student t-test. A Wilcoxon matched pairs signed rank test was used for comparison of the 24-hourly means of blood pressure and heart rate.

A P value of <0.05 was selected as the level of statistical significance.

RESULTS

The mean of casual office blood pressures and heart rates during captopril and enalapril treatment were not significantly different (Table 1).

The 24-hourly means of systolic and diastolic pressures are shown in Fig. 1. In the presence of captopril or enalapril, diurnal variations in blood pressure and heart rate were maintained. However, systolic and diastolic pressures were significantly lower during enalapril than during captopril treatment (systolic pressure: $P<0.0015$, diastolic pressure: $P<0.003$). Heart rate showed no difference between the two treatment groups.

DISCUSSION

This study compared the effects of captopril and enalapril treatment in patients with essential hypertension using a randomised, crossover, single-blind study design. The principal measure of treatment efficacy was its effect on 24-hour blood pressure profiles using an ambulatory non-invasive blood pressure measuring device. Unexpectedly, the results of this study demonstrated better blood pressure control with once-daily enalapril than with twice-daily captopril. Thus, using the doses, equipment and regimens studied in this small pilot trial, enalapril appeared to be superior to captopril. Enalapril caused a more sustained and consistent blood pressure reduction than captopril, particularly during late afternoon and evening. The dose of captopril used and the twice-daily administration were, however, sufficient to reduce blood pressure at night and during the (early) morning, 12 hours after the last dose. Looking at the 24-hour blood pressure curves, the given impression is that the effects of the evening dose of captopril (around 18.00 hours) are postponed until the evening.

How can this 'delay' in efficacy be explained? The present study gives no definite answer because no measurements were made of plasma drug levels or of plasma converting enzyme activity. However, it is tempting to relate this temporary lack of blood pressure control to an effect of food intake on the amount and/or rate of drug absorption (in Holland, the principal meal is the evening meal). It has been shown by Singhvi et al (1982) that food can reduce the

Table 1 Results of measurements of casual office blood pressure (by the London School of Hygiene sphygmomanometer) and heart rate (n=10, mean ± SEM)

	Captopril	Enalapril	P value
Systolic arterial pressure (mmHg)	134 (5)	129 (4)	NS
Diastolic arterial pressure (mmHg)	85 (4)	85 (3)	NS
Heart rate (beats/min)	78 (3)	78 (3)	NS

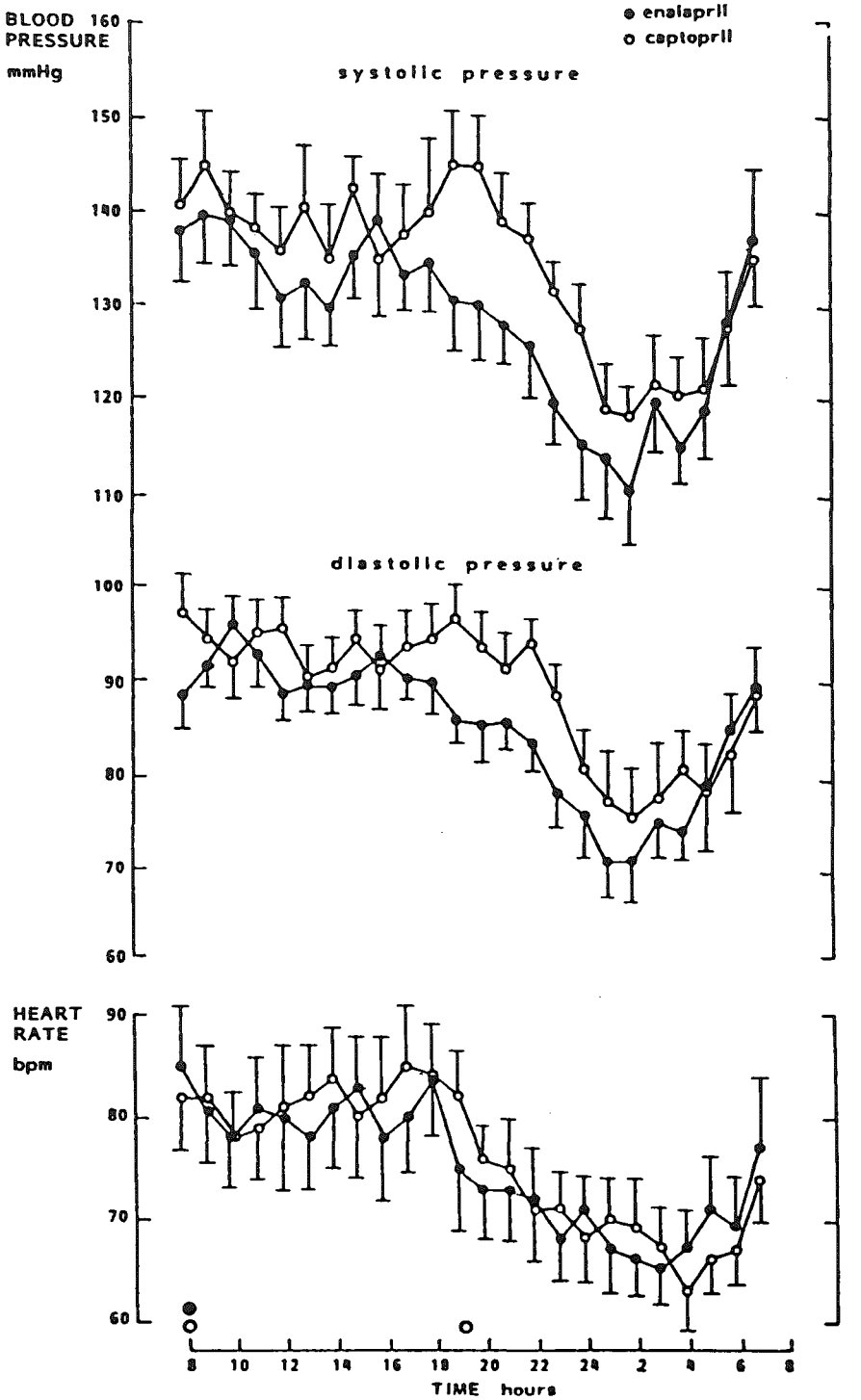

Fig. 1 Twenty-four hour blood pressure profiles during captopril and enalapril treatment. Data are hourly means ± SEM.

absorption of isotope-labelled captopril by 35–40% Diminished or delayed absorption could be of critical importance, especially when using a low-dose regi men, as in the present study. Öhman et al (1985) studied the effects of concomitant food intake on pharmacokinetics and blood pressure control during chronic low-dose captopril administration. Peak captopril plasma concentrations and area under the curve of total and non-protein bound captopril were clearly reduced with food intake, but this did not affect blood pressure control. However, the patients in this study were also treated with bendroflumethiazide which may have obscured the relationship between plasma captopril concentrations and blood pressure reduction. Salvetti et al (1985) showed that, in patients with uncomplicated essential hypertension, the timing of captopril administration (one hour before or immediately after eating) did not modify the haemodynamic and humoral effects of chronic captopril (50 mg twice daily). However, measurements were only made at a single moment in time, 12 hours after the last dose of captopril, which could be insufficient.

How can our findings be related to other comparative studies of captopril and enalapril? A review of the literature revealed seven papers. However, these reports cannot easily be compared with the present study because of methodological differences (parallel group design, no fixed dosage ratio's of the two agents, ambulatory blood pressure monitoring in only one small study). Chrysant et al (1985), Lewis et al (1985), and Thind et al (1985) followed more or less the same parallel group study design in which captopril (25 mg thrice daily) or enalapril (5 mg twice daily) was added (double-blind) to existing therapy with hydrochlorothiazide. Thind et al (1985) found lower blood pressures during enalapril treatment, but this observation was not confirmed by the other two groups. Vlasses et al (1986) compared a high ceiling dose of captopril (200 mg twice daily) with enalapril (20 mg twice daily). They found similar antihypertensive effects. Co-administration of captopril and enalapril led to no further reduction in blood pressure, which is not surprising in view of the high doses used.

As in the present study, Karlberg et al (1986) used a randomised, crossover design for comparison of captopril (twice daily 25 mg, 50 mg) with enalapril (once daily 20 mg, 40 mg). Enalapril caused a more sustained and consistent blood pressure reduction than captopril. Our ambulatory blood pressure data confirm and extend these casual blood pressure measurements. Shionoiri et al (1987) are the only authors who recorded ambulatory intra-arterial pressures before and during therapy with captopril or enalapril. As could be expected, both drugs lowered blood pressure, but the design of the study and the small number of patients included do not allow comparison between captopril and enalapril.

In conclusion, 24-hour blood pressure monitoring can unmask differences which can not be detected by casual readings. In the doses used in this study, enalapril clearly resulted in a better 24-hour control of blood pressure than did captopril. With captopril, vigilance regarding minimal effective dosage intervals should be maintained.

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CHAPTER 6

SEROTONIN, KETANSERIN AND BLOOD PRESSURE

Treatment of hypertension with ketanserin, a new selective 5-HT_2 receptor antagonist.

Wenting GJ, Man in 't Veld AJ, Woittiez AJJ, Boomsma F, Schalekamp MADH.
British Medical Journal 1982; **284**: 537-539.

Chronic effect of ketanserin in mild to moderate essential hypertension.

Woittiez AJJ, Wenting GJ, Van den Meiracker AH, Ritsema van Eck HJ, Man in 't Veld AJ, Zantvoort F, Schalekamp MADH.
Hypertension 1986; **8**: 167-173

Treatment of hypertension with ketanserin, a new selective 5-HT_2 receptor antagonist

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Abstract

The new selective 5-HT_2 receptor blocking agent ketanserin was given in a dose of 10 mg intravenously to 12 patients with essential hypertension. It caused a distinct fall in supine systemic arterial, right atrial, pulmonary artery, and pulmonary capillary "wedge" pressures. Cardiac output, renal blood flow, and glomerular filtration rate showed no persistent changes. Thus 5-HT_2 receptor blockade caused dilatation of both the resistance and capacitance vessels and of the renal vascular bed. Heart rate and plasma concentrations of renin and noradrenaline rose after ketanserin.

These data suggest that 5-HT may have a role in maintaining high blood pressure.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has long been recognised as a potent vasoactive agent.[1] It appears to act mainly as an amplifier of other vasoconstricting agents such as noradrenaline and angiotensin II.[2] 5-HT may be implicated in the pathogenesis of hypertension, but its role is obscured by its multifaceted actions in healthy individuals.[1] Furthermore, the lack of specific antagonists has hampered the precise delineation of 5-HT's

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role in blood pressure regulation. Recently Peroutka and Snyder, using radioligand studies, distinguished two distinct 5-HT receptors.[3] Binding to the 5-HT_1 receptor has so far not been related to any effect of 5-HT, but binding to the 5-HT_2 receptor correlates with both in-vitro and in-vivo pharmacological and physiological effects of 5-HT.[3 4] A specific 5-HT_2 receptor antagonist, ketanserin, is now available which in therapeutic doses does not block the effect of amines other than 5-HT. It appears to be free of the central side effects associated with less specific 5-HT antagonists.[5] This has prompted us to investigate the haemodynamic effects of this new compound in patients with essential hypertension.

Patients and methods

Twelve patients were studied, eight men and four women, aged 59 ± (SEM) 4 years (range 40 to 77 years). The diagnosis of essential hypertension was established by routine screening, including intravenous urography. Antihypertensive treatment, if any, was stopped three weeks before the study. The use of a new, intravenous antihypertensive agent was explained to the patients, who all gave their informed consent to the study.

They were admitted to a metabolic ward and received a diet with a constant sodium (40-50 mmol/day) and potassium (70-100 mmol/day) content. When blood pressure and sodium balance were stable the patients were investigated in the cardiovascular laboratory after an overnight fast. A Swan-Ganz catheter was introduced percutaneously in an antecubital vein and positioned in the pulmonary artery. A short polyethylene catheter was placed in a radial artery. A peripheral vein in the opposite arm was cannulated for renal function studies. Baseline haemodynamic measurements and blood sampling were started at least 90 minutes later. Ketanserin, 10 mg in 20 ml of saline, was infused in the right atrium in three minutes and the effects were followed for two hours. Right atrial pressure, pulmonary artery pressure, arterial pressure, and heart rate were continuously monitored and recorded. Mean pressures were obtained by electronic integration, and the mid-axillary line was defined as the zero-pressure reference level. The patients remained supine throughout the study. Cardiac output and mean pulmonary capillary "wedge" pressure were measured before, five minutes after the end of the ketanserin infusion, and then every 30 minutes. Triplicate measurements of cardiac output using the thermodilution technique (10 ml 5% dextrose in water at 1-2°C) were averaged. The following haemodynamic values were derived: total peripheral vascular resistance = (mean arterial pressure − right atrial pressure)/cardiac output (kPa/s/l); pulmonary vascular resistance = (mean pulmonary artery pressure − mean pulmonary capillary wedge pressure)/cardiac output (kPa/s/l). Using a continuous infusion technique[6] we estimated the effective renal plasma flow and glomerular filtration rate by clearance of ^{131}I-hippuran and ^{125}I-thalamate respectively. Renal blood flow was calculated using the

venous packed cell volume and assuming 75% renal extraction of hippuran. Renal vascular resistance was calculated as the mean arterial pressure/renal blood flow (kPa/s/l). Well-established methods were used to measure plasma concentrations of active renin[7] and noradrenaline.[8] Statistical analyses were performed using Student's *t* test for paired data.

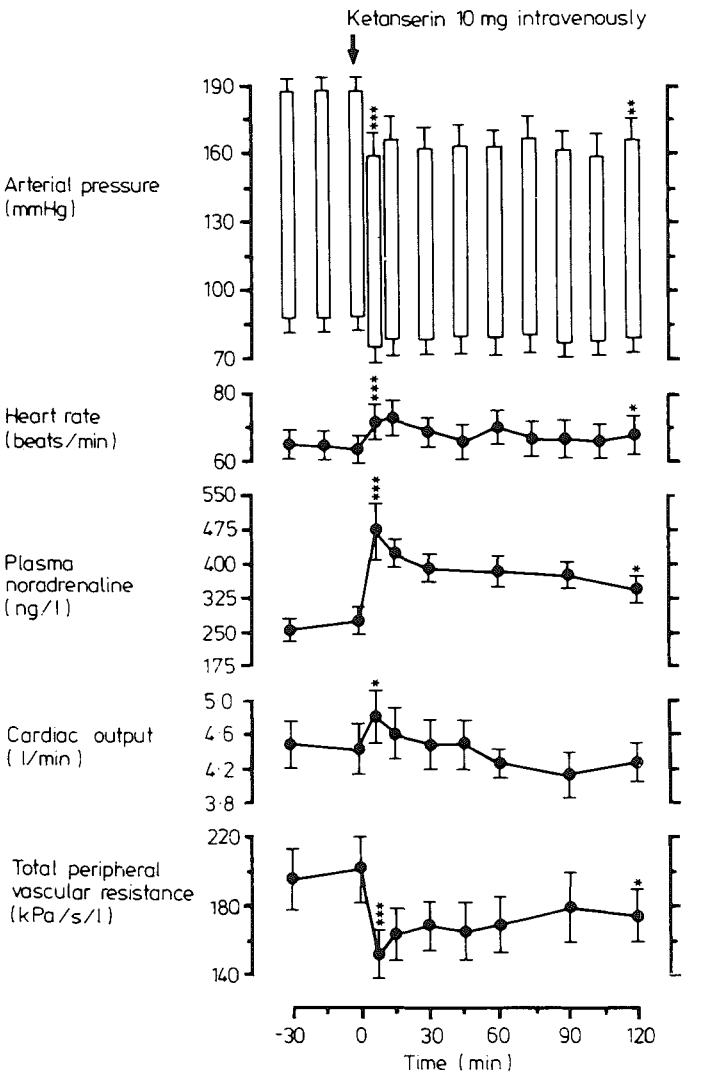

FIG 1—Effects of ketanserin on arterial pressure, heart rate, plasma noradrenaline, cardiac output, and total peripheral vascular resistance in 12 patients with essential hypertension.
*$p<0{\cdot}05$; **$p<0{\cdot}01$; ***$p<0{\cdot}001$.

Conversion: SI to traditional units—Vascular resistance: 1 kPa/s/l $\approx$ 10 dyn/s/cm^5.

Effects of ketanserin on mean arterial pressure and renal function in 12 patients with essential hypertension

	Baseline	After ketanserin infusion			
		1 h	p Value	2 h	p Value
Mean arterial pressure (mm Hg)	121 ± 5	102 ± 7	< 0·001	106 ± 7	< 0·001
Glomerular filtration rate (ml/min)	90 ± 5	92 ± 5	NS	90 ± 5	NS
Effective renal plasma flow (ml/min)	302 ± 18	290 ± 17	NS	296 ± 21	NS
Renal vascular resistance (kPa/s/l)	1368 ± 102	1202 ± 97	< 0·05	1284 ± 110	NS
Plasma active renin (mU/l)*	9·5 ± 2·0	14·9 ± 3·4	< 0·01	15·3 ± 2·8	< 0·01

*Normal range 15-45 mU/l.
Conversion: SI to traditional units—Vascular resistance: 1 kPa/s/l ≈ 10 dyn/s/cm^5.

Results

Systolic and diastolic pressures fell from 188 ± 6/89 ± 5 mm Hg to 159 ± 9/75 ± 6 mm Hg five minutes after the end of the infusion of ketanserin (fig 1). Arterial pressure remained significantly below baseline level during the two-hour observation period. One patient had symptomatic hypotension; his arterial pressure fell from 177/85 mm Hg to 82/43 mm Hg within five minutes, but by 30 minutes it had risen to 105/57 mm Hg. Both the initial and the sustained fall in arterial pressure were due to a drop in total peripheral vascular resistance. Heart rate and cardiac output rose, but in contrast to heart rate, the cardiac output increased only transiently (fig 1). The rise in heart rate was accompanied by an increase in plasma noradrenaline concentration. Ketanserin caused a fall in the filling pressures of both ventricles (fig 2). Pulmonary artery pressure also fell, but this was probably not due to a direct vasodilating effect of ketanserin on the pulmonary vascular bed, as pulmonary vascular resistance did not change. The effects of ketanserin on renal function are shown in the table. Renal vascular resistance fell significantly, but there were no major changes in effective renal plasma flow and glomerular filtration rate. Although basal active renin concentration was low it rose significantly.

Discussion

5-HT is released by aggregating platelets during clotting and it contributes to local vasoconstriction.[2] It is also released by enterochromaffin cells, where it increases blood flow to exocrine glands of the gut.[9] Apart from such local actions endogenous 5-HT has not been thought to have systemic haemodynamic effects.[1] This view, however, is now being challenged by preliminary results with ketanserin, a newly developed specific 5-HT_2 receptor antagonist.[10]

Our data show that ketanserin exerted its hypotensive action by lowering total peripheral vascular resistance. The fall in right atrial pressure suggested that it also had an effect on venous tone. The responses of the heart rate and plasma noradrenaline concentrations were probably baroreflex-mediated. Reflex sympathetic stimulation may also have caused the transient rise in cardiac output. This effect might have been overruled by the fall in venous return and the reduction of preload. Vasodilatation also occurred within the kidneys, and, despite a fall in arterial pressure, the glomerular filtration rate did not diminish. The anti-

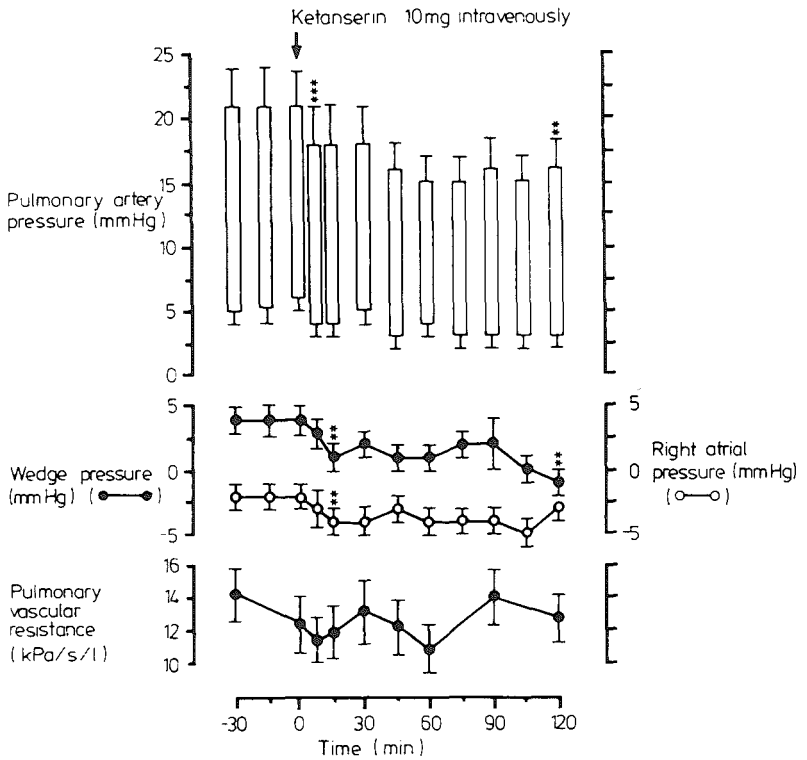

FIG 2—Effects of ketanserin on pulmonary artery pressure, wedge pressure, right atrial pressure, and pulmonary vascular resistance in 12 patients with essential hypertension.
**p < 0·01; ***p < 0·001.
Conversion: SI to traditional units—Vascular resistance: 1 kPa/s/l ≈ 10 dyn/s/cm^5.

hypertensive action of ketanserin is thus characterised by a favourable haemodynamic profile. The observation that ketanserin lowered not only systemic arterial pressure but also cardiac filling pressures suggests that this balanced vasodilatation may be of particular interest for the treatment of congestive heart failure. Indeed, a favourable response to ketanserin in this condition has been reported recently.[11]

5-HT not only acts as a direct vasoconstrictor, but it also amplifies the vasoconstrictor responses to agents such as noradrenaline and angiotensin II.[2] 5-HT is released by aggregating platelets in atherosclerotic arteries, which are abnormally responsive to this amine.[12] Ketanserin antagonises not only the direct vasoconstrictor effect of 5-HT but also its amplifying effects on other vasoactive substances.[5] These mechanisms may all be implicated in the haemodynamic effects of 5-HT$_2$ receptor

blockade by ketanserin. This compound is thus a new therapeutic tool for investigating the role of 5-HT in the pathogenesis of various forms of hypertension. Experience so far warrants further assessment of its place in the management of hypertension.

We thank Dr J Symoens of Janssen Pharmaceutica for generous supplies of ketanserin.

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Chronic Effect of Ketanserin in Mild to Moderate Essential Hypertension

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SUMMARY **Ketanserin, an antagonist highly selective for 5-hydroxytryptamine (serotonin) type 2 (S_2) receptors, was given as monotherapy in a dose of 40 mg b.i.d. to 24 subjects with mild to moderate essential hypertension. Its effects were evaluated in a placebo-controlled double-blind crossover study. The effect on blood pressure in 18 subjects was monitored by 24-hour ambulatory intra-arterial measurements. Systolic and diastolic intra-arterial pressures were significantly lowered by ketanserin both during the day and at night, whereas heart rate was unchanged. Cuff pressure readings (triplicate measurements) with the London School of Hygiene sphygmomanometer and an automatic device (12 measurements in 1 hour) in the outpatient clinic also showed a significant effect on both supine and standing pressures. No postural hypotension was noted. Ketanserin had no effect on endogenous creatinine clearance, serum cholesterol levels, and the plasma levels of norepinephrine, renin, and aldosterone. The only side effect that was significantly more common with ketanserin than with placebo treatment was an increase in body weight. Ketanserin may have a place in the treatment of mild to moderate essential hypertension. (Hypertension 8: 167–173, 1986)**

KEY WORDS • serotonin • blood pressure • ketanserin • ambulatory monitoring

SEROTONIN has been implicated in the regulation of blood pressure and pathogenesis of hypertension for more than 2 decades, but its role is still controversial.[1,2] Although central serotoninergic neurons are likely to be involved in blood pressure control, surgical and pharmacological manipulations of these central serotoninergic pathways have produced conflicting results.[3] The effect of intravenously administered serotonin on blood pressure is also notoriously variable because activation of vascular serotoninergic receptors can elicit constriction, dilatation, or a biphasic response, depending on the type of blood vessel, its anatomical location, the animal species, and the concentration of the monoamine. The vascular response is also dependent on sympathetic tone.[4]

Radioligand binding studies on brain tissue have led to a subdivision of S_1- and S_2-serotoninergic receptors.[5] A vascular S_2-receptor has been shown to mediate serotonin-induced constriction. This receptor also appears to be involved in the so-called amplifying effect of serotonin on the pressor responses to norepinephrine and angiotensin II.[6–8] Ketanserin, a serotonin antagonist that is highly selective for S_2-receptors, is now available for clinical research. Herein, we report on a double-blind placebo-controlled crossover study of the blood pressure lowering effect of ketanserin in 24 subjects with mild to moderate essential hypertension. In 18 of these subjects, the effect was evaluated by 24-hour ambulatory intra-arterial monitoring of blood pressure.

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Subjects and Methods

Trial Design

The study comprised 17 men and 7 women, aged 50 ± 3 years (mean ± SEM; range, 24–69 years). A diagnosis of essential hypertension was made after routine screening, which included isotope renography. Antihypertensive treatment, if any, was tapered off 3 weeks before the study began and was replaced by placebo. Hypertension was defined as a cuff measurement of blood pressure in excess of 95 mm Hg diastolic on two or more consecutive visits to the outpatient clinic. After a single-blind placebo run-in period of 4 weeks, the subjects were randomly allocated to receive either placebo or 40 mg of ketanserin

at 0800 and 1800 for 8 weeks, followed by an identical crossover period. The randomization schedule was kept in the hospital pharmacy, and unmarked formulations were provided by Janssen Pharmaceutica (Beerse, Belgium). Neither the investigator nor the subjects were aware of who was receiving the assigned drug.

Throughout the study the subjects were seen in the outpatient clinic at 2-week intervals between 0900 and 1100. Side effects, both volunteered and elicited by direct questioning, were recorded, and compliance was checked by tablet counting. In the last week of the two treatment periods ambulatory blood pressure was monitored by 24-hour continuous intra-arterial measurement. The recordings were taken while the subjects were hospitalized, which allowed their environmental and living conditions to be relatively well standardized, particularly with regard to physical activity, timing of meals, afternoon nap, and night rest. Apart from these periods, the subjects were free to move around. During the hospital stay, blood was taken, with the subjects in the supine position, 1 to 2 hours after the morning dose of placebo or ketanserin for biochemical determinations, including plasma levels of ketanserin and hormones.

The protocol was approved by the Hospital Ethical Review Committee. Informed consent was obtained from all subjects.

Blood Pressure Measurements

Office readings were made at each visit to the outpatient clinic, with the London School of Hygiene sphygmomanometer.[9] Blood pressure was also recorded for 1 hour in each subject with an automatic device (Accutorr TM1, Datascope, Paramus, NJ, USA). Direct 24-hour intra-arterial ambulatory recordings, using the Oxford system,[10] were made in 18 of the 24 subjects.

Measurements with the London School of Hygiene sphygmomanometer were made after 5 minutes of supine rest. Korotkoff phase V was taken as diastolic pressure. Readings were taken at 1-minute intervals, and the arithmetic mean of three measurements was recorded. Measurements were repeated with the subject in the upright position. Pulse rate was counted for 30 seconds in both positions. Mean arterial pressure was calculated as diastolic pressure plus one third of the pulse pressure.

The Accutorr noninvasive blood pressure monitor has been designed for measuring systolic, mean, and diastolic pressure according to the oscillometric method. The microprocessor computes the displayed variables as follows. The systolic pressure is the preset cuff pressure at which the pressure oscillations begin to increase in amplitude during deflation. The mean pressure is the lowest preset cuff pressure at which the oscillations are maximal. The diastolic pressure is the preset cuff pressure at which the oscillations stop decreasing in amplitude. Twelve consecutive measurements were made at 5-minute intervals with the subject in the supine position. The accuracy of the Accutorr was assessed by comparison with direct intra-arterial measurement. Results are shown in Figure 1. In the pressure range tested, the Accutorr slightly underestimated intra-arterial systolic pressure; the mean difference was 7.5 mm Hg (SD = 7.1). The Accutorr-measured diastolic pressure was consistently higher than the intra-arterial value; the mean difference was 11.7 mm Hg (SD = 6.6). These results are comparable with data obtained with the Dinamap oscillometric device.[11]

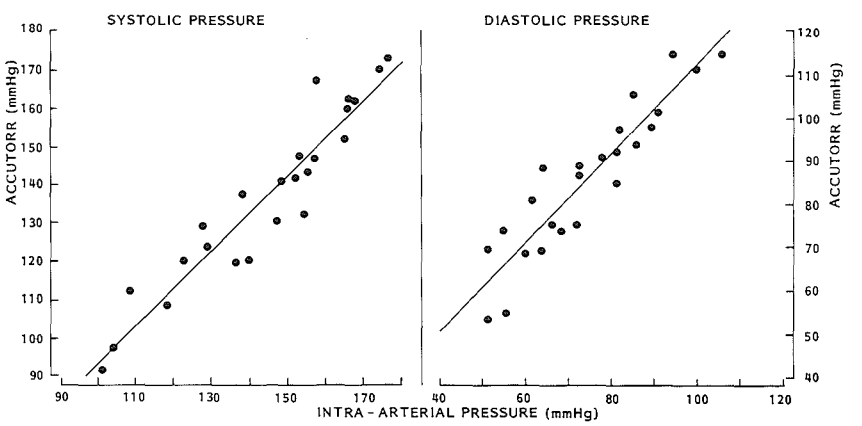

FIGURE 1. *Comparison between blood pressures simultaneously determined by Accutorr and intra-arterial measurement in 24 subjects. Data were analyzed by linear regression; for systolic pressure:* $y = 0.98x - 4.77$, $r = 0.95$, $p < 0.01$; *for diastolic pressure:* $y = 1.04x + 9.14$, $r = 0.92$, $p < 0.01$.

Reproducibility of blood pressure readings obtained with the Accutorr at weekly intervals was assessed by comparing pressures obtained during Weeks 7 and 8 of placebo treatment. The correlation coefficient (linear regression) for 12 consecutive measurements of systolic pressure in individual subjects ranged from an r value of 0.78 to 0.92 (mean, 0.84; SEM = 0.02; n = 24; $p < 0.01$). The correlation coefficient for 12 consecutive measurements of diastolic pressure ranged from an r value of 0.64 to 0.78 (mean, 0.72; SEM = 0.03; n = 24; $p < 0.01$).

The Oxford system was used for continuous intra-arterial ambulatory blood pressure monitoring. Its accuracy and the reproducibility of 24-hour blood pressure profiles have been reported by others.[10] Briefly, a small (1-mm diameter) catheter (Seldicath, Plastimed, Saint-Leu-La Foret, France) was inserted into the non-dominant brachial artery after local anesthesia with 2% lidocaine solution and connected to a transducer-perfusion unit suspended in front of the subject's chest. Signals from the transducer and from two thoracic electrocardiographic electrodes were registered on a portable minicassette tape recorder (Medilog 2, Oxford Instruments, Oxford, UK). Calibration was performed before and after registration. The analogue pressure signal was digitized with a sample frequency of 33⅓ samples per second of real time and fed into a Hewlett-Packard 2113-E computer (Palo Alto, CA, USA). A computer-based program was used to preprocess the signal, which was scrutinized for beat loss, damping, clipping, and movement artifacts, but the final editing of distorted tracings was done after visual inspection. For each 24-hour tracing, the number of beats that had to be excluded from the final analysis was less than 10% of the total. The computer determined systolic, mean, and diastolic pressure and pulse interval and stored this information for each beat. Blood pressure and heart rate obtained during placebo and with ketanserin treatment were examined in two ways: as hourly means and as 6-hour means.

Blood Chemistry

The plasma levels of active renin (normal range, 6–52 μU/ml, unrestricted sodium intake) and aldosterone (normal range, 40–180 pg/ml) were measured by radioimmunoassay.[12, 13] Plasma norepinephrine (normal range, 150–400 pg/ml) was measured by a radioenzymatic technique.[14] The plasma level of ketanserin was measured by high-performance liquid chromatography.[15]

Statistical Analysis

Data are given as means ± SEM. Mean values of renin were calculated after logarithmic transformation. Treatment-order interactions were tested by analysis of variance. The differences between placebo and ketanserin therapy were assessed by the two-tailed paired Student's t test. Chi-square test was used for calculating differences in the number of subjects having side effects and in the number of subjects who responded to treatment. Subjects, in whom supine diastolic pressure, as measured by the London School of Hygiene sphygmomanometer, fell by more than 10% after 8 weeks of treatment from the last measurement in the placebo run-in period were considered to be responders.

TABLE 1. *Clinical Characteristics of Subjects at the Time of Randomization*

Variable	Ketanserin to placebo	Placebo to Ketanserin
Number of subjects	11	13
Men/women	6/5	10/3*
Age (yr)	48 (33–63)	51 (24–69)
Blood pressure (mm Hg)†	161 ± 10/104 ± 5	174 ± 6/109 ± 4
Duration of hypertension (mo)	61 (20–200)	74 (15–200)
Weight (kg)†	72.4 ± 3.1	75.3 ± 2.8
Serum creatinine (mg/dl)†	0.94 ± 0.04	1.08 ± 0.07

Range is given in parentheses.
*$p < 0.05$, compared with ketanserin-to-placebo value.
†Values are means ± SEM.

Results

The study began with 29 subjects. During the run-in phase, three subjects appeared to be normotensive and one had to be withdrawn because of concomitant disease (sarcoidosis). Six weeks after randomization one patient withdrew because she moved to another city. Of the remaining 24 subjects, 11 were first treated with ketanserin and 13 were first treated with placebo. Both groups were reasonably matched for blood pressure and age (Table 1).

Blood Pressure

As shown in Figure 2, supine systolic and diastolic cuff pressures, as measured with the London School of Hygiene sphygmomanometer, were significantly reduced by ketanserin treatment in both groups. Similar results were obtained for standing blood pressures measured with the London School of Hygiene sphygmomanometer and for supine 1-hour blood pressure measurements obtained with the automatic device, Accutorr. Since a significant carryover effect of ketanserin was observed in the first 2 weeks after switching to placebo, the data obtained from the two groups after more than 2 weeks are combined in Table 2. Based on the criterion described in Methods, 11 subjects (46%) were considered to be responders to ketanserin, whereas three subjects (12%) responded to placebo ($p < 0.05$). Ketanserin had no effect on heart rate. None of the subjects showed a more than 10 mm Hg drop in systolic or diastolic pressure on standing.

Eighteen subjects (3 women), aged 49 ± 3 years, were studied by using continuous 24-hour intra-arterial measurement of pressure. Ketanserin lowered systolic

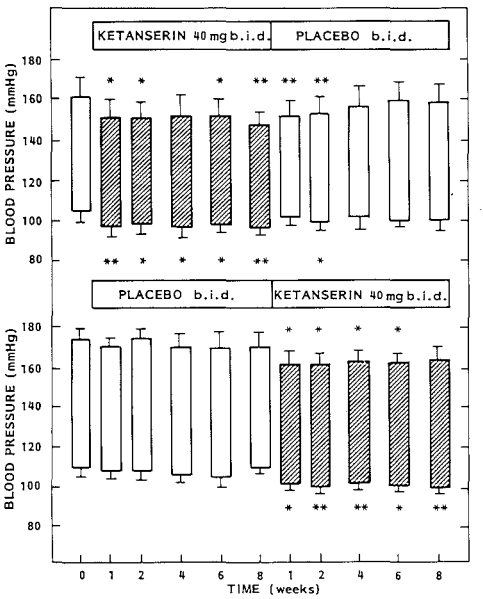

FIGURE 2. *Effect of ketanserin on supine blood pressure in 24 subjects, as measured with the London School of Hygiene sphygmomanometer.* *p < 0.05, **p < 0.01, *for difference from the last week of the placebo run-in period (Week zero).*

and diastolic pressure both at night and during the day (Figure 3, opposite). The effect on intra-arterial pressure in the late morning was comparable to the effect on cuff pressure measured at about the same time of day in the outpatient clinic (Tables 2 and 3). The blood pressure patterns in our subjects showed a dip between 1300 and 1500 that corresponded with the time of the afternoon nap.

Humoral and Biochemical Parameters

Ketanserin had no effect on the serum levels of urea, uric acid, and cholesterol. Endogenous creatinine clearance and the plasma levels of norepinephrine, renin, and aldosterone were unchanged (Table 4). Sodium excretion on the day that blood samples for hormone measurements were taken was 133 ± 13 mEq/24 hr during placebo treatment and 144 ± 15 mEq/24 hr during ketanserin treatment; the difference was not statistically significant. The values for serum electrolytes, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glutamic-oxaloacetic transaminase and lactate dehydrogenase, and white blood cell and platelet counts were also unchanged.

TABLE 3. *Effect of Ketanserin on Ambulatory Intra-arterial Pressure in 18 Subjects*

Blood pressure (mm Hg)	Time of day	Placebo	Ketanserin
Systolic	0600–1200	149 ± 2	145 ± 3*
	1200–1800	153 ± 3	150 ± 2
	1800–2400	155 ± 3	145 ± 3†
	0000–0600	132 ± 1	123 ± 2†
Diastolic	0600–1200	98 ± 2	91 ± 2†
	1200–1800	99 ± 2	94 ± 1‡
	1800–2400	99 ± 3	90 ± 2†
	0000–0600	81 ± 1	75 ± 1‡

Values are means ± SEM.
*$p < 0.05$, †$p < 0.001$, ‡$p < 0.01$, compared with placebo value.

TABLE 2. *Effect of Ketanserin on Cuff Blood Pressure in 24 Subjects*

Variable	Run in (4 wk)	Placebo 4 wk	Placebo 6 wk	Placebo 8 wk	Ketanserin 4 wk	Ketanserin 6 wk	Ketanserin 8 wk
LSH							
Supine							
Systolic BP (mm Hg)	168 ± 5	164 ± 6	162 ± 5	164 ± 6	158 ± 5	158 ± 5	158 ± 5*
Diastolic BP (mm Hg)	107 ± 3	104 ± 3	101 ± 2	104 ± 3	99 ± 3*	99 ± 2	98 ± 2†
Heart rate (beats/min)	72 ± 2	74 ± 2	71 ± 2	73 ± 2	72 ± 2	70 ± 2	70 ± 2
Standing							
Systolic BP (mm Hg)	160 ± 5	160 ± 5	156 ± 5	158 ± 5	157 ± 5	154 ± 5	154 ± 4
Diastolic BP (mm Hg)	109 ± 3	107 ± 4	104 ± 3	112 ± 3	104 ± 3	102 ± 2	104 ± 2‡
Heart rate (beats/min)	82 ± 2	81 ± 2	81 ± 2	84 ± 2	82 ± 3	80 ± 2	80 ± 2
Accutorr (1-hr measurement)							
Systolic BP (mm Hg)	149 ± 4	152 ± 4	152 ± 4	152 ± 5	148 ± 5	144 ± 4‡	149 ± 4
Diastolic BP (mm Hg)	101 ± 3	102 ± 3	100 ± 2	101 ± 3	96 ± 2†	95 ± 2‡	95 ± 2‡
Heart rate (beats/min)	74 ± 2	74 ± 2	73 ± 2	72 ± 2	71 ± 2	71 ± 2	71 ± 2

Values are means ± SEM. LSH = London School of Hygiene sphygmomanometer; BP = blood pressure.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$, as compared with 8-week placebo value.

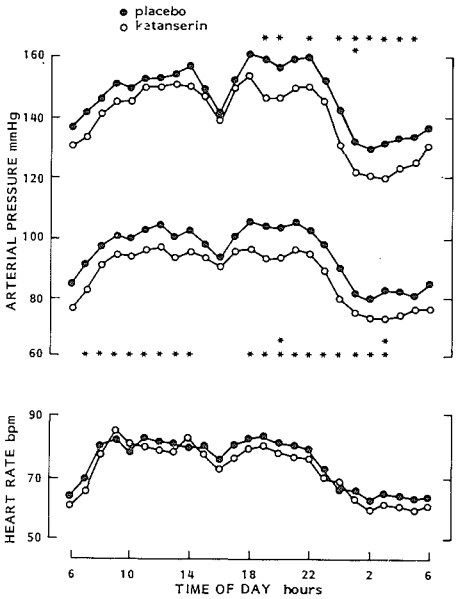

FIGURE 3. *Effect of ketanserin on ambulatory 24-hour systolic and diastolic intra-arterial pressure and heart rate in 18 subjects. Each circle represents the mean value of 1-hour continuous measurement. Ketanserin, 40 mg, was given at 0800 and 1800. Note the dip in pressure during the afternoon nap and during the night. *p < 0.05, **p < 0.01, for difference from placebo; bpm = beats per minute.*

Subject Compliance and Side Effects

Subject compliance was excellent. Less than 5% of the tablets to be taken were returned. Also, the plasma level of ketanserin was 71.9 ± 7.1 ng/ml, which is in the upper therapeutic range.[15, 16] Ketanserin caused an increase in body weight, and this effect was sustained for the full 8 weeks of treatment (see Table 4). Drowsiness was reported by 50% of the subjects at some time during the active treatment period and by 25% of the subjects receiving placebo (Figure 4). The difference was not statistically significant. Other side effects also were not significantly more common with ketanserin than with placebo treatment.

Discussion

Long-term oral treatment with ketanserin, 40 mg b.i.d., significantly reduced blood pressure. Intra-arterial 24-hour measurements showed a 5% fall in mean arterial pressure as compared with results of placebo treatment. Since these measurements were made in the hospital, they might not have been representative for daily practice; however, the effect on intra-arterial pressure in the hospital was comparable to the effect on the office cuff pressure readings (see Tables 2 and 3).

A reduction of approximately 10% in cuff pressure has been reported in a double-blind placebo-controlled study of 10 patients using ketanserin, 20 mg t.i.d.,[16] in a similar study of 8 patients using ketanserin, 40 mg b.i.d.,[17] in a dose-finding study of 16 patients using ketanserin in an average daily dosage of 91 mg,[18] and in a single-blind study of 13 patients receiving 40 mg once daily and 12 patients receiving 40 mg b.i.d.[19] A somewhat lower pressure reduction has been observed in a double-blind placebo-controlled crossover study of 14 patients receiving ketanserin, 40 mg t.i.d.; systolic and diastolic pressures fell by 6%,[20] which agrees with our findings. We chose a dosage of 40 mg b.i.d. because the terminal plasma half-life of ketanserin has been reported to be approximately 10 hours.[19] Although this fixed dose may not have been optimal in all subjects, the continuous blood pressure measurements showed an effect that lasted for a large proportion of the 24-hour period, if not for the full period.

About half of the subjects receiving ketanserin showed a more than 10% decrease in diastolic pressure

TABLE 4. *Effect of Ketanserin on Body Weight, Biochemical Variables, and Renal Function in 24 Subjects*

Variable	Run in (4 wk)	Placebo (8 wk)	Ketanserin (8 wk)
Weight (kg)	76.8 ± 2.3	76.7 ± 2.4	78.0 ± 2.4*
Plasma			
Norepinephrine (pg/ml)	340 ± 34	284 ± 25	314 ± 23
Renin (μU/ml)	10.5 (8.9–12.4)	9.8 (8.2–11.6)	9.2 (7.8–10.8)
Aldosterone (pg/ml)	115 ± 11	110 ± 16	103 ± 13
Ketanserin (ng/ml)	0	0	71.9 ± 13
Serum			
Urea (mg/dl)	31.8 ± 1.8	33.0 ± 2.4	32.4 ± 1.8
Uric acid (mg/dl)	6.0 ± 0.3	6.0 ± 0.3	5.9 ± 0.3
Cholesterol (mg/dl)	205 ± 8	213 ± 8	205 ± 12
Endogenous creatinine clearance (ml/min)	136 ± 14	112 ± 13	118 ± 17

Values are means ± SEM. Range is given in parentheses.
*$p < 0.01$, compared with placebo value.

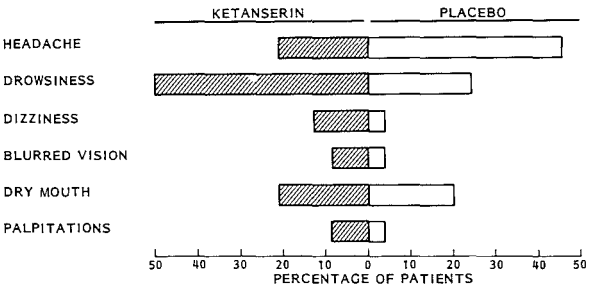

FIGURE 4. *Incidence of side effects during 8 weeks of treatment with ketanserin, 40 mg b.i.d., in 24 subjects. None of the side effects was significantly more frequent with ketanserin than with placebo treatment.*

as compared with the pressure at the end of the placebo run-in period. A similar response rate has been observed with thiazide diuretic or β-adrenergic receptor antagonist treatment.[21, 22] In our series, the responders could not be distinguished from the nonresponders with respect to age or initial blood pressure.

Ketanserin did not modify the circadian blood pressure pattern, since the blood pressure lowering action of the drug was at least as strong during the night as during the day. It is difficult to draw conclusions from these observations as to ketanserin's mechanism of action. Various drugs, such as diuretics, β-adrenergic antagonists, α_1-adrenergic receptor antagonists, and calcium entry blockers, have also been found to exert little influence on the circadian blood pressure variations.[22-26]

Heart rate and plasma norepinephrine levels were not altered by ketanserin, and these findings certainly contrast with the reduction in these parameters that occurs during treatment with a centrally acting drug, such as clonidine.[27] Animal experiments, in which ketanserin was injected into the cerebral ventricles, have provided evidence that the drug does not lower blood pressure by a central action.[28]

Previous studies have shown that the fall in blood pressure after an intravenous injection of 10 mg of ketanserin is associated with a small and transient rise in heart rate and plasma norepinephrine levels.[29] No such changes were observed in the present study, possibly because the effect on blood pressure was more gradual, thereby allowing the baroreflex to be reset.

The pharmacological evidence now available indicates that ketanserin selectively antagonizes the S_2-mediated peripheral pressor action of serotonin but is not fully specific for S_2-receptors. It also has some affinity for α_1-adrenergic receptors. Intravenous ketanserin, however, appears to be capable of lowering blood pressure in hypertensive subjects independently of α_1-adrenergic receptor blockade.[30, 31] Steady state plasma levels of ketanserin in these intravenous studies[19, 31, 32] were about 80 ng/ml, which is comparable to the peak levels measured after oral intake in the present study.

Ketanserin did not cause postural hypotension in our subjects. Body weight had increased by 1.3 kg after both 4 and 8 weeks of ketanserin treatment. This increase may have been caused by renal sodium and water retention, as is often seen during treatment with vasodilator drugs, but we did not perform balance studies to prove this possibility. Among other minor side effects, none were significantly more frequent with ketanserin than with placebo treatment.

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CHAPTER 7

KETANSERIN, MECHANISM OF ACTION

5-HT, alpha-adrenoreceptors, and blood pressure. Effects of ketanserin in essential hypertension and autonomic insufficiency.

Wenting GJ, Woittiez AJJ, Man in 't Veld AJ, Schalekamp MADH. Hypertension 1984; **6**: 100-109.

Role of α-adrenergic blockade in the cardiovascular actions of ketanserin: studies in patients with essential hypertension, autonomic insufficiency, and Raynaud's phenomenon.

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5-HT, Alpha-Adrenoceptors, and Blood Pressure

Effects of Ketanserin in Essential Hypertension and Autonomic Insufficiency

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SUMMARY **The serotonin (5-hydroxytryptamine, 5-HT) antagonist, ketanserin, has a high affinity for 5-HT_2-receptors but it also binds to α_1-adrenoceptors. The compound (10 mg i.v.) lowered mean arterial pressure by 22% ± 2% (mean ± SEM, $p < 0.001$) in 30 patients with essential hypertension. Measurements of heart rate, cardiac output, cardiac filling pressures, forearm blood flow, renal blood flow, and glomerular filtration rate revealed a hemodynamic pattern compatible with vasodilation of both resistance and capacitance vessels. This was accompanied by moderate reflex cardiostimulation. Ketanserin did not alter the pressor effect of bolus injections of (-)-phenylephrine hydrochloride (25, 50, 100, and 200 μg i.v.). Ketanserin also had a distinct hypotensive effect in four normotensive patients with autonomic insufficiency due to an efferent sympathetic lesion, who were unresponsive to phentolamine (20 mg i.v.). Thus, ketanserin in the dose we have used appears to lower blood pressure independently of α_1-adrenoceptor blockade. On the other hand, in patients with essential hypertension the antihypertensive effect of ketanserin was blunted by pretreatment with prazosin (12 mg/day). Therefore, a certain degree of α_1-adrenergic tone seems to be required for the compound to exert its full antihypertensive action. The findings are indirect evidence for a role of 5-HT in the maintenance of increased vascular resistance in essential hypertension. This may be related, at least in part, to the alleged amplifying effect of 5-HT on α_1-adrenoceptor-mediated vasoconstriction. (Hypertension 6: 100–109, 1984)**

KEY WORDS • **α-adrenoceptors** • **blood pressure** • **5-hydroxytryptamine** • **ketanserin** • **prazosin**

ALTHOUGH 5-hydroxytryptamine (5-HT, serotonin) was among the first vasoactive amines to be discovered and synthesized, its function in blood pressure regulation is still unclear. Recently, a differentiation between two subtypes of 5-HT-receptors has been made on the basis of radioligand-binding studies using membranes prepared from rat frontal cortex.[1] Although specific functions associated with these brain receptors have not been identified, it has been shown that the 5-HT-receptors subserving contraction of vascular smooth muscle cells are of the 5-HT_2-subtype.[2-7]

A 5-HT_2-receptor antagonist, ketanserin (3-2-[4-(-fluorobenzoyl)-1-piperidinyl]ethyl-2,4-[1H,3H] quinazolinedione, Janssen Pharmaceutica, Beerse, Belgium), devoid of agonist activity, is now available for clinical investigation.[4-6] Ketanserin, however, is not fully specific for 5-HT_2-receptors, since it also binds to α_1-adrenoceptors. High concentrations of ketanserin have an antagonistic effect on α_1-adrenoceptor-mediated contractions of isolated arteries and veins.[4]

Experiments in animals[4] and preliminary data in humans[8, 9] have shown that ketanserin has antihypertensive properties. This may, at least in part, depend on blockade of α_1-adrenoceptors. Indeed, it has been reported that hypotensive doses of ketanserin abolished the pressor response to α_1-agonists in the pithed rat, the anesthetized normotensive rat, and the conscious spontaneously hypertensive rat.[10, 11]

This paper describes the hemodynamic profile of ketanserin's antihypertensive action in patients with essential hypertension. The possibility that the antihypertensive effect of ketanserin depends on interference with α_1-adrenoceptor-mediated vasoconstriction was tested by comparison of the pressor effects of the α_1-adrenoceptor agonist, phenylephrine, before and after ketanserin and by assessment of the antihypertensive effect of ketanserin after administration of the α_1-adre-

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noceptor antagonist, prazosin. The cardiovascular effects of ketanserin were also studied in a small group of patients with autonomic insufficiency, who were unresponsive to the hypotensive action of the nonselective α-adrenoceptor antagonist, phentolamine.

Methods

Patients

Thirty hypertensive subjects were studied, 21 men and nine women, aged 55 ± 2 years (mean ± SEM; range, 38 to 77 years). The diagnosis of essential hypertension was made by routine screening including intravenous urography. Antihypertensive treatment, if any, was stopped 3 weeks before the study. The patients were admitted to a metabolic ward and received a diet with a fixed sodium and potassium content (50–70 mEq/day and 70–100 mEq/day, respectively). When blood pressure and sodium balance were stable, the patients were investigated in the cardiovascular laboratory after an overnight fast. The use of a new antihypertensive agent was explained to the patients. They all gave their consent to participate in the study. The study protocol was approved by the local hospital ethical committee.

Four patients aged 40–76 years, three women and one man, with chronic autonomic failure of the peripheral type, were also studied. Two patients had idiopathic autonomic neuropathy, one patient had primary amyloidosis, and the remaining patient had amyloidosis of the hereditary type. Clinical signs of involvement of the central nervous system were absent in all. Diagnostic aspects of these patients have been described in detail elsewhere.[12, 13] Incapacitating orthostatic hypotension was the presenting symptom. The systolic pressure overshoot in the Valsalva maneuver was absent, and heart rate did not increase after atropine (2 mg i.v.). Plasma norepinephrine was below the detection limit of 20 pg/ml in two patients and it was 40 and 135 pg/ml in the remainder. It was unresponsive to head-up tilting in all. Thus, the patients had combined efferent sympathetic and parasympathetic lesions. At the time of the study, they had not been on any drug for at least 3 weeks.

Hemodynamic Measurements

Arterial pressure was measured directly in a radial artery with a P 23 D Statham transducer and recorded on a direct-writing Hewlett-Packard multigraph. Heart rate was determined from the simultaneously recorded ECG signal. Ketanserin, 10 mg in 20 ml of saline, was infused intravenously in 2 to 3 minutes, and the effects were followed for 2 hours. In the patients with autonomic insufficiency, the effects of ketanserin were compared with those of phentolamine, 20 mg i.v. Ketanserin and phentolamine were given on different occasions at least 2 weeks apart.

More detailed data on central and renal hemodynamics were obtained in 12 hypertensive patients. A Swan-Ganz thermodilution catheter was introduced percutaneously in an antecubital vein and positioned in the pulmonary artery. Triplicate cardiac output (CO) measurements were performed at frequent intervals and integrated mean values for systemic arterial pressure (MAP), right atrial pressure (RAP), pulmonary arterial pressure (PAP), and pulmonary capillary wedge pressure (PCWP) were determined. Pressures and cardiac output were measured with the patients in a supine position, and the transducers were zeroed at midthoracic level. Cardiac output was corrected for body surface area and is expressed per 1.73 m^2. The following variables were derived: total peripheral resistance is TPR = (MAP − RAP)/CO and pulmonary vascular resistance is PVR = (PAP − PCWP)/CO.

A peripheral vein was cannulated for renal function studies using a constant infusion technique. Effective renal plasma flow and glomerular filtration were estimated by the clearances of ^{131}I-hippuran and ^{125}I-thalamate, respectively. Hemodynamic measurements were made after a 90-minute equilibration period. Renal blood flow (RBF) corrected for 1.73 m^2 body surface area was calculated using central venous packed cell volume and assuming 75% renal extraction of hippuran.[14] Renal vascular resistance was derived as RVR = MAP/RBF.

The effects of ketanserin on peripheral hemodynamics were studied in a subgroup of eight hypertensive patients. Measurements were taken with the patients supine after resting for at least 30 minutes at a room temperature of 23°–25°C and in an air humidity of approximately 50%. Forearm blood flow including hand flow was measured semicontinuously by means of an ECG-triggered venous occlusion plethysmograph (Janssen Scientific Instruments). A mercury-in-Silastic strain gauge was placed around the midforearm, and the arm was elevated so that venous pressure approached zero. Venous occlusion was achieved within 50 msec by inflation of a sphygmomanometer cuff wrapped around the upper arm and attached to a container of compressed air with a pressure valve preset at 50 mm Hg. This occlusion pressure was intermittently applied for a period of three heart beats, with a recovery period of two heart beats. To obtain synchronization between cuff pressure and flow pulse, the occlusion pressure was regulated by means of electromagnetic valves that were triggered by properly delayed impulses derived from the R-top of the electrocardiogram. Forearm blood flow was calculated from the change in forearm circumference during occlusion and was expressed as ml per 100 ml of tissue per minute. Rectal temperature and skin temperature of the forehead and distal, volar surface of digits no. 2, 3, and 5 of the nonoccluded arm were recorded by means of telethermometers (Yellow Springs Instruments, Cleveland, Ohio).

Phenylephrine Injections

In seven patients with hypertension, bolus injections of (-)-phenylephrine hydrochloride (25, 50, 100, and 200 μg), in random order, were flushed into the circulation through a cannula in an antecubital vein, before and during ketanserin infusion. Intraarterial pressure

and the electrocardiogram were simultaneously recorded on a multichannel ink-jet writer (Mingograph Siemens, Elema, Solna, Sweden) at a paper speed of 100 mm/sec. The interval between the injections was 10 minutes. After the first series of injections had been finished, a loading dose of 10 mg ketanserin was infused intravenously in 2 or 3 minutes followed by a sustaining infusion of 2 mg/hour. According to the manufacturer, in this way a stable plasma level of about 80 ng/ml is reached within 15 minutes. A second series of phenylephrine bolus injections was then given. The 200 μg dose of phenylephrine was used for determination of baroreflex sensitivity. Measurements covered the period from the onset of the rise in arterial pressure until pressure had reached its peak level. Baroreflex sensitivity was expressed as the slope of the regression line relating RR-interval to the systolic pressure of the preceding heart beat.[15] The result was accepted only when the correlation coefficient was greater than 0.75, with a p value of less than 0.05. In one patient these conditions were not met.

A third series of phenylephrine injections was given to five patients after they had been treated with prazosin, 4 mg orally three times a day for 2 days. Phenylephrine was injected 60–90 minutes after the morning dose of prazosin.

Pretreatment with Prazosin and Furosemide

Fourteen patients with essential hypertension, in whom the effects of a first dose of ketanserin (10 mg i.v.) had been followed for 2 hours, were randomly assigned to two treatment modalities. Eight patients were treated with prazosin (4 mg three times a day) for 1 week, and the effects of a second dose of ketanserin (10 mg i.v.) were studied 60–90 minutes after the morning dose of prazosin. Six patients were treated with furosemide (40 mg daily) and after 1 week a second dose of ketanserin (10 mg i.v.) was administered.

Analytical Procedures

For determinations of plasma renin and aldosterone, arterial blood was collected in chilled tubes containing disodium ethylenediaminetetraacetate (EDTA) in a final concentration of 2 mg/ml of blood, and stored on ice for no longer than 60 minutes until centrifugation at 5,000 rpm for 15 minutes at 4°C to separate the plasma. Plasma was stored at −20°C. Active renin was measured by radioimmunoassay as described previously.[16] Semipurified human kidney renin (MRC standard no. 68/356, WHO International Laboratory for Biological Standards and Control, London, England) was used as a standard, and results are expressed as microunits of this standard per milliliter of plasma.

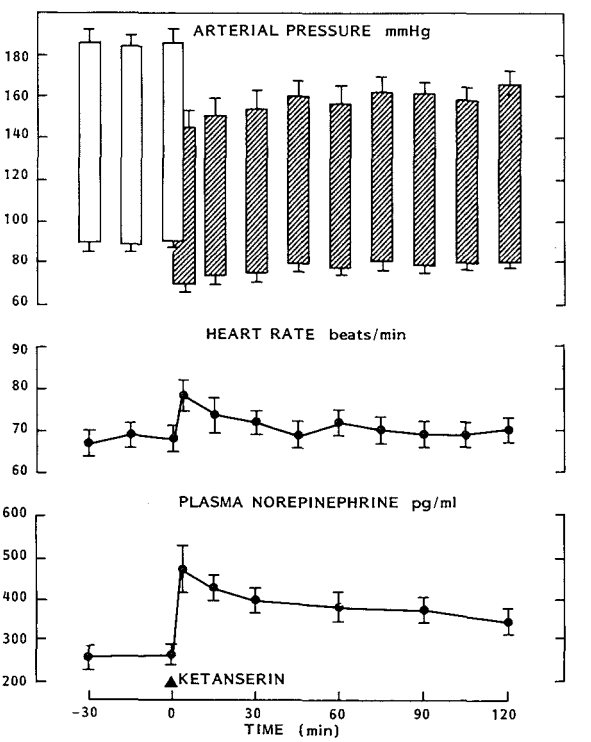

FIGURE 1. *Responses of systemic arterial pressure, heart rate, and plasma norepinephrine to a single dose of ketanserin (10 mg i.v.) in 20 patients with essential hypertension. Changes in arterial pressure and norepinephrine were significant* ($p < 0.01$) *throughout the observation period. Heart rate had returned to baseline after 45 minutes.*

The normal range in our laboratory is 15–40 μU/ml. Plasma aldosterone was also measured by radioimmunoassay.[17] The normal range is 40–180 pg/ml. For determination of plasma norepinephrine, 10 ml of arterial blood was collected into a chilled tube containing 143 USP units lithium heparin and 15 mg glutathione in 200 μl distilled water. After centrifugation, the plasma was deproteinized with trichloroacetic acid. The precipitate was removed by centrifugation at 8000 rpm for 15 minutes and the supernatant was stored at −20°C. Norepinephrine was measured by a radioenzymatic method, in which norepinephrine is quantitatively converted to ^{3}H-epinephrine in the presence of phenylethanolamine-N-methyltransferase using ^{3}H-S-adenosyl-L-methionine as a ^{3}H-methylgroup donor.[18] All samples were assayed in duplicate, both with and without the addition of norepinephrine as the internal standard. The normal range is 150–450 pg/ml.

Statistical Analysis

Data are given as means ± SEM. Plasma levels of renin were not distributed normally. Mean values and standard errors were calculated after logarithmic transformation. Student's *t* tests for paired and unpaired data were used. Statistical significance was taken to be $p < 0.05$.

Results

Studies in Essential Hypertension

Systemic, Central, and Peripheral Hemodynamics and Plasma Norepinephrine

In 30 patients, arterial pressure fell from 177 ± 5/86 ± 3 mm Hg before ketanserin to 139 ± 6/68 ± 3 mm Hg ($p < 0.001$) after ketanserin injection. Heart rate rose from 68 ± 2 to 78 ± 3 bpm ($p < 0.01$). Fifteen minutes after ketanserin, arterial pressure was still low, 147 ± 6/72 ± 3 mm Hg ($p < 0.001$ for difference from baseline), but heart rate had returned to 73 ± 3 bpm. The effects on arterial pressure, heart rate, and plasma norepinephrine, which were followed for 2 hours in 20 patients, are shown in Figure 1. After 2 hours, mean arterial pressure was still significantly below baseline ($p < 0.05$) and norepinephrine was still elevated ($p < 0.05$), whereas heart rate had already returned to baseline.

The fall in arterial pressure after ketanserin was caused by a fall in total peripheral resistance (Figure 2). Cardiac output rose from 4.35 ± 0.26 liter/min before ketanserin to 4.68 ± 0.26 liter/min ($p < 0.01$) 5 minutes after the ketanserin injection. Thereafter, cardiac output fell, and after 90 minutes it was 4.08 ± 0.23 liter/min, which was even below baseline ($p < 0.05$). Stroke volume was 68 ± 4 ml before ketanserin. It was not altered 5 minutes after ketanserin but it fell to 61 ± 3 ml after 90 minutes ($p < 0.01$ for difference from baseline). Right atrial pressure, pulmonary arterial pressure, and pulmonary capillary wedge pressure were also lowered (Figure 3). Pulmonary vascular resistance was not altered. Forearm blood flow and digital skin temperature increased markedly after ketanserin (Table 1). Rectal temperature and skin temperature of the forehead remained constant.

Renal Hemodynamics, Plasma Renin, and Aldosterone

The pattern of flow and resistance changes induced by ketanserin in the renal vascular bed resembled the changes in the systemic circulation (Figure 4). Renal blood flow was 736 ± 44 ml/min before ketanserin and rose to 767 ± 51 ml/min ($p < 0.05$) 15 minutes after the drug. Glomerular filtration rate was 90 ± 5 ml/min before ketanserin and remained unchanged de-

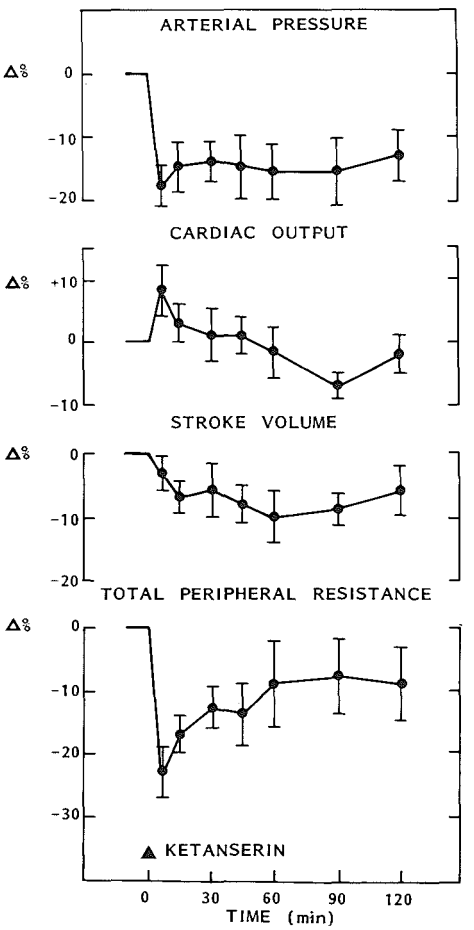

FIGURE 2. *Effects of ketanserin (10 mg i.v.) on systemic hemodynamics in 12 patients with essential hypertension. The decrease in arterial pressure was due to a decrease in total peripheral resistance. After 120 minutes, arterial pressure and total peripheral resistance were still below baseline* ($p < 0.05$).

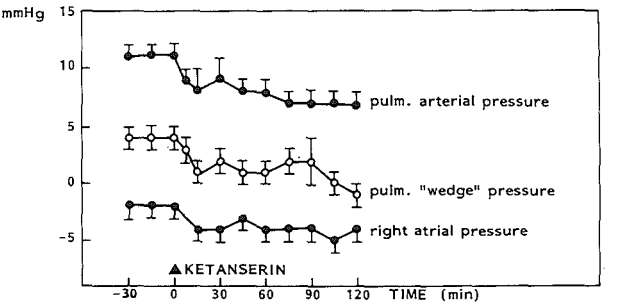

FIGURE 3. *Effects of ketanserin (10 mg i.v.) on central hemodynamics in 12 patients with essential hypertension. After 120 minutes, all changes were still significant at* $p < 0.05$.

spite the decrease in arterial pressure. Plasma renin rose from 9.4 μU/ml (antilog of arithmetic mean after logarithmic transformation of data) before ketanserin to 17 μU/ml ($p < 0.01$) 30 minutes after the drug. Plasma aldosterone did not change; it was 102 ± 15 pg/ml before ketanserin and 92 ± 14 pg/ml after 30 minutes.

Responses to Bolus Injections of Phenylephrine

From the start of the injection until changes had returned to baseline pulsatile and mean arterial pressures and the RR-interval of the ECG were analyzed beat to beat. Figure 5 shows an example. Mean arterial pressure and the subsequent RR-interval (pulse interval) were used for constructing log dose-response curves. As shown in Figure 6, the changes in mean arterial pressure and pulse interval were not modified by ketanserin. However, treatment with prazosin competitively antagonized the responses, as indicated by a parallel shift of the dose-response curve to the right (Figure 7). Baroreflex sensitivity was not modified by ketanserin. It was 8.82 ± 1.71 msec/mm Hg before ketanserin and 9.03 ± 1.52 msec/mm Hg after the drug.

Effects of Pretreatment with Prazosin or Furosemide

Mean arterial pressure was 114 ± 8 mm Hg in the patients who were to be treated with prazosin and 116 ± 6 mm Hg in the patients to be treated with furosemide. After 1 week of treatment, mean arterial pressure was 103 ± 6 mm Hg in the prazosin group ($p < 0.05$ for difference from the value before treatment) and 102 ± 4 mm Hg in the furosemide group ($p < 0.05$). Body weight did not change with prazosin and fell by 2.1 ± 0.3 kg with furosemide ($p < 0.01$). The blood pressure response to ketanserin was blunted by pretreatment with prazosin and was not affected by pretreatment with furosemide (Figure 8).

Studies in Autonomic Insufficiency

Arterial pressure fell from 128 ± 8/66 ± 3 mm Hg before ketanserin to 95 ± 4/56 ± 3 mm Hg ($p <$

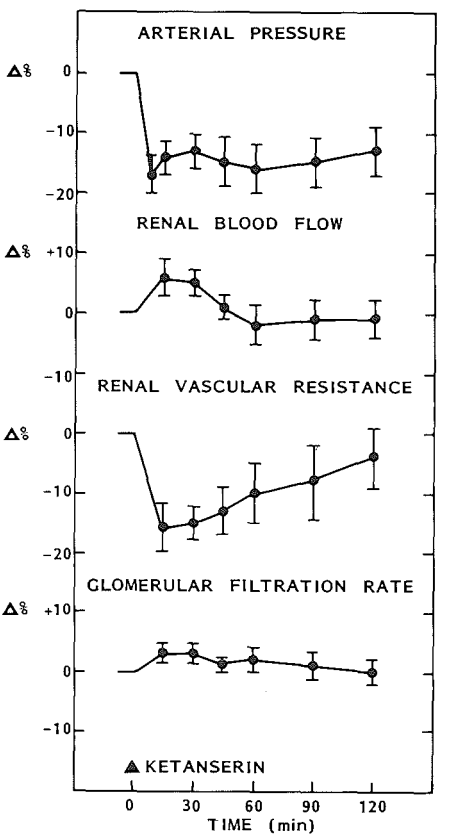

FIGURE 4. *Effects of ketanserin (10 mg i.v.) on renal hemodynamics in 12 patients with essential hypertension. Note the decrease in renal vascular resistance* ($p < 0.01$ *after 15, 30, and 45 minutes).*

TABLE 1. *Effects of Ketanserin (10 mg i.v.) on Systemic and Regional Hemodynamics* (mean ± SEM) *in Eight Patients with Essential Hypertension*

	Time (min) before and after ketanserin administration					
	−5	0	2.5	5	10	15
Mean arterial pressure (mm Hg)	111±6	115±5	99±6†	101±6†	105±6*	106±6*
Heart rate (bpm)	65±4	65±4	72±3†	70±3*	67±4	65±4
Forearm blood flow (ml/min/100 ml)	3.6±0.2	3.7±0.2	6.5±0.5†	6.3±0.6†	5.4±0.5*	4.4±0.6*
Digital skin temperature (°C)	31.3±0.8	31.2±0.8	31.8±0.9	33.2±0.6*	33.3±0.6*	33.6±0.8*

*$p < 0.05$.
†$p < 0.01$.

0.001) after the ketanserin injection (Table 2). In contrast, 20 mg phentolamine i.v. had no effect on arterial pressure in these patients. With both drugs, heart rate did not change.

Discussion

Cardiovascular and Hormonal Effects of Ketanserin

The profile of ketanserin's hemodynamic effects is compatible with combined arteriolar and venous dilatation. Systemic arterial pressure, total peripheral resistance, and cardiac filling pressures were lowered. Part of the decrease in total peripheral resistance was caused by renal vasodilatation. Forearm resistance was also reduced, and this reduction appeared out of proportion as compared to the overall reduction in vascular resistance. The hand was included in our semicontinuous measurements of forearm flow so that changes in hand skin flow have influenced the results. Indeed, our thermographic studies showed that digital skin flow was increased by ketanserin, presumably by opening of arteriovenous shunts, which are densely innervated by sympathetic nerves[19] and possibly also by serotonergic fibers.[20] It is worth mentioning that forehead skin flow, as estimated by skin temperature

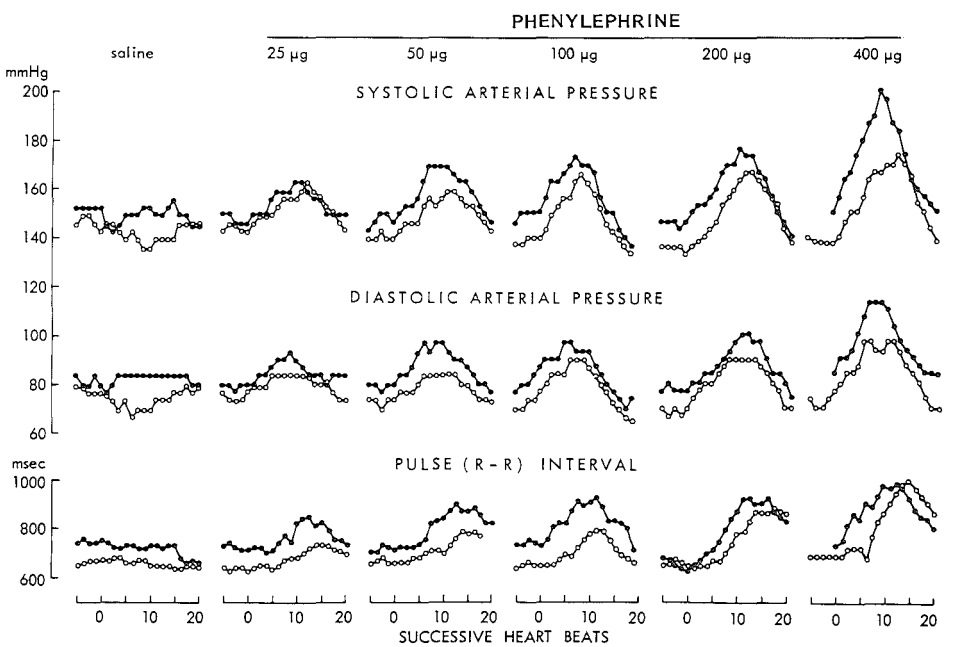

FIGURE 5. *Effects of bolus injections of phenylephrine in a 38-year-old mildly hypertensive patient before (black circles) and during (open circles) ketanserin infusion.*

measurements, was not increased by ketanserin. This contrasts with the rise of forehead temperature after hydralazine.[21]

The fall in arterial pressure was associated with increments in heart rate and cardiac output, probably by baroreflex-mediated withdrawal of vagal tone and increase in sympathetic activity.[22] The increased sympathetic activity was reflected by a rise in plasma norepinephrine. Despite continued sympathetic stimulation, cardiac output returned to its initial level. This may have been due to the gradual decrease in cardiac filling pressures. For a given effect on arterial pressure, the increase in heart rate after ketanserin was less than with drugs like hydralazine and diazoxide,[21, 22] which have their predominant effect on the resistance vessels.

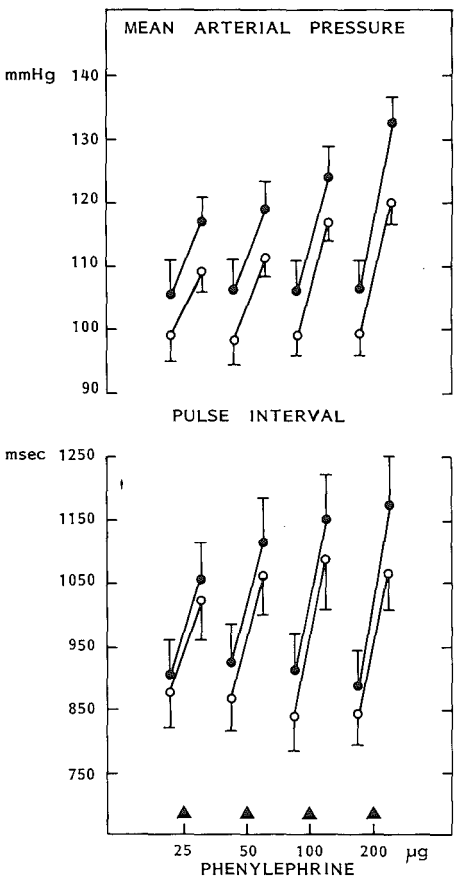

FIGURE 6. *Peak responses to bolus injections of phenylephrine in seven patients with essential hypertension before (black circles) and during (open circles) ketanserin infusion.*

Sympathetic stimulation may have contributed to the observed rise in plasma renin. It may be of interest that plasma aldosterone did not change after ketanserin. From studies with isolated rat glomerulosa cells it is known that 5-HT has direct aldosterone-stimulating properties.[23] Suppression of aldosterone was found in patients with idiopathic aldosteronism after administration of the antiserotonergic agent cyproheptadine.[24] The divergent responses of plasma renin and aldosterone after ketanserin in our patients suggest a suppressive effect of 5-HT blockade on aldosterone secretion but further studies are needed to clarify this point.

Mechanism of Antihypertensive Action of Ketanserin

Ketanserin has been characterized as a selective ^{3}H-ligand for 5-HT$_2$-binding sites in brain.[2] These binding sites probably differ[7] from the D- and M-types of 5-HT-receptors postulated by Gaddum and Picarelli.[25] The close connections between the central serotonergic neuronal system and the pathways of catecholaminergic neurons suggest a role for 5-HT in cardiovascular control, but both pharmacological and surgical

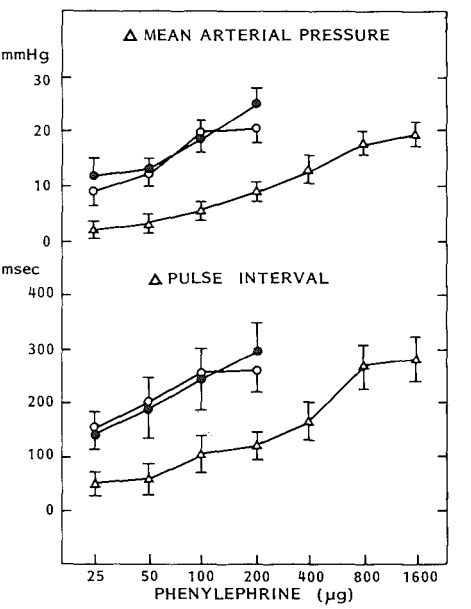

FIGURE 7. *Dose-response curves for phenylephrine in seven patients with essential hypertension before (black circles) and during (open circles) ketanserin infusion. In five patients, phenylephrine injections were also given after treatment with prazosin (open triangles). Baseline values for mean arterial pressure were 106 ± 5 mm Hg before ketanserin, 96 ± 3 mm Hg during ketanserin, and 97 ± 6 mm Hg after prazosin.*

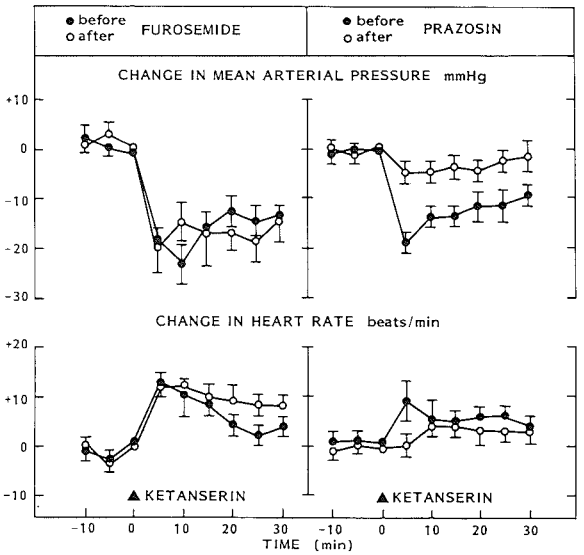

FIGURE 8. *Effects of pretreatment with furosemide or prazosin on the antihypertensive action of ketanserin (10 mg i.v.) in, respectively, six and eight patients with essential hypertension. The decrements in arterial pressure after ketanserin were smaller ($p < 0.01$) throughout the observation period in the prazosin-treated patients.*

TABLE 2. *Effects of Ketanserin (10 mg i.v.) and Phentolamine (20 mg i.v.) on Systemic Arterial Pressure and Heart Rate in Four Patients with Autonomic Insufficiency*

	Case no.	Ketanserin time (min)						Phentolamine time (min)					
		−5	0	2.5	5	10	15	−5	0	2.5	5	10	15
Systolic arterial pressure (mm Hg)	1	117	115	90	91	89	90	121	123	125	125	123	121
	2	111	112	94	92	90	90	119	119	121	123	120	120
	3	147	143	107	113	108	109	165	163	167	169	161	162
	4	139	142	90	88	86	89	137	135	135	142	140	140
	mean	129	128	95*	96*	93*	95*	136	135	137	140	136	136
	± SEM	9	8	4	6	5	5	11	10	10	11	9	10
Diastolic arterial pressure (mm Hg)	1	60	60	53	51	49	50	61	60	63	63	61	60
	2	68	66	51	52	51	53	61	63	61	65	65	64
	3	60	61	60	60	50	52	75	75	79	81	79	78
	4	73	75	61	59	58	61	73	73	73	75	73	71
	mean	65	66	56*	56*	52*	54*	68	68	69	71	70	68
	± SEM	3	3	3	2	2	2	4	4	4	4	4	4
Heart rate (bpm)	1	73	74	72	71	70	71	69	69	70	69	69	70
	2	93	95	90	92	90	91	98	95	95	95	96	95
	3	69	68	65	65	66	66	70	70	71	71	73	71
	4	56	55	54	52	50	52	52	54	52	50	55	55
	mean	72	73	70	70	69	70	72	72	72	71	73	73
	± SEM	8	8	8	8	8	8	10	9	9	9	9	8

*$p < 0.01$.

manipulations of this central serotonergic system have yielded conflicting results.[26] From animal studies, ketanserin appears to be more potent as a serotonin antagonist in the periphery than in the central nervous system.[6]

The peripheral effects of 5-HT are notoriously variable.[27–29] 5-HT can cause either contraction or relaxation of vascular smooth muscle cells depending on the anatomical origin of the blood vessel studied, the species, experimental conditions, and concentration of the monoamine. Apart from its direct vasoconstrictor effect, 5-HT also amplifies the responses to endogenous vasopressor substances such as norepinephrine and angiotensin II.[30] This could be a conceptual framework for visualizing how 5-HT may contribute to the elevated vascular resistance in essential hypertension. The available pharmacological evidence, which includes experiments using ketanserin as a 5-HT-antagonist, indicates that the 5-HT-receptors subserving vascular smooth muscle contraction are of the 5-HT_2-receptor subtype.[3–7] This is not only true for the direct vasoconstrictor effect of 5-HT but probably also for its indirect effect through amplification of the responses to norepinephrine.[4]

In anesthetized normotensive rats and in conscious spontaneously hypertensive rats, doses of ketanserin required to lower blood pressure were 25–100 times higher than those required to inhibit the blood pressure response to 5-HT.[10, 11] At such high doses, however, the pressure responses to α_1-adrenoceptor agonists were also blocked. It is unlikely that ketanserin (10 mg i.v.) caused α_1-adrenoceptor blockade in our patients, since the pressor responses to bolus injections of phenylephrine were not altered. This contrasted with the shift of the dose-response curve to the right that was observed after treatment with the α_1-adrenoceptor antagonist, prazosin. Heart rate was reduced by bolus injections of phenylephrine due to a baroreflex-mediated increase in vagal tone.[31, 32] The relationship between the responses of pressure and heart rate to phenylephrine was not altered by previous administration of ketanserin, which suggests that the sensitivity of the baroreflex was not affected by this drug.

That ketanserin can lower blood pressure independently of α-adrenoceptor blockade was substantiated in our patients with autonomic insufficiency. The nonselective α-adrenoceptor antagonist phentolamine (20 mg i.v.) had no effect on blood pressure and heart rate in these patients, whereas this dose is known to cause hypotension and tachycardia in normal individuals. This confirmed the presence of an efferent sympathetic lesion in our patients resulting in a low occupancy of the α_1- and α_2-adrenoceptors. Still, ketanserin had a distinct hypotensive effect in these patients.

Pretreatment with prazosin attenuated the antihypertensive effect of ketanserin in our hypertensive patients. Pretreatment with furosemide, which had an effect on blood pressure comparable to that of prazosin, did not affect the response to ketanserin. Perhaps a certain degree of endogenous tone on vascular α_1-adrenoceptors is required for ketanserin to exert its full antihypertensive action. This is in accord with the contention that 5-HT_2-receptor stimulation is capable of amplifying the pressor response to norepinephrine.[4]

We conclude that the antihypertensive action of ketanserin that we have observed is not caused by blockade of α_1-adrenoceptors. The pharmacological evidence obtained so far supports that this effect depends on blockade of vascular 5-HT_2-receptors. Our findings are therefore indirect evidence for a role of 5-HT in the maintenance of an increased vascular resistance in essential hypertension.

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Role of α-Adrenergic Blockade in the Cardiovascular Actions of Ketanserin: Studies in Patients with Essential Hypertension, Autonomic Insufficiency, and Raynaud's Phenomenon

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Summary: It remains to be established whether ketanserin's antihypertensive effect is caused by S_2-serotonergic blockade, by α_1-adrenergic blockade, or by a combination of both. Ketanserin, 10 mg i.v. or 40 mg t.i.d. orally, blocked the serotonin-induced contractions of hand veins in patients with essential hypertension. Intravenous ketanserin had no effect on the venoconstrictor action of noradrenaline. The increase in digital blood flow after i.v. ketanserin, as measured by the change in digital skin temperature in patients with Raynaud's phenomenon, was not blocked by phentolamine or pretreatment with prazosin. Intravenous ketanserin also lowered arterial pressure in patients with autonomic insufficiency who were unresponsive to phentolamine. These observations suggest that α-adrenergic blockade is not the sole mechanism of ketanserin's cardiovascular actions in humans. **Key Words:** α-Adrenoceptors—Autonomic Failure—Hypertension—5-HT_2 receptors—Ketanserin—Raynaud's phenomenon.

The mechanism by which the S_2-serotonergic antagonist ketanserin lowers blood pressure in humans is not fully understood. Ketanserin, in higher concentrations than required to inhibit S_2-serotonergic receptors, also binds to α_1-adrenergic sites and antagonizes α_1-adrenoceptor-mediated contractions of isolated arteries and veins (1). It remains to be established whether ketanserin's antihypertensive effect is caused by serotonergic blockade, by α-adrenergic blockade, by a combination of both, or by some other action (2). Here we report some studies in patients with hypertension, indicating blockade of serotonin-induced constriction of hand veins by ketanserin; we also report studies on patients with Raynaud's syndrome and those with autonomic insufficiency, indicating that ketanserin is capable of causing vasodilation independently of α-adrenergic blockade.

PATIENTS

Ketanserin, 10 mg i.v., was given to 30 patients (21 men, 9 women, aged 55 ± 2 years, mean ± SEM, range 38–77 years) with essential hypertension, to four patients (three women, one man, aged 40–76 years) with chronic autonomic failure of the peripheral type, and to 11 patients (nine women and two men, aged 37 ± 4 years) with primary Raynaud's phenomenon.

The diagnosis of essential hypertension was made after routine screening, which included i.v. urography. These patients were admitted to the hospital and received a diet with fixed sodium content. Antihypertensive treatment had been stopped 3 weeks before the study. Patients were studied when blood pressure and sodium balance were stable.

Patients with chronic autonomic insufficiency suffered from incapacitating orthostatic hypotension. All patients had combined efferent sympathetic and parasympathetic lesions of the baroreflex arc. Plasma noradrenaline was abnormally low in all patients and was unresponsive to head-up tilting. A detailed description of these patients has been given elsewhere (3). At the time of the study, they had not used any drug for at least 3 weeks.

The diagnosis of Raynaud's phenomenon was made according to the criteria proposed by Allen and Brown (4). Secondary Raynaud's phenomenon was excluded by history, physical examination, and laboratory screening. All drugs were discontinued 2 weeks prior to the study.

The use of a new drug was explained to the patients, and all gave their consent to participate in the study. The study protocols were approved by the Hospital Ethical Review Committee.

MEASUREMENT OF BLOOD PRESSURE

Blood pressure was measured directly from a radial artery with a P23 ID Statham transducer and recorded on a direct-writing Hewlett–Packard multigraph. Heart rate was determined from the simultaneously recorded ECG signal. Ketanserin, 10 mg in 20 ml of saline, was infused intravenously in 3 min.

In 20 patients with essential hypertension, the effects of

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ketanserin on blood pressure and heart rate were followed for 2 h. After this investigation, 14 patients were randomly assigned to two treatment modalities. Eight patients were treated with prazosin (4 mg t.i.d) and six patients were treated with furosemide (40 mg daily). In both groups, after 1 week of treatment, a second dose of ketanserin (10 mg i.v.) was administered.

In patients with autonomic insufficiency, the effects of ketanserin (10 mg i.v.) were compared with those of phentolamine (20 mg i.v.). Ketanserin and phentolamine were given on different occasions at least 2 weeks apart.

HAND VEIN SENSITIVITY TEST

In six men with essential hypertension, the effect of ketanserin on serotonin-induced venoconstriction was studied. The hand vein sensitivity test was performed during saline infusion, during infusion of ketanserin (10-mg bolus injection dissolved in 20 ml of saline in 3 min, followed by constant infusion of 2 mg/h), and after 3 days of oral treatment with ketanserin (40 mg t.i.d.).

An antecubital vein of the right arm was cannulated for infusion of ketanserin or matching placebo (saline) infusion. The left hand was supported on an arm rest at midchest position, with the patient supine. A 22-gauge needle (Quick-Cath, Travenol Laboratories, Castlebar, Ireland) with extension with "T" (Abott, Sligo, Ireland) and short polyethylene tubing was inserted into a dorsal hand vein of the left hand. The needle in the hand vein was connected to a Gould Statham P23 ID pressure transducer with a sterile diaphragm dome and an infusion pump (Perfusor, Braun, Melsungen, West Germany) delivering saline at a constant infusion rate of 55 ml/h. Venoconstriction, locally induced in the hand vein, will increase resistance to flow and thereby cause a rise in pressure. The pressure signal was recorded on a direct-writing Hewlett–Packard multigraph. Serial dilutions of serotonin (100–10,000 ng/ml) and noradrenaline (100–5000 ng/ml) were prepared. Incremental doses of serotonin and noradrenaline (volume: 1 ml) were injected in the hand vein at regular intervals. Contractions induced in the vein produced elevations of pressure, which returned to baseline in 10–15 min. The area under the curve was taken as a measure of the venous response. Recordings of a hand vein sensitivity test are shown in Fig. 1.

For studying the effect of ketanserin on the venoconstrictor action of serotonin and noradrenaline, identical doses of each amine were given during infusion of saline, during infusion of ketanserin, and after oral treatment with ketanserin. Venoconstriction pressure curves are influenced by the diameter and filling of the vein and by the position of the hand. As a consequence, the potency of serotonin and noradrenaline to produce venoconstriction showed considerable variance when studied at different occasions. For studying the effect of oral treatment with ketanserin, the results were standardized by taking the ratio between the responses to serotonin and noradrenaline.

MEASUREMENT OF DIGITIAL BLOOD FLOW

In 11 patients with primary Raynaud's phenomenon, the effect of ketanserin (10 mg i.v.) on digital blood flow was followed. The skin temperature of the volar portions of digits I, III, and V of the right hand was measured with thermistors (Yellow Springs Instruments, Cleveland, Ohio, U.S.A.) and recorded semicontinuously. Seven measurements per finger per minute were taken. After 1 h of supine rest in a climatized room (temperature: 22–24°C), ketanserin (10 mg dissolved in 20 ml of saline) was injected in an antecubital vein of the left arm. The effect of ketanserin on digital skin temperature was followed for 30 min. In a subgroup of patients, a second injection of ketanserin (10 mg i.v.) was given after pretreatment with the nonselective α-antagonist phentolamine (20 mg i.v.) and after 2 weeks of oral treatment with prazosin (2 mg t.i.d for the first week, followed by 4 mg t.i.d. for the second week).

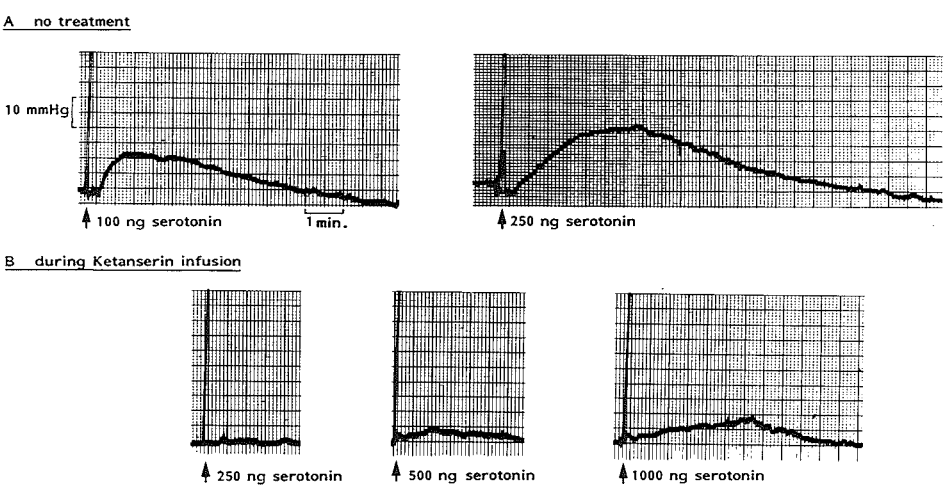

FIG. 1. Serotonin-induced venoconstriction. **A:** Response of hand veins without pretreatment. **B:** Attenuated response to serotonin during infusion with ketanserin.

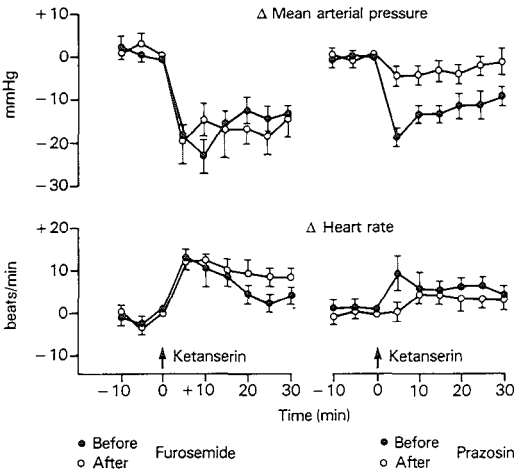

FIG. 2. Effects of pretreatment with furosemide or prazosin on the hypertensive action of ketanserin (10 mg i.v.) in patients with essential hypertension. Mean arterial pressure was 114 ± 8 mm Hg in the patients who were to be treated with prazosin and 116 ± 6 mm Hg in patients to be treated with furosemide. After 1 week of treatment, mean arterial pressure was 103 ± 6 mm Hg in the prazosin group and 102 ± 4 mm Hg in the furosemide group. (From ref. 3, with permission of the American Heart Association.)

RESULTS

Effect on blood pressure

In 30 patients with essential hypertension, arterial pressure fell from 177 ± 5/86 ± 3 mm Hg before ketanserin to 139 ± 6/68 ± 3 mm Hg ($p < 0.001$) after ketanserin injection. Heart rate rose from 68 ± 2 to 78 ± 3 beats/min ($p < 0.01$). After 2 h, mean arterial blood pressure was still below baseline. After pretreatment with prazosin, the blood pressure response to ketanserin was blunted (Fig. 2), whereas pretreatment with furosemide did not affect the response to ketanserin.

In patients with autonomic insufficiency, arterial pressure fell from 128 ± 8/66 ± 3 mm Hg before ketanserin to 95 ± 5/54 ± 2 mm Hg ($p < 0.001$) after ketanserin injection (Fig. 3). In contrast, phentolamine (20 mg i.v.) had no effect on blood pressure in these patients. Neither ketanserin nor phentolamine had an effect on heart rate in these patients.

Hand vein sensitivity test

Intravenous infusion of ketanserin significantly reduced the serotonin-induced contraction of the hand veins of patients with essential hypertension, whereas it had no effect on noradrenaline-induced venoconstriction (Figs. 1 and 4). In four patients the constrictor effect of serotonin on hand veins was also studied after oral treatment with ketanserin (40 mg t.i.d. for 3 days). Oral treatment with ketanserin caused an attenuation of the response to serotonin in these patients (Table 1).

Effect on digital blood flow

After acclimatization for 1 h at an ambient temperature of 22–24°C, the mean temperature of the fingers of 11

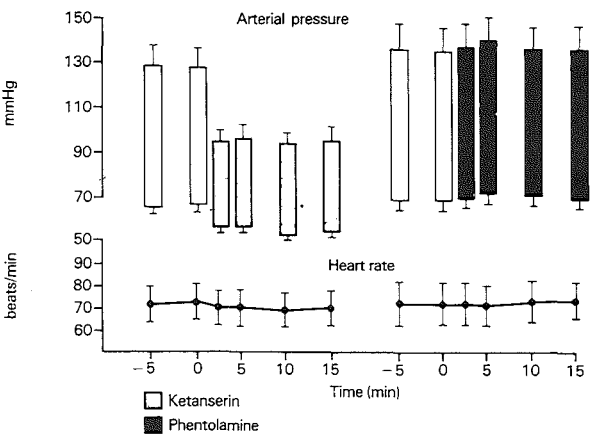

FIG. 3. Effects of ketanserin (10 mg i.v.) and phentolamine (20 mg i.v.) on arterial pressure and heart rate in four patients with autonomic insufficiency. (From ref. 5, with permission of Raven Press.)

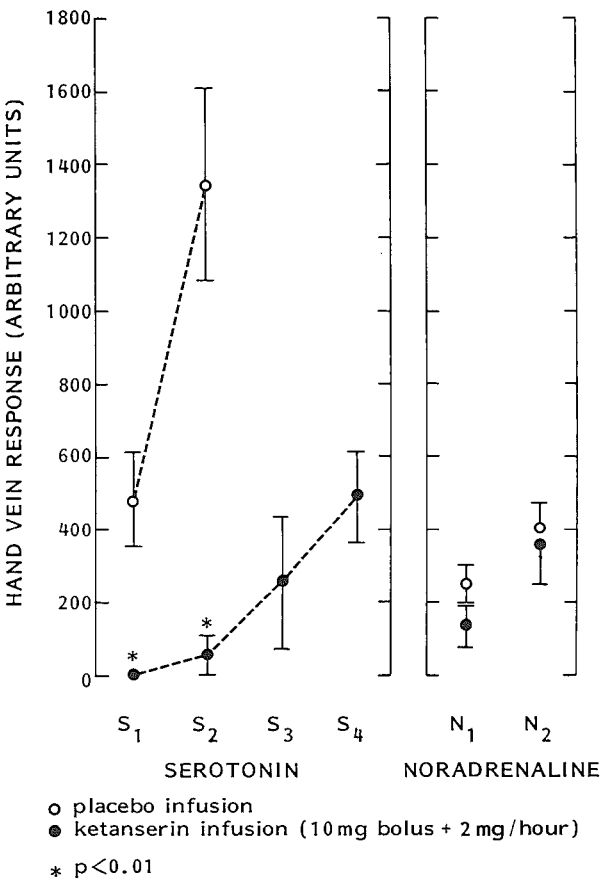

FIG. 4. Constrictor responses of hand vein to serotonin **(left)** and noradrenaline **(right)** in six patients with essential hypertension.

patients with Raynaud's phenomenon was 24.2 ± 0.8°C. Ten minutes after injection of ketanserin (10 mg i.v.), this pathologically low skin temperature rose to 31.5 ± 0.7°C ($p < 0.001$). The nonselective α-antagonist phentolamine (20 mg i.v.) induced a small increase in digital skin temperature, after which ketanserin (10 mg i.v.) caused an additional rise in digital temperature. Chronic oral pretreatment with prazosin did not affect basal digital skin temperature. The rise in digital skin temperature after injection of ketanserin was comparable

TABLE 1. *Effect of ketanserin on serotonin-induced contractions of the dorsal hand vein*

Patient no.	No treatment		Ketanserin intravenously				Ketanserin orally	
	S1:1/2N	S2: 1/2N	S1: 1/2N	S2: 1/2N	S3: 1/2N	S4: 1/2N	S1: 1/2N	S2: 1/2N
1	0.7	8.7	0	0	2.8	26.7	0.7	1.2
2	1.8	3.3	0	0	0	3.6	0.3	0.5
3	2.3	5.7	0	0	0.2	2.8	0.6	7.8
4	1.4	5.2	0	0.5	1.8	—	0.1	1.2

The response to different doses of serotonin (S1, S2, S3, and S4) was divided by the mean response (1/2N) to two doses of noradrenaline (N1 and N2) (see Fig. 4). The response of the hand vein to serotonin was reduced both after intravenous and oral administration of ketanserin.

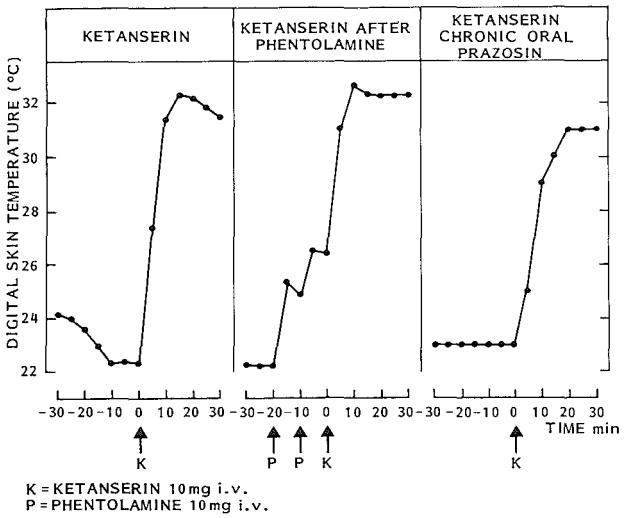

FIG. 5. Effect of ketanserin on digital flow in a patient with Raynaud's phenomenon without pretreatment **(left)**, after phentolamine (20 mg i.v., **middle**), and after chronic oral treatment with prazosin (4 mg t.i.d., **right**).

to the rise in temperature without pretreatment with prazosin. An example of the effect of ketanserin on digital flow is shown in Fig. 5. More detailed results of this study have been submitted for publication.

Additional evidence that ketanserin may increase digital blood flow independently of an α-adrenoceptor-mediated effect was obtained in a patient who underwent a complete unilateral (left-sided) sympathectomy. Increase in digital skin temperature after administration of ketanserin was more pronounced and of longer duration in the hand devoid of sympathetic tone (Fig. 6).

DISCUSSION

In the present study, i.v. ketanserin blocked the response of hand veins to serotonin. Serotonin-induced

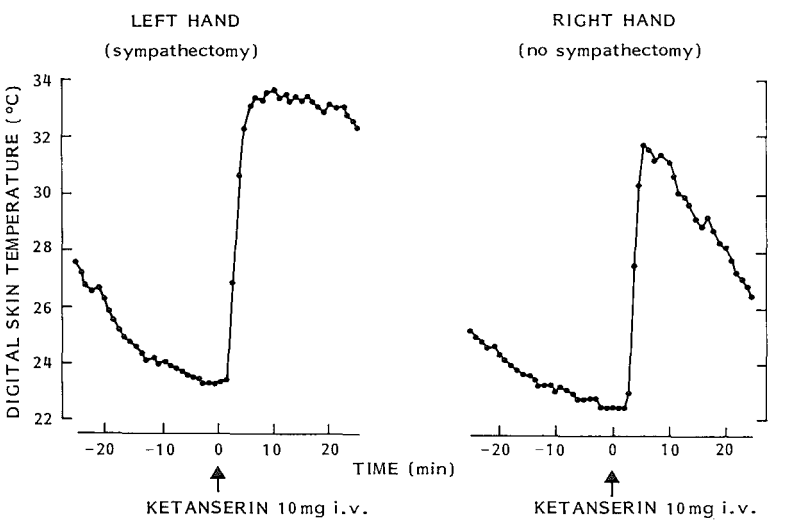

FIG. 6. Effect of ketanserin (10 mg i.v.) on digital skin temperature in a 24-year-old man who underwent a left-sided cervical sympathectomy. The increase in digital flow was more pronounced and of longer duration in the hand without sympathetic tone.

venoconstriction was also attenuated during oral treatment with ketanserin. This effect of ketanserin seems to be independent of α-adrenoceptors, since i.v. ketanserin did not affect the venoconstrictor response to noradrenaline.

Also, the increase of digital flow (at least part of it) after administration of ketanserin does not seem to be mediated by α-adrenoceptors, since the response to ketanserin was not blocked by pretreatment with phentolamine or prazosin. Furthermore, our observation that ketanserin had a hypotensive effect in patients with autonomic insufficiency, who were unresponsive to phentolamine, is an indication that ketanserin is capable of lowering arterial blood pressure independently of α-adrenergic blockade.

The above findings do not exclude that α-adrenoceptor antagonism is involved in the blood pressure lowering effect of ketanserin in normal and hypertensive subjects. In fact, our previous observations that the antihypertensive effect of ketanserin was blunted by pretreatment with prazosin may suggest an α-adrenoceptor-mediated component (3). However, the findings we report here suggest that α-adrenergic blockade is not the sole mechanism of ketanserin's cardiovascular actions.

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CHAPTER 8

SEROTONIN, KETANSERIN AND RAYNAUD'S PHENOMENON

Acute effects and mechanisms of action of ketanserin in patients with Raynaud's phenomenon.

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ACUTE EFFECTS AND MECHANISM OF ACTION OF KETANSERIN IN PATIENTS WITH PRIMARY RAYNAUD'S PHENOMENON

SUMMARY

This study evaluates the vasoactive effects and mode of action of ketanserin, a selective $5HT_2$ receptor antagonist, on digital circulation in 11 patients with primary Raynaud's phenomenon. Reflex digital vasoconstriction was induced by moderate body cooling. We found that ketanserin, 10 mg i.v., normalized digital skin temperature and digital blood flow estimated by venous occlusion plethysmography and laser-Doppler flowmetry. These effects were much more pronounced than with administration of a high dose of the non-selective alpha- adrenoceptor antagonist phentolamine. In order to differentiate between effects of ketanserin on capillary nutritional flow and on arteriovenous (AV) shunt flow, digital blood flow was also assessed by transcutaneously measured oxygen pressure ($TcPO_2$). Effects of ketanserin on $TcPO_2$ were modest. This could imply a preferential effect of ketanserin on AV shunt flow. Pretreatment with high doses of the $alpha_1$-selective antagonist prazosin and with phentolamine did not diminish the effects of ketanserin on digital blood flow. Ketanserin caused a small drop in systemic blood pressure in these normotensive subjects. This effect was blunted by pretreatment with the alpha-adrenoceptor antagonists. We conclude that $5HT_2$ receptors are present in digital blood vessels and that the principle mechanism of digital vasodilatation after ketanserin is blockade of these receptors. In primary Raynaud's phenomenon activation of these receptors plays an important role in cold induced digital vasoconstriction.

INTRODUCTION

Primary Raynaud's phenomenon is characterized by episodic reversible vasospasms of blood supply to fingers and toes, triggered by cold exposure or emotional stress.(1-4) The often severe vasoconstriction has been suggested to be a consequence of overactivity of the sympathetic nervous system,(5) but therapy with alpha-adrenoceptor blocking agents has been disappointing.(6)

The circulation of the human digit is complex in that two vascular beds exist in parallel. There are large numbers of arteriovenous anastomoses (AVA), on the finger tip in particular.(7,8) These AVA's play an important role in the temperature regulation of the fingers; in a warm environment, they are the major pathway of digital blood flow while they are almost closed during cold exposure. The capillary vascular bed supplies the nutritional digital blood flow, which must be maintained even during extreme cold exposure. If the temperature drop is sufficiently drastic and prolonged, mechanisms promoting a decrease in flow through vasoconstriction can be overcome by factors tending to cause "cold" vasodilatation.(9,10)

Unfortunately our knowledge about this system of multiple interacting and opposing forces is very limited. It has been shown that an AVA receives a substantial amount of innervation by adrenergic sympathetic nerves (11) but relatively little information is available concerning the contribution of other vasoconstrictor and vasodilator mechanisms.(12-15) Moreover much of our thinking about arteriovenous shunt flow in the digital vascular bed is based on the difference between total digital blood flow as measured by capacitance plethysmography and the clearance rate of subcutaneous deposits of $Na^{131}I$- or $^{22}Na^{+}$.(16,17) The fairly constant clearances of $Na^{131}I$- or $^{22}Na^{+}$, in the face of a wide range of plethysmographically determined digital flows, have led to the assumption that these substances measure capillary blood flow but the evidence for this is rather circumstantial. Both substances are electrically charged and chemically active and, at high flow rates, their clearance is diffusion limited, which makes it hazardous to assume that they really measure skin capillary blood flow.(18)

In vitro isolated arteries and veins from the hand of healthy humans are contracted by serotonin with an intrinsic activity approximately equal to that of noradrenaline and adrenaline.(19) Direct infusion of serotonin into the brachial artery of humans caused a drop in the digital temperature and induced the characteristic sequential skin discoloration of Raynaud's phenomenon.(20,21) To date it is unknown whether these effects are mediated by changes of arteriovenous shunt flow or capillary blood flow.

Recently three major subclasses of serotonin receptors haven been identified.(22) Contraction of vascular smooth muscle is induced by activation of $5HT_2$ receptors.(23) Ketanserin is a selective $5HT_2$ receptor antagonist.(24) Unlike most other serotonergic antagonists, it has no partial agonist activity. During its initial clinical evaluation as an antihypertensive agent, ketanserin appeared to improve symptoms in patients with Raynaud's phenomenon(25-27), raising the possibility of a role for serotonin in this condition. However, in addition to its serotonergic antagonist properties, ketanserin inhibits alpha-adrenergic receptors.(28) Although it has been shown that ketanserin is capable of lowering systemic blood pressure independently of alpha-adrenoceptor blockade, blockade of these receptors is likely to contribute to its hypotensive effect. Thus it is possible that the beneficial effect of ketanserin on digital blood flow is also related to blockade of alpha-receptors rather than $5HT_2$ receptors.

The purpose of the present study was to explore the mode of action of ketanserin in patients with primary Raynaud's phenomenon. Changes of digital blood flow after ketanserin administration were studied in the presence and absence of $alpha_1$- and $alpha_{1+2}$-adrenoceptor blockade. In addition, and maybe more importantly, we have tried to determine whether ketanserin's actions concerned arteriovenous shunt flow or capillary nutritional blood flow (Fig.1). For this purpose the effects of ketanserin on total finger blood flow (digital skin temperature, venous occlusion plethysmography) were compared with skin blood flow measurements (laser-Doppler flow, transcutaneous measured oxygen pressure).

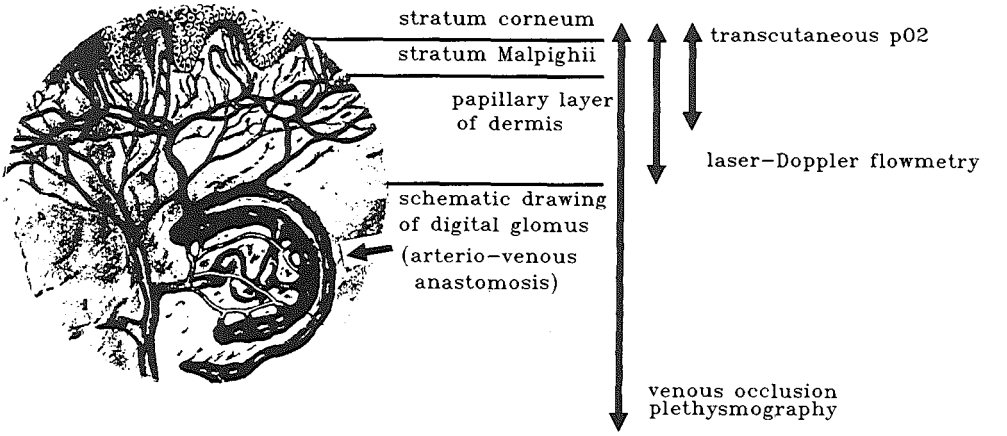

Figure 1.
Schematic drawing of digital cutis with different layers and a glomus, the arteriovenous anastomosis that connects subpapillary arterioles and venules. This specialized vascular structure is supplied from the subpapillary plexus, which runs in parallel with the skin surface. Venous occlusion plethysmography obviously measures total digital blood flow. Laser light, after penetrating the layer of keratin, problably will reach the arteriovenous anastomes. The papillary layer of the dermis is scanned by the transcutaneous oxygen monitor.

PATIENTS AND METHODS

PATIENT SELECTION

Eleven patients with primary Raynaud's phenomenon, nine women and two men, aged 37± 4 years (mean±SEM), consented to participate in the study which was approved by the local Hospital Ethical Review Committee. They were selected from the outpatient clinics of the departments of internal medicine and dermatology at the university hospital "Dijkzigt". They all had reversible vasospastic attacks in response to cold. History revealed that symptoms existed for more than 3 years. After cold provocation all subjects had a lower digital skin temperature and longer temperature recovery time than healthy volunteers. They fulfilled the clinical criteria of Allen and Brown (29) for classification as primary Raynaud's phenomenon. In addition, they had normal physical examinations and normal results were obtained by laboratory screening including the absence of serum antinuclear antibodies and serum cryoglobulin. Medical treatment, if any, was stopped three weeks before the study. The use of a new vasoactivedrug was explained to the patients, who all gave their informed consent to the study.

STUDY DESIGN

Patients were seen at three separate sessions, 7-9 days apart, during the period from december to march. All investigations were carried out in the morning. Patients came to the cardiovascular laboratory after a light breakfast. They had refrained from smoking and drinking coffee since the preceding evening. An antecubital vein of the left arm was cannulated. Telethermometers and the equipment (see methods) for measurements of digital blood flow and transcutaneous oxygen tension were attached to the fingers of the right hand. Studies were performed after acclimatization for at least one hour in a 20-21°·C room. The patients were lightly clothed and lay supine on bed. The right hand was held stable on a sand bag slightly above heart level. Upon achieving stable baseline values 20 ml of saline solution (9 g/l) was infused as placebo followed about ten minutes later by 10 mg ketanserin dissolved in a volume of 20 ml *(study 1)*. A week later the same protocol was followed but now the injection of ketanserin was preceded by an infusion of phentolamine, 20 mg dissolved in a volume of 20 ml *(study 2)*. Six patients were then given prazosin, t.i.d. orally, for at least one week in increasing maximal tolerable doses and then restudied with ketanserin 2 to 3 hours after the last dose of prazosin, which always was 4 mg *(study 3)*.

MEASUREMENTS

Temperature.

Digital skin temperature was measured at the volar side of the distal phalanx of digits I, II and V by three copperconstantan thermocouples (Telethermometers, Yellow Spring Instruments, Cleveland, USA). Seven measurements per finger per minute were taken and averaged.

Venous occlusion plethysmography (VOP).

Total finger flow was measured by a venous occlusion plethysmograph (Periflow) developed by Janssen Scientific Instruments (Beerse, Belgium). The venous occlusion cuff was placed just distal of the metacarpophalangeal joint of digit III and intermittently triggered by an electrocardiographic signal to inflate to a venous occlusion pressure of 60-80 mmHg (2 heart beats occlusion, 1 beat release).
A mercury - in - silastic strain gauge was placed just distal to the proximal interphalangeal joint. Flow readings were estimated from 10 successive pulses.

Transcutaneous oxygen tension ($TcPO_2$).

Oxygen diffusing through the skin of the volar surface of digit IV was measured by a polarographic technique using a transcutaneous PO_2 sensor (Clark-type electrode) with a temperature- regulated heating element (Radiometer TCM-i, monitor, Radiometer, Copenhagen, Denmark). The probe was calibrated according to the manual. The skin at the measurement site was prepared by rubbing with alcohol and stripping off the stratum corneum with adhesive tape. The probe was mounted on the skin via a double-sided adhesive ring and a drop of contact gel(Radiometer). The skin below the electrode was heated to 44°C and heat consumption of the probe was measured. At least 30 min was allowed to permit $TcPO_2$ to stabilize.

Laser-Doppler blood flow (LDF) .

LDF was measured with a Periflux PF 1c laser-Doppler flow meter (Perimed KB, Stockholm, Sweden). Briefly, the method involves conducting a coherent 2-mW light from a He-Ne laser via a fiber optic light guide to the tissue surface. Reflected light is gathered by another set of light guides and processed. This procession involves analog computation of the power spectral density of shifted light which, in theory, is a linear function of the average velocity of moving cells within the tissue. The LDF probe was positioned over the distal volar surface of the index finger and held by a black plastic disk supplied by the manufacturer. The disk, in turn, was affixed to the skin by an adhesive ring. Special care was taken to support the fiber optic cable and to keep the arm stationary. The same gain (x5), electronic zero (0 mV, by shining the laser probe on a stationary surface), upper frequency cutoff (4 kHz), and time constant (3 s) were used throughout the study. Data were expressed as arbitrary LDF units (AU), which represent percent values of full-scale deflection on the instruments meter.

STATISTICAL ANALYSIS

Data are given as means ± SEM. Evaluation of results was done with the t-test for paired data (study 1) or the Wilcoxon signed-rank test. Statistical significance was set at the level of 0.05, with two tailed values for p.

RESULTS

Study 1 Effects of ketanserin (n=11).

Moderate body cooling, i.e. one hour at an ambient temperature of 20-21°C, induced severe digital vasoconstriction in our patients. Skin temperature of the digits was low, 22.8 ± 0.5°C, and measurements of finger blood flow by venous occlusion plethysmography and laser-Doppler flowmetry showed that hardly any blood was entering the digits. Compromised skin perfusion was also reflected by low levels of transcutaneous oxygen tension ($TcPO_2$), 19 ± 3.4 mmHg (normal: 44 ± 5.2 mmHg, n=14).Ketanserin infusion but not saline had a pronounced effect on digital circulation. An example of a typical registration is shown in Fig. 2. It illustrates the discrepancy between huge increases of temperature and flow in comparison with the modest rise of $TcPO_2$.

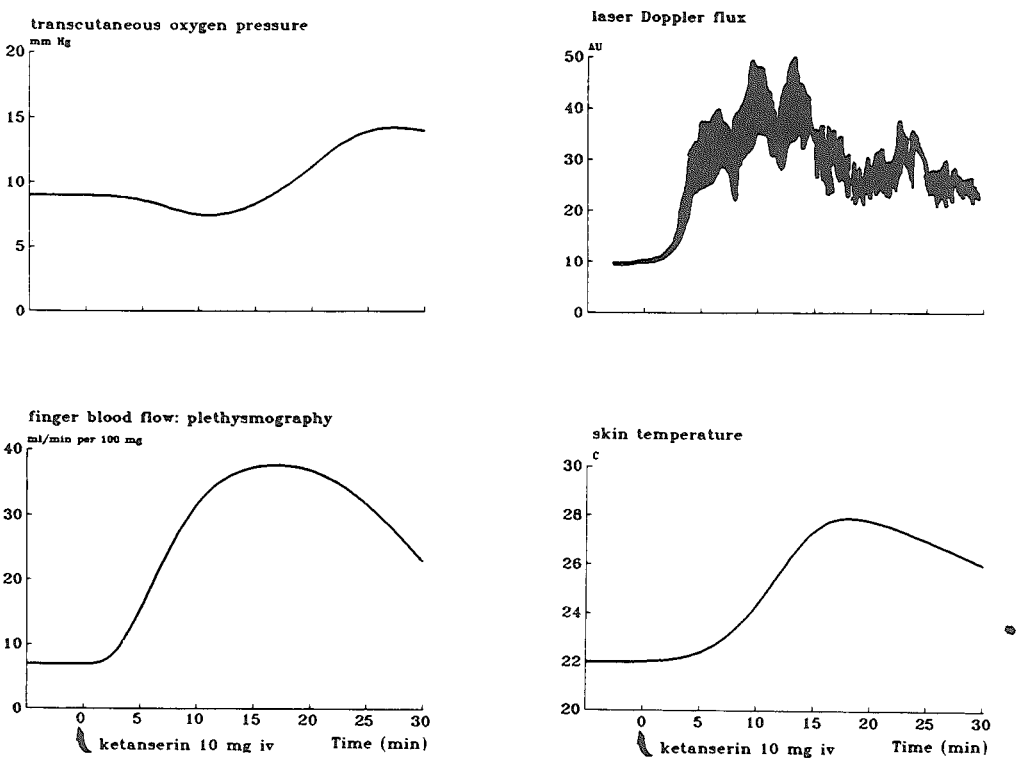

Figure 2. Responses of finger skin temperature, finger blood flow (plethysmography, laser-Doppler flux) and transcutaneous oxygen pressure after intravenous infusion of 10 mg ketanserin are shown for one patient. Digital vasoconstriction was provoked by moderate body cooling (room 20°C). Note that transcutaneous oxygen pressure remained low despite a pronounced increase in digital perfusion.

Fig. 3 summarizes the effects of ketanserin on digital skin temperature of the eleven patients studied. Fingertip temperature recovered from 22 ± 0.5 to 31 ± 0.7°C ($p < 0.001$) and the color of the digits changed from white-blue to pink red.

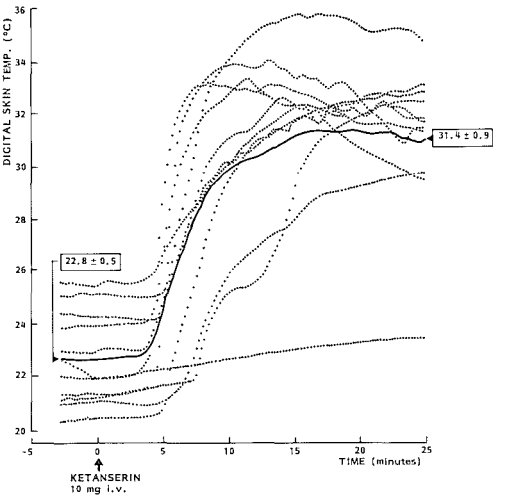

Figure 3. Response of individual finger skin temperatures to a single intravenous dose of 10 mg ketanserin in 11 patients with primary Raynaud's phenomenon. Each line represents the mean of 3 simultaneous readings (fingers I, II and V).

Fig. 4 depicts group averages of changes in digital blood flow and $TcPO_2$. Total finger blood flow rose from 8 ± 0.9 to 31 ± 2.2 ml/min per 100 ml tissue ($p < 0.001$) and subpapillary blood flow (laser-Doppler) increased from 9±2 to 45 ± 4 AU. ($p < 0.01$). In contrast, changes of $TcPO_2$ were modest. Thirty minutes after ketanserin $TcPO_2$ was not significantly higher compared to baseline values (19 ± 3.2 versus 23 ± 5.1 mmHg).

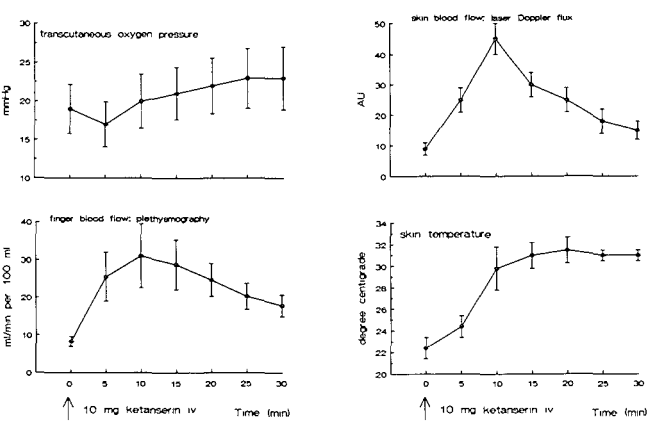

Figure 4. The effects of intravenous ketanserin (10 mg) on finger skin temperature, skin blood flow, total digital blood flow and transcutaneous oxygen in 11 patients with primary Raynauds' phenomenon. Changes in temperature, laser-Doppler flux and plethysmographic flow were significant ($p < 0.01$) throughout the observation period. The slow rise in transcutaneous oxygen pressure did not reach statistical significance.

Pronounced differences in response between individual patients were noted (Fig. 5).

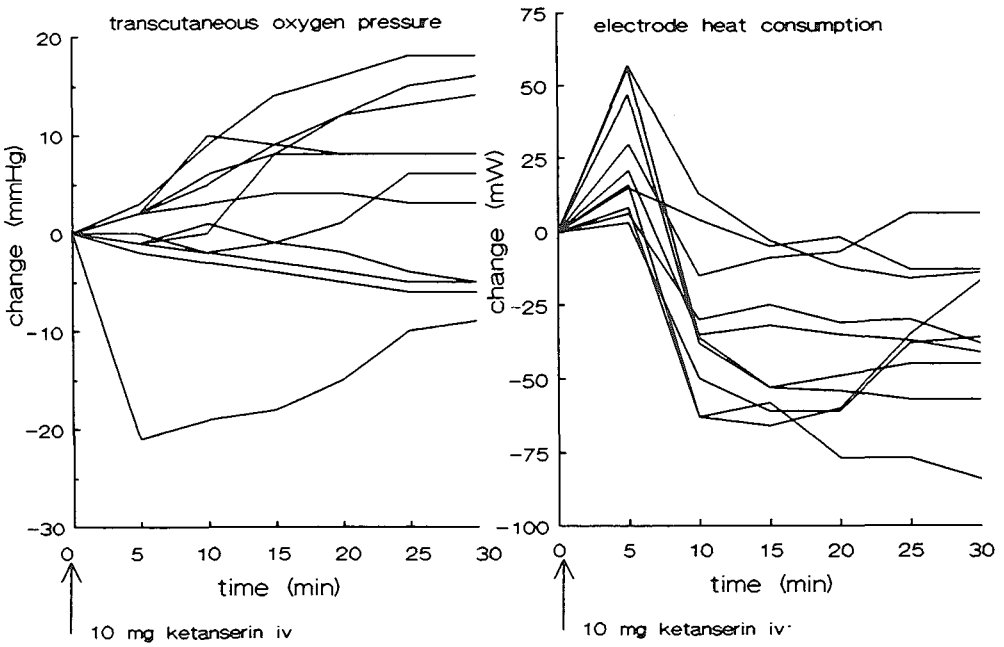

Figure 5. Individual values for changes in transcutaneous oxygen pressure and heat consumption of the Clark-type electrode after administration of ketanserin. Note the biphasic response of heat consumpt

However a consistent finding was that after ketanserin heat consumption of the oxygen probe first rose and then fell below baseline (Fig. 5). There was no correlation between $TcPO_2$ and the maximum responses of digital blood flow measured either by occlusion plethysmography or by temperature change. After ketanserin systemic blood pressure fell from $114 \pm 4 / 71 \pm 3$ to $108 \pm 4 / 66 \pm 2$ mmHg ($p < 0.01$) and heart rate rose from 69 ± 2 to 76 ± 2 bpm ($p < 0.01$).

Study 2 Effects of ketanserin during phentolamine infusion (n=6).

During the pre-drug period, i.e. during body cooling, digital skin temperature and total finger blood flow did not differ significantly from baseline values measured during study 1 (Table 1). High doses of phentolamine caused significant digital temperature and blood flow changes (Table 1, Fig. 6). However these effects were modest as compared to the effects of ketanserin in study 1. After phentolamine ketanserin further increased finger temperature and flow to the level seen during study 1. However during phentolamine the effects of ketanserin on systemic blood pressure and heart rate were blunted.

Study 3 Effects of ketanserin after pretreatment with prazosin (n=6).

Pretreatment with high oral doses of prazosin did not prevent digital vasoconstriction produced by body cooling (Table 1). Ketanserin, 10 mg i.v., under these circumstances increased digital skin temperature and flow to the levels seen during studies 1 and 2 (Fig. 6). In the presence of prazosin ketanserin had no effect on systemic blood pressure or heart rate.

Table 1. Comparison of measurements made in the six patients who participated in all three studies.

	digital skin temp. (°C)	digital blood flow (ml/min per 100ml)	blood pressure systolic (mmHg)	blood pressure diastolic (mmHg)	heart rate (bpm)
study 1					
placebo	25 ± 1.2	8 ± 4	115 ± 3.9	72 ± 2.7	69 ± 3.4
ketanserin	32 ± 0.5*	33 ± 6*	110 ± 4.4*	66 ± 2.6*	71 ± 3.3*
study 2					
placebo	24 ± 1.4	9 ± 4	111 ± 4.2	69 ± 4.2	66 ± 3.6
phentolamine	27 ± 0.8#	18 ± 5#	105 ± 4.7#	64 ± 3.5#	76 ± 5.2#
ketanserin	30 ± 0.9*¤	31 ± 6*¤	105 ± 6.0*	64 ± 3.2*	80 ± 6.2*¤
study 3					
prazosin	25 ± 0.8	9 ± 4	113 ± 5.4	71 ± 3.2	73 ± 4.1
ketanserin	31 ± 0.9+	34 ± 7+	113 ± 6.0	71 ± 3.5	73 ± 3.5

* p <0.05 ketanserin vs placebo
\# p <0.05 phentolamine vs placebo
¤ p <0.05 ketanserin vs phentolamine
\+ p <0.05 ketanserin vs prazosin

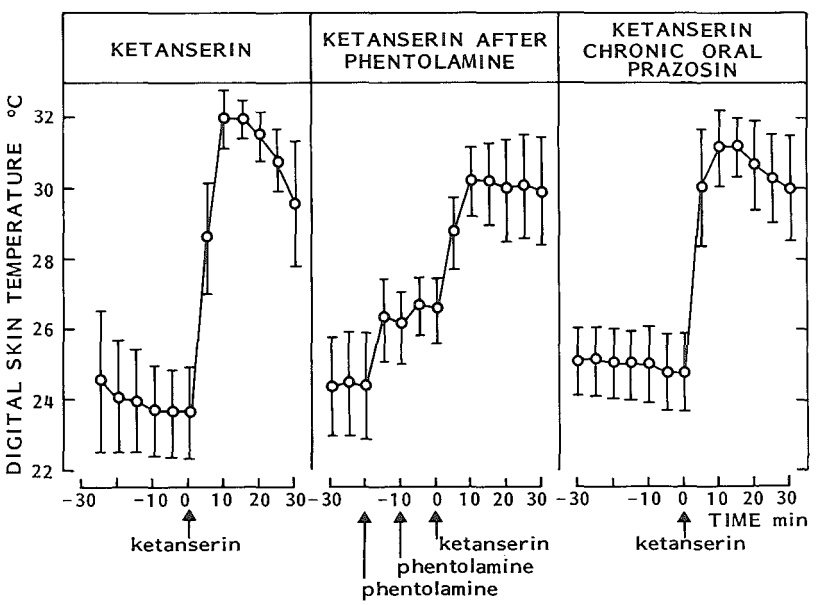

Figure 6. Effects of ketanserin infusion on digital skin temperature in the presence or absence of selective and nonselective alpha-adrenoceptor blockade (n=6). For statistical evaluation see Table 1.

DISCUSSION

Ketanserin: serotonergic or alpha-adrenoceptor blockade?

In agreement with earlier data obtained by us (30) and by others, (25,31-33) the present study shows that ketanserin given intravenously has marked effects on digital blood flow in patients with Raynaud's phenomenon. In previous work, however, the relative contributions of the $5HT_2$-serotonergic and $alpha_1$- adrenergic antagonistic properties to the vasodilatory effect of ketanserin have not been assessed. The present study explores this issue. We deliberately chose body cooling in the presence or absence of alpha-adrenoceptor blockade as the stimulus to decrease digital blood flow. It has been shown that body cooling leads to a strong reflex sympathetic vasoconstriction in this particular vascular bed.(12) These effects of the sympathetic nervous system are mediated on specialized vascular structures in the digital skin, namely arteriovenous anastomoses (AVA). AVA's play an important role in temperature regulation of the body and fingers; in a warm environment, they may be the pathway of greater than 80% of the digital blood flow while in a cool environment they are almost closed. An AVA and its associated related vessels and nerves constitutes a "glomus". Microscopically an AVA can be easily differentiated from other vessels by its thick wall surrounding a barely perceptible lumen.(8) By comparison a collecting venule is thin walled with a large lumen. The absence of elastic membranes in the AVA problably makes that an AVA can close completely. Adrenergic nerves are densely spread on the outer surface of the glomus cell layer and their innervation resembles a thread densely wound around a bobbin.(11)

Under conditions of reflex sympathetic vasoconstriction, however, the increase in digital blood flow seen with $alpha_1$- or $alpha_{1+2}$- adrenoceptor antagonists is sometimes not very large and seldom reaches the flow seen in warm, vasodilated subjects.(34) The present study confirms this. Administration of the non-selective alpha-adrenoceptor antagonist phentolamine in a dose considered to cause virtually complete alpha-blockade had a modest effect on digital blood flow as compared to ketanserin. In contrast, with ketanserin the low temperature of the fingers of our patients, during body cooling, returned to normal and the white-blue discoloration of the digits changed into pink-red. It is unlikely that these effects of ketanserin are due to the weak $alpha_1$-adrenoceptor antagonistic property of the drug. This conclusion is supported by the fact that ketanserin was able of causingvasodilatation also when $alpha_1$- adrenoceptors were blocked by high doses of prazosin. In addition, ketanserin caused a pronounced further increase in digital skin temperature in the presence of $alpha_{1+2}$-adrenoceptor blockade with phentolamine.

Recently, the differential contribution of $alpha_1$-and $alpha_2$-adrenoceptors to contractile responses to norepinephrine in human limb arteries was assessed.(35) Proximal (dorsalis pedis artery of the foot and superficial palmar arch of the hand) and distal (digital arteries of the hand and foot) arteries were obtained from patients undergoing amputation for reasons other than vascular disease. In proximal vessels, the $alpha_1$-adrenergic antagonist prazosin was more potent at inhibiting the responses evoked by high concentrations of norepinephrine compared to the $alpha_2$-adrenergic antagonist rauwolscin. In distal blood vessels, the responses to norepinephrine were not affected by either prazosin or rauwolscine, but were depressed by the combination of the two antagonists. Moreover, it has been shown that local conditions can alter the relative importance of the subtypes of alpha- adrenoreceptors.(36) For example, cooling caused an instantaneous increase in the affinity of postjunctional adrenoceptors for the agonist. This involved however mainly $alpha_2$-adrenoreceptors. Thus, from a physiological standpoint the postjunctional $alpha_2$-

adrenoreceptor may be more important in mediating vasoconstrictor responses during prolonged, intense, cold-induced, sympathetic nerve activity than the alpha$_1$-adrenoreceptor.

Ketanserin is certainly not an alpha$_2$-adrenoceptor antagonist. It is a selective 5HT$_2$-receptor antagonist with some alpha$_1$-blocking properties. Therefore our results indicate and confirm that 5HT$_2$-serotonergic receptors are present in the digital vasculature. Their blockade by ketanserin in "reflex" vasoconstricted patients leads to an important increase in digital blood flow. Digital alpha-adrenergic blockade seems not to be essential for this action of ketanserin.

Along other lines of evidence Arneklo-Nobin and Owman (37) came to the same conclusion. They tested isolated hand arteries from healthy human subjects for their contractile response to noradrenaline and serotonin. Serotonin was as potent on hand arteries as noradrenaline, ED50 being similar for the two substances. Ketanserin counteracted the contractions induced by serotonin in a dose-dependent manner but did not shift the dose-response curve of noradrenaline, indicating that, at the level of digital arteries, ketanserin does not possess affinity for alpha-adrenoceptors.

In contrast to the effects of ketanserin on the digital vascular bed, pretreatment with prazosin or phentolamine abolished the modest blood pressure lowering effect of ketanserin in our normotensive Raynaud patients. This is in agreement with our previous findings in hypertensive patients.(38) In the systemic circulation a certain degree of alpha$_1$-adrenoceptor blockade may contribute to the hyprtensive effect of ketanserin. However, ketanserin is also capable of lowering blood pressure independently of its effects on alpha-adrenoceptors.In the finger, blockade of serotonergic receptors appears to be the mechanism for its vasodilating effect.

Taken together, our findings indicate that serotonin probably plays an important role in cold-induced digital vasoconstriction. The source of serotonin causing this effect is not known. Serotonergic nerves like those present in cerebral blood vessels could be involved (39) but a more likely source of serotonin appears to be the aggregating blood platelet.(40) It has been shown that spiral strips of human digital arteries contract vigorously when exposed to a suspension of platelets aggregated by thrombin.(41) Pretreatment of the arteries with ketanserin nearly abolished the contractile response to platelets but did not affect the contractile response to potassium chloride. In normal volunteers Cowley et al (42) found a rather close correlation between the maximum reduction of forearm blood flow in response to cold and the concentration of sodium arachidonate that was required to induce platelets from each individual to aggregate and to liberate serotonin, indicating that cold exposure somehow had altered, at least temporarily, platelet behavior. In addition, cold-induced reduced blood flow and diminished serotonin degradation could contribute to high local serotonin concentrations.

Ketanserin: AV shunt flow but not nutritional blood flow?

In contrast to the large increments of digital skin temperature and digital blood flow after ketanserin, $TcPO_2$, which was very low in most patients, did not reach normal values. In some patients $TcPO_2$ even fell despite a huge increase in digital blood flow. How to explain this discrepancy and for what does it stand? It could mean that ketanserin did have a much more pronounced effect on arteriovenous shunt flow than on capillary blood flow. However before we accept this explanation, we should know what we actually were measuring with the Clark-type electrode. The most important determinants of the relationship between PO_2 and the transcutaneous PO_2 measured by the probe are 1) the

degree of arterialization of the capillary blood in the skin, i.e. the ratio between the mean capillary and arterial PO_2, 2) the diffusion gradient in the epidermal skin layer between the capillaries and the surface of the probe, and 3) the rate of O_2 consumption in this layer.(43) At a probe and skin temperature of 37°C the transcutaneous PO_2 is much lower than the arterial value. However under normal operational conditions the skin below the probe is heated to about 44°C to produce full local vasodilatation. Problably because of complete thermic arterialization of capillary blood, $TcPO_2$ approaches PaO_2. However, when skin circulation is compromised as in our patients, PaO_2 and $TcPO_2$ remain dissociated despite high probe temperature and full local vasodilatation.

By analysing the relation between $TcPO_2$ and perfusion pressure, Wyss et al (44) and Dowd (45) have shown that $TcPO_2$ falls in non-linear fashion with decreasing perfusion pressure; $TcPO_2$ is little affected by changes in the high perfusion range but much more so by changes in low perfusion pressures. In other words $TcPO_2$ is a sensitive indicator of low perfusion pressure.(46) It is this relationship between $TcPO_2$ and perfusion pressure we tried to explore in the assessment of ketanserin's mode of action. After ketanserin the heat consumption of the probe rose temporarily, indicating that heat under the probe was leaking away, problably as a consequence of an increase of skin blood flow. In addition, laser-Doppler flowmetry showed that blood flow in the subpapillary vascular bed of the skin did increase markedly after ketanserin. Despite these effects, the Clark-type electrode did not pick up more oxygen. A possible explanation could be that the huge increase in subpapillary blood flow after ketanserin somehow shunted the diffusion pathway of oxygen away from the electrode. Furthermore, the rise in skin temperature after ketanserin problably caused the epidermal oxygen consumption to increase. Unchanged values for $TcPO_2$ therefore do not indicate that no more oxygen was delivered but that rather the delicate balance between oxygen demand and supply was not altered by ketanserin.

Unfortunately our knowledge about the multiple factors that affect the measurement of $TcPO_2$ under the experimental conditions of the study is far too limited to draw a firm conclusion. Vital microscopy of nailfold capillaries during ketanserin infusion may give a better insight in what really is going on in the microcirculation of the finger. It has been shown in a small series of patients with primary and secondary Raynaud's phenomenon (47) that oral treatment with ketanserin did increase capillary red blood cell velocity before and after cold provocation. However this increase in capillary perfusion was found only in a minority of patients.

In conclusion, our findings are consistent with the hypothesis that in patients with primary Raynaud's phenomenon serotonin is involved in cold-induced digital vasoconstriction. The results of long-term placebo-controlled studies of the potential therapeutic usefulness of ketanserin in this condition are under way.

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SUMMARY AND CONCLUSIONS

Over the past decade, a great deal of clinical research of our group has been directed toward two major areas of the pathophysiology of hypertension: (1) the significance of the renin-angiotensin system; and (2) the role of serotonin. Serotonin was identified by Irvin Page as, in his words, a "nuisance factor" interfering with his attempt to isolate and identify angiotensin II,in which he fortunately succeeded. The first part of this thesis deals with clinical aspects of Page's second moiety, angiotensin II, and the enzymatic cascade involved in its formation. The second part of the thesis entertains the idea that the "nuisance factor" serotonin deserves more clinical attention than it had enjoyed in the past.

PART 1
ACE INHIBITION

CHAPTER 1

Chapter 1 of this thesis brings together studies which deal with the specificity of captopril's mode of action. We have tried to clarify whether or not pretreatment plasma renin is an important predictor of the blood pressure response to ACE inhibition. Renin has since long been suspected to play a role in the pathophysiology of hypertension, especially in patients with high levels of circulating renin and severe hypertension associated with renal and/or renovascular disease. Indeed captopril proved to be highly effective in this category of patients (Atkinson 1980). Unexpectedly, captopril was also effective in patients with milder forms of hypertension including patients with low levels of circulating renin (De Bruyn 1981, Vidt 1982, Veterans Administration Cooperative Study Group 1983).These findings have aroused many questions.

On theoretical grounds it seems more logical to use the plasma level of angiotensin II rather than renin as a measure of the activity of the renin-angiotensin system in vivo. Unfortunately, the plasma levels of angiotensin II are very low, a few picograms per ml, and the antibodies that are used in the radioimmunoassay crossreact with degradation products of angiotensin II. Angiotensin II measurements are therefore difficult to perform, and, until recently, unreliable in the low range.

In clinical practice plasma renin activity (PRA) is generally accepted as a measure of the formation of angiotensin II in vivo. In the PRA assay, plasma of the patient is incubated at 37°C. During this incubation in vitro, renin is catalysing the generation of angiotensin I from renin substrate. Plasma however has angiotensinase activity, by which angiotensin I is hydrolysed. This degradation of angiotensin I can be prevented by the addition of angiotensinase inhibitors. These substances, however, also inhibit angiotensin converting enzyme. The reaction stops therefore at the generation of angiotensin I. Angiotensin I is measured by radioimmunoassay. Under normal circumstances the plasma concentration of renin substrate (angiotensinogen) equals the Km value, which means that the generation of angiotensin I not only depends on the concentration of renin but also on the concentration of renin substrate.

The interpretation of PRA measurements is difficult in circumstances where the concentration of substrate is not normal (nephrectomized subjects, patients with heart and/or liver failure). Techniques were therefore developed, in which the reaction velocity in vitro is independent of the substrate concentration. In these assays endogenous substrate is selectively destroyed and an excess of purified substrate is added. The generation of angiotensin I then only depends on the concentration of renin.

The quantities of angiotensin I generated in this type of assay of plasma renin concentration (PRC) appeared to be much greater than with the PRA method. In the PRC method the plasma is acidified (pH 3) in order to destroy the endogenous substrate. The increase in renin activity with acidification appeared to depend on the activation of an enzymatically inactive form of renin. It has been suggested that this socalled inactive renin is a proenzyme (prorenin) and that the activation is caused by pH dependent limited proteolysis. It appeared that about 90 percent of renin in plasma is in the inactive form, which is now known to be the biosynthetic precursor of renin, prorenin. These observations have important consequences for both the technical procedure and interpretation of plasma renin determinations. In our studies we report on active renin (or renin) and total renin (renin plus prorenin) concentration.

We found that plasma converting enzyme activity is inhibited by more than 90 percent from 60 minutes up to four hours after a single oral dose of 50 mg of captopril. Active renin rose within 60 minutes after the first dose,and it remained high with chronic treatment. In contrast, prorenin fell initially and rose after four hours. Blood pressure reduction was maximal 60-90 minutes after captopril both in patients with essential hypertension and in renovascular hypertension. A similar time course of blood pressure response was observed in patients with chronic heart failure (very high plasma levels of active renin). Blood pressure came also down in fluid-depleted nephrectomized subjects (active renin approximately zero,inactive renin approximately normal), although the response rate was more sluggish.

The percent changes in blood pressure after ACE inhibition were not much different in these groups of patients despite large differences in the level of plasma active renin. Weak correlations of acute changes in bloodpressure with pretreatment plasma renin were found but they were absent with chronic treatment. The relation between renin and changes in resistance was sligthly better but clinically of no importance.

Most of the circulating angiotensin II originates from angiotensin I-II conversion by ACE that is located at the luminal surface of endothelial cells lining the capillaries, particularly those in the lung. Conversion also occurs in circulating plasma. From our clinical observations we conclude that inhibition of ACE at these two sites is not the only mechanism by which captopril and ramipril exert their antihypertensive action. Originally ACE inhibitors were thought to act mainly through blockade of the circulating renin-angiotensin system. Our observations, however, indicate that other mechanisms must play a role.

A likely alternative explanation for the antihypertensive effect of ACE inhibitors is inhibition of ACE at the tissue level, resulting in diminished local formation of angiotensin II. Such inhibition has been demonstrated in animals to occur in blood vessels, kidneys, adrenals and the brain. Recently, the complete renin-angiotensin system has been demonstrated in several cell cultures, including neuroblastoma, juxtaglomerular cells, adrenal cells, endothelial cells and vascular smooth muscle. Our studies contribute to the growing evidence that local angiotensin II formation within some critical tissues is involved in blood pressure regulation. However, effects of ACE inhibitors on other systems

than the renin-angiotensin system, e.g. the prostaglandin- and the .kallikrein-kinin systems, cannot be excluded.

CHAPTER 2

According to Page good research often originates from shrewd clinical observation. We would like to agree with him. Looking at relationships between pretreatment plasma active renin and the haemodynamic response to ACE inhibition, we were struck by the beneficial haemodynamic effect of a single gift of captopril in hypertensive patients who appeared to be in some state of heart failure (Swan-Ganz catheter positioned for other reason, i.e. measurement of cardiac output). This prompted us to go to heart failure. The results of an open study with captopril in patients with severe acute and treatment resistant chronic heart failure are presented in this chapter. We showed the sustained effectiveness of captopril in these patients. Cardiac output was raised and heart rate, arterial, pulmonary-wedge and pulmonary arterial pressure were reduced. Clinical improvement was often impressive. Nowadys ACE inhibition is considered to be a major advance in the treatment of heart failure.

CHAPTER 3

In the first paper of this chapter we presented evidence that prorenin is a biosynthetic precursor of active renin and that, at least under certain circumstances, a major proportion of circulating prorenin originates from the kidney. In addition we found that changes in release of prorenin and active renin after ACE inhibition did not run in parallel. The findings indicate that prorenin and renin are secreted via different pathways. The renin pathway is rapidly responsive to the stimulation of ACE inhibition (interruption of the negative feedback by angiotensin II), whereas the prorenin pathway is slowly responsive to this stimulus. This is compatible with the view that an acute stimulus only causes the release of intrarenally stored renin, whereas chronic stimulation causes in addition increased intrarenal synthesis and activation of renin. While the relative amounts of active and inactive renin released were variable, and conversion of inactive to active renin was also variable, our findings indicate that the classical "PRA" as such, which does not take into account concentrations of substrate or prorenin, has lost much of its meaning.

In the last two papers of chapter 3 we present evidence that angiotensin II, either circulating or locally formed within the kidney, has an important function in maintaining a normal glomerular filtration, at least in patients with renovascular hypertension. After blocking the formation of angiotensin II by ACE inhibition, glomerular filtration rate of the stenotic kidney fell in all patients. In some of them the affected kidney even stopped filtering. Fortunately, function of the affected kidney returned on discontinuing treatment.

Thus, we are left with a rather unexpected if not totally new perspective. ACE inhibitor therapy has originally been advocated for those forms of hypertension where circulating renin is high, i.e. renovascular hypertension. However, it appears that in these circumstances ACE inhibitor therapy can have major deleterious effects on renal function. Fortunately this is an infrequent and reversible complication. On the other hand we now know, in part because of our own studies, that ACE inhibitors are very effective in essential hypertension, despite that the levels of circulating renin and angiotensin II are normal or even low.

CHAPTER 4

Evidence concerning the clinical utility of a single dose captopril in the diagnosis of renovascular hypertension was evaluated in the first paper of this chapter. Our study included 179 consecutive hypertensive patients who were less than 40 years old with a diastolic blood pressure of greater than 120 mmHg or who had hypertension that was uncontrolled (greater than 100 mmHg) on at least three drugs. Sodium intake was controlled and antihypertensive medication was discontinued for at least two weeks. Patients received 50 mg of captopril after one hour in a recumbent position and had renin samples taken at -30, 0, 60, 120, and 240 minutes. All patients had an arteriogram after the captopril study. Definition of a positive captopril test was determined retrospectively by finding the best balance between sensitivity and specificity of the various criteria from an ROC curve.

Using this method an abnormal test was defined as a plasma active renin concentration of greater than 200 μU/ml one hour after captopril. The sensitivity and specificity of this definition were calculated as 0,93 and 0,84, respectively. We concluded that a one hour post 50 mg captopril renin is a useful criterion for selecting patients for angiography, at least in a hypertension referral clinic where a population of patients is seen with a relatively high prevalence of renal artery stenosis and also with the proviso that the same inclusion criteria for testing are used as in our study.

The second paper of this chapter deals with the diagnostic value of renal vein renin sampling. We used a new immunoradiometric assay to measure renin and we stimulated the secretion of renin prior to sampling by giving an ACE inhibitor. Our study included 116 patients with unilateral renal artery stenosis and 160 patients with normal renal arteries. The renal vein-to-artery renin ratios obtained with the new direct assay proved to be as reliable as those provided by the more laborious indirect assay. In our group of patients with renal artery stenosis more than 90% had a renal vein-to-artery renin ratio on the affected side of 1,5 or more. In patients with normal renal arteries the ratio was always < 1.5. Thus renal vein renin measurements appeared to have little additive diagnostic power over angiography, at least in patients with unilateral renal artery disease.

CHAPTER 5

The purpose of this study was to assess and to compare in the same patient, a twice-daily dosing regimen of captopril with a once-daily dosing regimen of enalapril. The principal measure of treatment efficacy was its effect on 24-hour blood pressure profiles using an ambulatory non- invasive blood pressure measuring device. The mean of casual office blood pressures and heart rates during captopril and enalapril treatment were not significantly different. Unexpectedly, 24-hour blood pressure profiles demonstrated that enalapril caused a more sustained and consistent blood pressure reduction than captopril, particularly during late afternoon and evening. The dose of captopril used and the twice-daily administration were, however, sufficient to reduce blood pressure at night and during the early morning. We related this temporary lack of blood pressure control in the late afternoon and evening with captopril to an effect of food intake on the amount and/or rate of drug absorption. We concluded that with captopril vigilance regarding the minimal effective dose and the dosage interval is inorder. The postulate that cumulative inhibition of tissue ACE allows dosing regimens independent of inhibition of serum ACE needs further clarification.

PART 2

SEROTONIN ANTAGONISM

CHAPTER 6

The first paper of chapter 6 did set the stage for our own "serotonin story". In a rather provocative study we reported that the selective 5-HT_2 receptor antagonist ketanserin, given intravenously, caused a distinct fall in supine systemic arterial, right atrial, pulmonary artery, and pulmonary capillary "wedge" pressures. Cardiac output, renal blood flow, and glomerular filtration rate showed no persistent changes. Thus the fall in elevated blood pressure after ketanserin was due to a fall in total peripheral resistance. We concluded, rather preliminary, that ketanserin may unmask a role for serotonin in maintaining high blood pressure.

The second paper examined long-term effects of ketanserin. We found that ketanserin lowered systolic and diastolic pressure during the day and at night, but did not modify the circadian blood pressure pattern. Ketanserin had no effect on heart rate. Although the fixed dose of 40 mg b.i.d. may not have been optimal in all patients, the continuous blood pressure measurements showed an effect that lasted for the full 24-hour period.If patients in whom diastolic pressure fell by more than 10% were considered to be responders, then about half of our patients were responders.

CHAPTER 7

The mechanism of action of ketanserin is the subject of the two papers of this chapter. We found that ketanserin, in a dose (10 mg intravenously) that lowered blood pressure, did not alter the pressor effects of bolus injections of the $alpha_1$-adrenoceptor agonist phenylephrine. Thus in man we could not confirm important $alpha_1$-adrenoceptor blockade by ketanserin. Furthermore, ketanserin had a distinct hypotensive effect in 4 normotensive patients with autonomic insufficiency due to an efferent sympathetic lesion, who were unresponsive to alpha-adrenoceptor blockade.

On the other hand, it appeared that the antihypertensive effect of ketanserin was blunted by pretreatment with high doses of prazosin but not by frusemide. Therefore, we proposed that a certain degree of $alpha_1$-adrenergic tone seems to be required for ketanserin to exert its full antihypertensive action. This may be related to the alleged amplifying effect of serotonin on $alpha_1$-adrenoceptor mediated vasoconstriction.

CHAPTER 8

Rather by serendipity we found that ketanserin appeared to improve digital blood flow in a few patients with hypertension and concomitant Raynaud's phenomenon. We confirmed this beneficial effect in a later study including 11 patients with primary Raynaud's disease. We found that ketanserin given intravenously in a dose of 10 mg normalized digital skin temperature and digital blood flow as estimated by venous occlusion plethysmography and laser-Doppler flowmetry. Effects however on transcutaneously measured oxygen tension were modest. This could imply a preferential effect of ketanserin on arterio-venous shunt flow rather than on nutritional blood flow.

Pretreatment with high doses of selective and non- selective alpha-adrenoceptor antagonists did not abolish the effects of ketanserin on digital perfusion. We concluded that serotonergic type 2 receptors are present in the digital vasculature. In patients with Raynaud's disease, their activation plays an important role in cold- induced digital vasoconstriction.

CONCLUSION

The aim of this thesis was to answer the question of how angiotensin converting enzyme inhibitors and the serotonin antagonist ketanserin contribute to blood pressure normalisation. However it must be apparent from our clinical pharmacological data that the role of angiotensin and serotonin in the cardiovascular system is far more complex than ever imagined. Our clinical experiments do not allow us to draw sweeping, straightforward, conclusions as to the exact roles of these alleged bloodborne agents in the pathogenesis of hypertension.

The fact is that our pharmacological probes were no razorblade-like knifes for dissecting the numerous physiological mechanisms involved. In spite of this difficulty, our results have contributed to a better insight in these mechanisms. Particularly, these studies have shown that the levels of the circulating components of the renin-angiotensin are not the only determinants of the hypertensive action of this system. In addition the studies have demonstrated the effectiveness of angiotensin converting enzyme inhibitors in hypertension and heart failure. Our studies were also useful in highlighting some unexpected effects of ACE inhibitors on the kidney, effects that can be made use of in the diagnostic work-up of renovascular hypertension.

Our experience with the serotonin antagonist ketanserin suggests a role for serotonin in essential hypertension. It is problably the combined antagonistic action of ketanserin on 5-HT_2- and α_1-receptors that is responsible for its blood pressure lowering effect. Furthermore, our results indicate that ketanserin is a promising drug for treating Raynaud's phenomenon.

We should remember that in the past we could not find out how diuretics and betablocking agents reduce blood pressure. Today, we have to admit that the exact mode of action of ACE-inhibitors and serotonin antagonists also rests on the knees of the gods.

SAMENVATTING EN CONCLUSIES

INLEIDING

Al bijna honderd jaar is bekend dat in ons bloed stoffen circuleren die bloeddrukverhogend werken. Tigerstedt en Bergman (1898) maakten waterige extracten van nieren van konijnen en spoten het verkregen extract in bij nierloos gemaakte andere konijnen. De mode van hun tijd volgend, waren zij op zoek naar een soort verjongingselixer maar vonden dat hun niersap bloeddrukverhogend werkte. Zij noemden hun extract renine.

Wij weten nu dat dit uit de nier afkomstige, in het bloed circulerende, renine als zodanig geen effect heeft op de bloeddruk maar een onderdeel is van een ingewikkeld enzym systeem dat uiteindelijk verantwoordelijk is voor de vorming van het sterk bloeddrukverhogende eiwit angiotensine II. Deze uitvinding werd tegelijkertijd, maar onafhankelijk van elkaar, gedaan door de Amerikaanse dokter Irvin Page en de Argentijnse fysioloog Braun-Menendez.

Bij zijn speurtocht naar angiotensine had Page veel last van de storende invloed die gestold bloed, serum, had op zijn experimenten. Gebruik van ontstold bloed, plasma, gaf geen problemen. Daarom isoleerde hij eerst de storende, bij stolling vrijkomende, faktor en noemde de gevonden stof serotonine. Kort daarop ontdekte hij het eindprodukt van de renine cascade, angiotensine. De afgelopen 40 jaar nam onze kennis over het renine-angiotensine systeem exponentieel toe. Met serotonine konden de onderzoekers na een ogenschijnlijk flitsende start niet goed uit de voeten.

VRAAGSTELLING

De in dit proefschrift gebundelde en reeds elders gepubliceerde artikelen ("publish or perish") gaan over de twee bloeddruk verhogende "tonines" van Irvin Page, angiotensine en serotonine. Onze klinische interesse in deze stoffen werd namelijk gewekt door het in de zeventiger en tachtiger jaren ter beschikking komen van geneesmiddelen die het mogelijk maakten om zowel in het renine- angiotensine als in het serotonine systeem in te grijpen.

Aan de introduktie van remmers van de aktiviteit van het renine angiotensine systeem ging grensverleggend industrieel-farmacologisch onderzoek, o.a. door Squibb Laboratories in de Verenigde Staten, vooraf. Het bleek mogelijk met listig "geconstrueerde" geneesmiddelen, waarvan de angiotensine converting enzyme (ACE) remmer captopril het prototype is, de vorming van angiotensine II in de renine-enzyme cascade te voorkomen.

Meer bij toeval, maar toch niet zonder verdienste, werd door Janssen Pharmaceutica een stof ontdekt, ketanserin genaamd, die ervoor bleek te kunnen zorgen dat het uit bloedplaatjes afkomstige serotonine een deel van zijn werk, vernauwen van bloedvaten, niet kan doen. Ketanserin werd farmacologisch gekarakteriseerd als een zgn. competatieve, selectieve, serotonine type 2 receptor antagonist.

Na uitvoerig overleg met de Ethische Commissie van ons ziekenhuis kregen wij

toestemming deze prototypes van nieuwe generaties van geneesmiddelen toe te dienen aan patiënten met uiteenlopende vasculaire pathologie. Het spreekt voor zich dat de betrokken patiënten tevoren duidelijk werden ingelicht.

Deel 1 van het proefschrift gaat over de ervaring die wij opdeden met verschillende ACE remmers. Wij trachten met deze geneesmiddelen als het ware te "sonderen" (geneesmiddel als onderzoeksinstrument) hoe aktief het renine-angiotensine systeem is bij patiënten met verschillende vormen van verhoogde bloeddruk en bij patiënten met insufficiënte hartswerking.

In het tweede deel van de thesis komt blokkade van de vasculaire serotonine receptor aan de orde. Met ketanserin probeerden wij de rol van serotonine in het cardiovasculaire systeem te ontmaskeren.

DEEL 1
ANGIOTENSINE CONVERTING ENZYME (ACE) REMMING

HOOFDSTUK 1
ACE REMMING EN PLASMA RENINE

Hoofdstuk 1 gaat over hoe de ACE remmer captopril de bloeddruk verlaagt. We gingen na of de activiteit van het renine-angiotensine systeem, zoals dat in een reageerbuis wordt gemeten, voorspellende waarde heeft voor de mate van bloeddrukdaling na inname van captopril. Wij bestudeerden patiënten met sterk uiteenlopende plasmaspiegels van renine: (1) haemodialysepatiënten zonder nieren, bij wie het plasma vrijwel geen enzymatisch actief renine bevat, (2) patiënten met essentiële hypertensie met lage tot normale plasma renine spiegels, (3) patiënten met hoge bloeddruk veroorzaakt door een vernauwing van de nierslagader met normale tot hoge renine spiegels en tenslotte, (3) patiënten met een ernstige storing van de hartfunktie bij wie plasma renine spiegels extreem verhoogd bleken.

Op theoretische gronden lag het meer voor de hand om niet de plasmaspiegel van renine maar die van angiotensine II te hanteren als maat voor de activiteit van het renine-angiotensine systeem in vivo. Immers, het is niet renine maar angiotensine II dat krachtige bloeddrukeffecten heeft. Praktisch stuitte dit echter op grote problemen omdat de concentratie van angiotensine II in plasma zeer laag is, enkele picogrammen per ml, en de bij de radio-immunoassay gebruikte antilichamen behalve met angiotensine II ook met de in het plasma aanwezige niet actieve afbraakproducten van dit peptide reageren.

Voor klinisch gebruik is de bepaling van de zogenaamde plasma renine activiteit (PRA) nog steeds de meest gebruikte maat voor de vorming van angiotensine II *in vivo*. Bij bepalingen van PRA wordt het plasma van een patiënt gedurende een bepaalde tijd geincubeerd bij 37°C. Tijdens deze incubatie *in vitro* wordt door de enzymatische werking van renine op renine substraat (angiotensinogeen) het biologisch niet actieve peptide angiotensine I gevormd. Plasma echter heeft angiotensinase activiteit, waardoor angiotensine I wordt afgebroken. Dit wordt voorkomen door remmers van angiotensinase aan het plasma toe te voegen. Deze stoffen remmen echter ook de omzetting van angiotensine I in angiotensine II, zodat de reactie stopt bij de vorming van angiotensine I. Het gevormde angiotensine I wordt bepaald met behulp van een radio-immunoassay. Het

zal duidelijk zijn dat wij de activiteit van het renine-angiotensine systeem dus slechts heel indirect konden benaderen.

Onder normale omstandigheden ligt de concentratie van renine substraat in plasma op het niveau van de K_m waarde, hetgeen betekent dat de snelheid waarmee angiotensine I wordt gevormd niet alleen afhankelijk is van de concentratie van renine maar ook van die van renine substraat. Dit zou geen probleem zijn wanneer de substraat concentratie altijd constant en in overmaat aanwezig was. Helaas bleek dit niet het geval. Bij patiënten zonder nieren is de substraatconcentratie sterk verhoogd, bij patiënten met hart- en leverfalen vaak zeer laag. Dit bemoeilijkt de interpretatie van PRA bepalingen.

Om de reaktiesnelheid van renine *in vitro* onafhankelijk van de substraatconcentratie te maken ontwikkelden wij, in navolging van anderen, bepalingsmethoden waarbij het endogene substraat wordt gedenatureerd. Hierna wordt een grote overmaat gezuiverd substraat toegevoegd, zodat het enzym renine verzadigd is met substraat. De concentratie van renine op deze wijze bepaald, PRC, leek een nauwkeuriger maat dan de PRA bepaling.

Het bleek echter dat bij de PRC bepaling meer angiotensine I wordt gevormd dan bij de PRA bepaling. Dit verschil bleek te berusten op de aanwezigheid in plasma van een enzymatische *inactieve vorm* van renine dat tijdens de bepaling ten dele geactiveerd wordt. Gaandeweg werd duidelijk dat ongeveer 90% van het bij gezonde personen in plasma aanwezige renine zich in de enzymatisch inactieve vorm (prorenine) bevindt en dat het renine angiotensine systeem in dit opzicht veel meer lijkt op andere enzymsystemen zoals het stollingssysteem dan op hormonale systemen waarbij het pro-hormoon niet in plasma circuleert. Het zal duidelijk zijn dat deze biochemische waarnemingen belangrijke consequenties hebben voor de klinicus practicus die op zoek is naar een relatie tussen de activiteit van het renine angiotensine systeem en het bloeddruk verlagend effect van een geneesmiddel dat de activiteit van dit systeem remt. In ons onderzoek werd zowel gekeken naar actief renine als naar prorenine.

Captopril bleek een effectieve remmer van de activiteit van het angiotensine converting enzyme (ACE). Na de orale inname van 50 mg captopril bleek reeds na 30 minuten belangrijke blokkade aantoonbaar, die enkele uren aanhield. Door het wegvallen van de vorming van angiotensine II steeg de concentratie aan actief renine (wegvallen van negatieve terugkoppeling) en deze concentratie bleef hoog tijdens chronische toediening. De stijging van actief renine ging aanvankelijk gepaard met een daling van prorenine, maar later steeg ook de concentratie van dit proenzyme. Merkwaardigerwijs verschilde de bloeddruk response op captopril niet veel tussen de verschillende groepen patiënten ondanks sterk uiteenlopende biochemische activiteit van het circulerende renine angiotensine systeem. Ook correlaties tussen daling in vaatweerstand na toediening van captopril en actief renine waren gering.

Wij trokken de conclusie dat het niet blokkade van het circulerend renine-angiotensine systeem is dat bepaald in hoeverre de bloeddruk daalt na ACE inhibitie, maar dat bijkomende mechanismen een rol moesten spelen. Mede geinitieerd door onze klinische bevindingen, maar ook door die van anderen, werd de afgelopen jaren intensief gezocht of componenten van het renine-angiotensine systeem ook buiten de bloedsomloop konden worden aangetoond. Dit bleek het geval. De hele renine cascade werd o.a. aangetroffen in de vaatwand, het hart, de nier en in de hersenen. Het zou goed kunnen zijn dat ACE remmers de bloeddruk verlagen door blokkade van dit weefsel renine-angiotensine systeem. Vooral in dié gevallen waarbij na ACE remming wel de bloeddruk daalt maar activiteit van het circulerend renine- angiotensine systeem nauwelijks aantoonbaar is (nierloze patiënten, patiënten met zgn. essentiële hypertensie).

HOOFDSTUK 2
ACE REMMING EN FALENDE HARTSWERKING

Page heeft eens gezegd dat nauwkeurige klinische observaties vaak ten grondslag liggen aan belangrijke vooruitgangen in de geneeskunde. Dat deze uitspraak koren op de molen is van deze medicus practicus promovendus hoeft geen betoog. Onze klinische researchgroep was even betrokken bij zo'n plotselinge vooruitgang. Tijdens het onderzoek met captopril bij patiënten met verhoogde bloeddruk maakten wij gebruik van een catheter die werd gelegd in de longcirculatie. Dit om vullingsdrukken van het hart te meten en het hartminuutvolume te bepalen teneinde te kunnen vaststellen wat het effect was van captopril op de totale vaatweerstand in het lichaam. Deze invasieve metingen maakten duidelijk dat sommige patiënten op de grens van hartfalen balanceerden zonder dat daar bij lichamelijk onderzoek veel aanwijzingen voor waren. Na toediening van captopril werd bij deze patiënten sterke verbetering van de hartswerking waargenomen.

Dit bracht ons ertoe captopril te geven aan patiënten met een veel ernstiger pompfunktiestoornis van het hart, al hoewel hartfalen toen nog niet als een indicatie voor ACE remming werd gezien. De studie werd uitgevoerd in nauwe samenwerking met de cardiologische staf van het Thoraxcentrum. De resultaten van dit onderzoek zijn beschreven in dit hoofdstuk. Captopril had een verrassend gunstig effect op de falende hartswerking van deze patiënten. Ook na langduriger toediening van captopril bleef deze verbetering bestaan.

Wij waren niet de enigen die dit verrassend effect van ACE remming waarnamen. Een stroom van publicaties volgde. De onze was er één van. ACE remming wordt nu als een belangrijke aanwinst gezien bij de vaak medicamenteus moeizame behandeling van hartfalen.

HOOFDSTUK 3
ACE REMMING EN DE NIER

In het eerste artikel van dit hoofdstuk wordt aannemelijk gemaakt dat inactief renine een biosynthetische voorloper is van actief renine, en dat, althans onder bepaalde omstandigheden, een belangrijk deel van het circulerend inactief renine (prorenine) afkomstig is uit de nier. Verder vonden wij dat tijdens blokkade van ACE de secretie van inactief en actief renine door de nier niet parallel verliep. Tevens bleek dat de nier waarschijnlijk in staat is om circulerend inactief renine om te zetten in de actieve vorm. Deze ingewikkelde, variabele, verhouding tussen actief en inactief renine maakte duidelijk wat een geringe betekenis moet worden toegekend aan de "gewone" renine bepaling (PRA) die geen rekening houdt met inactief renine.

In de laatste twee artikelen van dit hoofdstuk komen de effecten van ACE inhibitie bij patiënten met een éénzijdige vernauwing van de nierslagader aan de orde. Na ACE inhibitie stelden wij een belangrijk nierfunktieverlies vast van de aangedane nier. Wij kwamen dit nadelig effect op het spoor door gescheiden nierfunktieonderzoek te verrichten. Deze waarnemingen onderstrepen welk een belangrijke, lokaal-renale, rol angiotensine II speelt bij het instand houden van de filterfunktie van een nier die te lijden heeft van een verminderde bloeddoorstroming.

Deze gegevens openden een onverwacht paradoxaal perspectief. ACE remmers werden ontwikkeld voor die vormen van hoge bloeddruk, zoals tengevolge van een vernauwing van een nierslagader, waarbij het renine angiotensine zeer actief is. Juist in deze gevallen

stelden wij vast dat remming van ACE problemen met de nierfunktie kan geven. Daar staat tegenover dat ACE remmers zeer werkzaam bleken zonder dat van een uitgesproken activiteit van het circulerende renine angiotensine systeem sprake is. Er zal nog veel water door de Maas gaan voor deze paradox ten volle is begrepen.

HOOFDSTUK 4
ACE REMMING ALS DIAGNOSTICUM

Diagnostiek van renovasculaire hypertensie en de rol die ACE remming hierbij kan spelen is het onderwerp van de twee artikelen in dit hoofdstuk. Captopril bleek de renale renine secretie van patiënten met een nierarteriestenose in belangrijke mate te kunnen stimuleren. De na ACE remming gemeten perifere plasma renine spiegels bleken beter te kunnen voorspellen dat een patiënt lijdende is aan een vernauwing van een nierslagader dan mogelijk was op grond van niet gestimuleerde renine waarden. De sensitiviteit, specificiteit en voorspellende waarde van de "captopril renine test" (onderzoek bij 179 patiënten) bleken van dien aard dat de test een plaats verdient bij de diagnostiek van renovasculaire hypertensie.

Of het tevens noodzakelijk is voor diagnostiek bloed te bemonsteren voor een renine bepaling uit de niervenen van patiënten met een nierarteriestenose is het onderwerp van het tweede artikel. Bij dit onderzoek maakten wij gebruik van een nieuwe immunoradiometrische renine bepaling (monoclonaal antilichaam gericht tegen het actieve deel van het renine molecuul), die wij vergeleken met onze standaard bepaling.

Wij stimuleerden de renale renine secretie met een gift captopril. De nieuwe, snellere, bepaling bleek betrouwbaar maar niervene renine bemonstering als zodanig bleek weinig bij te dragen aan de diagnostiek van renovasculaire hypertensie.

HOOFDSTUK 5
ACE REMMING EN 24-UURS BLOEDDRUK EFFECT

De éne ACE remmer is de andere nog niet. Dat is het onderwerp van dit hoofdstuk. Wij vergeleken 24-uurs bloeddruk effect van captopril met dat van enalapril. Pharmacokinetisch zijn er belangrijke verschillen tussen beide medicamenten. Op grond van een korte eliminatiehalfwaardetijd werd aanvankelijk geadviseerd captopril 4 x daags te doseren, maar in de praktijk van alle dag leek het erop dat men met een aanmerkelijk langer doseringsinterval ook uitkwam. Deze modieuze trend (éénmaal daags móet kunnen) is echter niet grondig onderbouwd.

Wij registreerden nauwkeurig, en bij dezelfde patiënt, 24-uurs bloeddrukprofielen tijdens *2 x daags* 37,5 mg captopril en *1 x daags* 20 mg enalapril. Wij gebruikten een automatische bloeddrukrecorder die de patiënt met zich meedroeg en hem in staat stelde zijn gewone bezigheden te verrichten.

Tijdens gebruik van enalapril werd een wat gelijkmatiger 24-uurs bloeddrukcurve gezien dan tijdens gebruik van captopril. Vooral rond de avonddosering van captopril en enkele uren hierna bleek de bloeddruk onder captopril hoger dan tijdens gebruik van enalapril. Echter 's nachts en in de vroege ochtend konden wij geen verschil aantonen.

Het is bekend, maar op de achtergrond geraakt, dat voedsel inname de opname van captopril in het lichaam vertraagt. Onze patiënten namen de avonddosering captopril rond de (hoofd)maaltijd. Verminderde cq uitgestelde resorptie van captopril zou een verklaring kunnen vormen voor de tijdelijk gedaalde effectiviteit. Oppassen lijkt geboden wanneer

men laag en weinig frekwent doseert. Men heeft wel gesteld dat het bloeddrukverlagend effect van captopril onafhankelijk zou zijn van de bloedspiegel van dit medicament. Onze gegevens ondersteunen dit postulaat niet.

DEEL 2 BLOKKADE VAN SEROTONINE RECEPTOREN

HOOFDSTUK 6
SEROTONINE, KETANSERIN EN VERHOOGDE BLOEDDRUK

In hoofdstuk 6 komt de vraag aan de orde of toediening van ketanserin aan patiënten met verhoogde bloeddruk de bloeddruk verlaagt. Het antwoord bleek recht toe recht aan: ja, dat doet het middel. Wij interpreteerden de bloeddrukdaling op ketanserin als indirect bewijs voor een mogelijke rol van serotonine bij verhoogde bloeddruk. De twee artikelen deden nogal wat stof opwaaien. Het lijkt daarom nuttig om onze "ketanserin-serotonine-hypertensie story" in een wat breder kader te plaatsen.

Geschiedenis.

Al 40 jaar brengt serotonine (5-hydroxytryptamine; 5-HT) onderzoekers in vervoering maar vooral ook in verwarring. De verbinding begon haar loopbaan als een darmhormoon, enteramine. Dit enteramine bleek echter identiek aan de door Page ontdekte serum faktor, serotonine, dat vrijkomt als bloedplaatjes samenklonteren. Nadat de structuur was opgehelderd, werd serotonine ook in de hersenen aangetoond. Spannende tijden braken aan, maar teveel onduidelijke stukjes maakten de puzzel onlegbaar. In vergelijking met haar familieleden, de monoaminen noradrenaline, adrenaline en dopamine, leek voor serotonine geen glansrol weggelegd. Een echte Assepoester met een onduidelijke taak in darm en brein om van de bloedsomloop maar niet te spreken. Bloedspiegels van vrij circulerend serotonine bleken extreem laag en toegediend als synthetische stof gaf serotonine in het ene vaatgebied vasoconstrictie, in het andere vasodilatatie. Tot overmaat van ramp wisselden deze effecten ook nog van uur tot uur en kwamen er belangrijke species verschillen aan het licht. De naam serotonine, door Page gekozen in de veronderstelling met een hypertensieve faktor "par excellence" te maken te hebben, leek weinig gepast. Recent inzicht in de diversiteit aan serotonine receptoren bracht onze Assepoester weer in het centrum van de belangstelling.

Receptoren.

Bindingsstudies en andere farmacologische experimenten maakten aannemelijk dat er op zijn minst drie hoofdgroepen serotonine receptoren bestaan: 5-HT_1, 5-HT_2 en 5-HT_3. De 5-HT_1 receptor werd gevonden in het centraal zenuwstelsel maar ook op het endotheel van de vaatwand. Er bestaan waarschijnlijk meerdere 5-HT_1-achtige receptoren. Hun funktie is nog omstreden. Sceptici veronderstelden dat het slechts gaat om onduidelijk receptoren op zoek naar een ziekte. Recent werd echter aangetoond dat een subgroep van deze 5-HT_1 receptoren waarschijnlijk een beslissende rol speelt bij het ontstaan van migraine.

De 5-HT_2 receptor is wat beter gedefiniëerd. Hij wordt gevonden op de gladde spiercel van darm en bloedvaten maar ook in de hersenen, het hart en op de wand van bloedplaatjes. Prikkeling door serotonine van de 5-HT_2 receptor in de vaatwand leidt tot vaatvernauwing. De 5-HT_3 receptor is waarschijnlijk een echte zenuwreceptor.

Kortom, een veelheid dus aan receptoren met uiteenlopende en soms tegengestelde

funkties. Dit verklaart waarom de rol van serotonine zo lang onduidelijk bleef en nog steeds wel is.

Vasculaire effecten.

Synthese van serotonine vindt uitsluitend plaats in de enterochromafienne cellen van de darm en in het centraal zenuwstelsel. In de darm geproduceerd serotonine bereikt via de vena porta de lever waar de verbinding direct wordt afgebroken. Serotonine dat aan de lever ontkomt, wordt geneutraliseerd door het endotheel van de longvaten. Bloedplaatjes beschikken over een actief opname mechanisme voor serotonine waarbij zij de verbinding opslaan in zogenaamde "dense bodies". Eén en ander zorgt ervoor dat er praktisch geen vrij serotonine in het bloed circuleert. Men neemt aan dat de vasculaire effecten van serotonine worden veroorzaakt door serotonine dat ter plekke vrijkomt uit samenklonterende, uiteenvallende bloedplaatjes.

Op deze wijze vrijgemaakt serotonine heeft meerdere en uiteenlopende vasculaire effecten. Serotonine dat de 5-HT_1 endotheel receptor prikkelt, geeft vaatverwijding. Het gaat hierbij om een indirect effect, waarbij het endotheel wordt aangezet tot de produktie van een "relaxant factor". Serotonine dat via het endotheel de 5-HT_2 receptor op de gladde spiercel in de vaatwand bereikt, brengt deze spiercel en daarmee het bloedvat tot contractie. De 5-HT_2 receptor is ook betrokken bij het versterkend effect dat serotonine heeft op de bloedvaatvernauwing dat door het sympathisch zenuwstelsel wordt bewerkstelligd. Ook samenklonteren van bloedplaatjes, éénmaal in gang gezet, wordt door vrijgekomen serotonine verder versterkt via een 5-HT_2 receptor op het bloedplaatje.

Op zich is het dus niet onaannemelijk dat geneesmiddelen die een interactie aangaan met deze receptoren (stimulering van 5-HT_1 receptoren, blokkade van 5-HT_2 receptoren) van nut zouden kunnen zijn bij de behandeling van vasculaire pathologie.

Zoals eerder genoemd, de concentratie van vrij serotonine in de circulatie is echter extreem laag. Het is dan ook moeilijk voorstelbaar dat dit gehalte aan vrij serotonine verantwoordelijk is voor de verhoogde vaatweerstand waarmee essentiële hypertensie gepaard gaat. De situatie ligt anders voor het lokaal vrijgemaakt serotonine dat wel zeker de vaattonus beïnvloedt. Maar deze concentraties zijn moeilijk meetbaar. Wel zijn er aanwijzingen dat de bloedplaatjes van patiënten met hoge bloeddruk serotonine minder gemakkelijk opnemen en dat deze bloedplaatjes makkelijker tot samenklonteren overgaan.

De laatste jaren wordt steeds duidelijker dat het vaatwand bekledend endotheel geen indolente laag, maar metabool zeer aktief. Zo zijn er receptoren en metaboliserende enzymen gevonden voor serotonine, bradykinine, histamine, ADP, angiotensine etc. en produceert het endotheel ook zelf vaatverwijdende en vaatvernauwende stoffen. Na beschadiging, bijvoorbeeld door sterk verhoogde bloeddruk, herstelt endotheel betrekkelijk snel. Toch is er iets bijzonders aan de hand met "genezen" endotheel. Het reageert anders op prikkels. Druppelen van serotonine op zulke bloedvaten bleek in plaats van vaatverwijding (5-HT_1 effect) onverwacht sterke vasoconstrictie (5-HT_2 effect) te geven. Vastgesteld werd dat de gevoeligheid van de vaatverwijdende 5-HT_1 receptor op "genezen" endotheel sterk verminderd is. Onder deze omstandigheden komen de vaatvernauwende eigenschappen van serotonine dus bij uitstek tot expressie. Op theoretische gronden konden wij het bloeddrukverlagend effect van de 5-HT_2 receptor blokker ketanserin dus goed verklaren.

Bij onze patiënten met verhoogde bloeddruk was de bloeddrukdaling na langzame intraveneuse infusie van 10 mg ketanserin een gevolg van een daling van de totale perifere vaatweerstand. Activatie van de baroreflex zorgde voor een, geringe, stijging van de

hartfrekwentie. De reflexmatige toename in de sympathische zenuwactiviteit, zoals weerspiegeld in de stijging van de plasma noradrenaline concentratie, was waarschijnlijk ook verantwoordelijk voor de toename van het hartminuutvolume. Ondanks aanhoudende sympathische stimulatie bleek de hartminuutvolume stijging slechts van korte duur. Mogelijk als gevolg van dalende vullingsdrukken van beide hartkamers, daalde het hartminuutvolume later tot zelfs iets onder de uitgangswaarde. Deze gegevens pleitten ervoor dat ketanserin niet alleen een effect had op de weerstandvaten maar ook op die vaten die het aanbod van het hart reguleren, de zgn. capaciteitsvaten. Geringe toename van bloeddoorstroming bij uitgesproken vaatverwijding werd ook in de niercirculatie aangetoond. De weerstandsdaling in dit vaatgebied zorgde ervoor dat ondanks de dalende bloeddruk (nierperfusiedruk) de glomerulusfiltratie behouden bleef.

Zijn al deze effecten van ketanserin nu toe te schrijven aan blokkade van 5-HT_2 receptoren? Of is ketanserin toch niet zo selectief voor 5-HT_2 receptoren als werd gedacht en blokkeert de verbinding ook receptoren van het sympathisch zenuwstelsel? Dierexperimenten, door anderen verricht, gaven hier op een duidelijk antwoord. Ketanserin, zij het in hoge concentratie, blokkeert naast 5-HT_2 receptoren ook α_1-adrenoreceptoren. Maar geldt dit ook voor doseringen die bij de mens bloeddrukverlagend werkten? Deze vraag leek ons zo belangrijk dat wij hier een apart onderzoek naar deden.

HOOFDSTUK 7
HOE WERKT KETANSERIN?

De twee artikelen in dit hoofdstuk gaan over het werkingsmechanisme van ketanserin. Aan patiënten met licht verhoogde bloeddruk gaven wij opklimmende doseringen van de α_1-adrenoreceptor agonist fenylefrine. Dit voor en na toediening van ketanserin. De bloeddrukstijging en de polsvertraging na fenylefrine werden door ketanserin nauwelijks beïnvloed. Toediening van de α_1-adrenoceptor antagonist prazosin gaf wel een duidelijke verschuiving van de fenylefrine dosiswerkingcurve. Dit laatste viel te verwachten daar prazosin bekend staat als een competatieve α_1-receptor blokker. Blokkade van α_1-receptoren door ketanserin, althans bij de mens en in de gebruikte bloeddrukverlagende dosering, was dus niet erg evident.

Wij dienden ketanserin ook toe aan een zeldzaam groepje patiënten bij wie door ziekte het sympatisch zenuwstelsel onwerkzaam was geworden. Een α-adrenoceptor blokker had bij deze patiënten geen effect op de bloeddruk, ketanserin had dit duidelijk wel.

Het effect van ketanserin op de bloeddruk is dus uitsluitend toe te schrijven aan blokkade van 5-HT_2 receptoren? Wij deden een waarneming die hier ogenschijnlijk mee in strijd leek. Toen wij patiënten "voorbehandelden" met hoge doseringen van de α_1-adrenoceptor blokker prazosin en vervolgens ketanserin aan hen gaven, toen bleek het bloeddrukverlagend effect van ketanserin verdwenen. Dit in tegenstelling tot een controlegroep patiënten die met furosemide werden voorbehandeld; bij hen bleef het bloeddrukverlagend effect van ketanserin behouden. Hoe dit te verklaren? Hier gaf literatuur studie enige klaarheid. Naast eigen vasoconstrictoire effecten versterkt serotonine de werking van andere vasoactieve mediatoren zoals die van noradrenaline. Blokkeert men met prazosin de effecten van dit noradrenaline, dan valt er voor serotonine niets meer te "amplificeren". Serotonine blokkade onder deze condities heeft dan geen uitwerking meer. Het zou wel eens kunnen zijn dat de door ketanserin "ontmaskerde" amplificerende rol van serotonine belangrijker is dan de vasoconstrictoire effecten van het monoamine zelf. Ook komen er steeds meer aanwijzingen dat het ketanserin molecuul uniek is in die zin dat het in staat is

om een ingewikkelde onbegrepen interactie aan te gaan met zowel 5-HT_2- als met α_1-adreno receptoren.

HOOFDSTUK 8
SEROTONINE, KETANSERIN EN HET FENOMEEN VAN RAYNAUD

Min of meer bij toeval namen wij bij een patiënte met hoge bloeddruk én een doorbloedingsstoornis van de handen waar dat er een opvallende verbetering van de vingercirculatie optrad na intraveneuse toediening van ketanserin. De kleur van haar vingers veranderde in enkele minuten van wit-blauw in rose-rood. De patiënte was lijdende aan het zgn. primaire fenomeen van Raynaud. Deze onbegrepen, door koude en emotie geluxeerde, voorbijgaande vaatkramp in handen en voeten wordt wel toegeschreven aan een overactief sympathisch zenuwstelsel maar geneesmiddelen, die de sympathische zenuwactiviteit blokkeren, bleken weinig effectief.

Serotonine zou een rol kunnen spelen. Althans de condities zijn gunstig. Pathologisch endotheel, zeer trage bloeddoorstroming, "low-grade" bloedplaatjesaggregatie. En de receptoren voor serotonine zijn er. Wanneer men serotonine druppelt op geisoleerde digitale arteriëen dan trekken deze bloedvaatjes zich krachtig samen. Serotonine, rechtstreeks toegediend in een armslagader, geeft een belangrijke vermindering van de doorbloeding van de vingers. De kleurveranderingen, die dan aan de vingers zichtbaar worden, lijken sprekend op die van patiënten met het fenomeen van Raynaud.

Wij bestudeerden de effecten van ketanserin bij een serie Raynaud patiënten. Aan hun vingers maten wij temperatuur en bloeddoorstroming (veneuse occlusie plethysmographie, laser-Doppler flux). Ook probeerden wij een indruk te krijgenr over het zuurstof transport door de huid van de vingers. Na intraveneuse ketanserin toediening nam de bloedsomloop door de vingers sterk toe en normaliseerde de huidtemperatuur zich. Effecten op zuurstof transport echter waren gering. Deze gegevens zouden er op kunnen wijzen dat ketanserin in de vinger de rijkelijk aanwezige arterioveneuse shunts opent, maar weinig doet aan verbetering van de capillaire doorstroming. Een opvallende bevinding was tevens dat de effecten van ketanserin in dit vaatgebied niet geblokkeerd konden worden door voorbehandeling met hoge doses van selectieve en niet-selectieve α-adrenoceptor antagonisten. Wij trokken de conclusie dat serotonine type 2 receptoren aanwezig zijn in vinger circulatie en dat hun activatie mogelijk een rol speelt bij het optreden van het Raynaud fenomeen.

CONCLUSIE

Dit proefschrift verkent de mogelijkheden die prototypes van nieuwe generaties geneesmiddelen ons bieden bij de bestudering van bloeddruk regulatie mechanismen. Met behulp van angiotensine converting enzyme remmers probeerden wij de functie van het renine angiotensine systeem nader te karakteriseren. De rol van serotonine in het cardiovasculaire systeem sondeerden wij met de specifieke en selectieve serotonine antagonist ketanserin.

Onze klinisch-farmacologische onderzoekingen maakten duidelijk dat de rol van angiotensine en serotonine in de pathogenese van verhoogde bloeddruk aanmerkelijk complexer is dan ooit voor mogelijk werd gehouden. Tevens bleken de gebruikte geneesmiddelen geen vlijmscherpe onderzoeksinstrumenten te zijn. Haarfijne dissectie van

fysiologische mechanismen bleek met dit "gereedschap" niet mogelijk.

Toch hebben onze onderzoekingen ons inzicht verruimd. Analyse van het bloeddrukverlagend effect van angiotensine converting enzyme remmers bracht naar voren dat het niet alleen de circulerende componenten van het renine angiotensine systeem zijn die de hoogte van de bloeddruk bepalen. Ook bij geringe activiteit van het circulerende renine angiotensine systeem bleken ACE remmers effectief. Mede dankzij deze gegevens richt fundamenteel onderzoek zich nu op karakterisering van het renine angiotensine systeem zoals dat in de weefsels wordt gevonden. ACE remming bij onvoldoende hartswerking bleek onverwacht zo effectief dat bijna niemand zich de aarzelende start herinnert. Oppassen met ACE remming bij nierarteriestenose blijft geboden, maar diagnostiek van deze onderschatte aandoening is mede dankzij ACE remmers eenvoudiger geworden.

Onze ervaring met de serotonine antagonist ketanserin suggereert dat serotonine een rol speelt bij verhoogde bloeddruk en het Raynaud fenomeen. De gunstige effecten van ketanserin moeten vermoedelijk worden toegeschreven aan een ingewikkelde interactie die de verbinding aangaat met zowel serotonine als alpha-adrenoreceptoren.

Tot besluit, veel van het bloeddrukverlagend effect van de bestudeerde, in theorie zo specifiek werkende, geneesmiddelen bleef onverklaard. Daarmee is er niets nieuws onder de zon. Ook na vele jaren ervaring met diuretica en betablokkers weten wij nog niet exact hoe zij werken. Harvey (1628), de ontdekker van de bloedsomloop, formuleerde het zo:..."it is to be understood by God alone".

DANKWOORD

Het latijnse werkwoord "promoveo" staat voor "bevorderen" maar ook voor "opdrijven". Het moet toegegeven worden, deze doctorandus liet zich wel opdrijven maar niet in de richting van een dissertatie. Een klein geboortedefect, paralyse van de linker nervus abducens, stelde hem in staat steeds een andere kant uit te kijken als het onderwerp ter sprake kwam. Toen echter de reukzin van omstanders in het geding kwam - langdurig werkzaam zijn in een universiteitsziekenhuis als publicerend maar niet gepromoveerd academicus schijnt een soort lijklucht te verspreiden - moest het er maar van komen. Hooggeschatte promotor, beste Maarten, je wordt bedankt voor je acceptatie en uiteindelijk snelle correctie van mijn opthalmologische deviatie. Ik voel mij bevoorrecht dat ik vanaf onze gemeenschappelijke start in Dijkzigt, nu al weer 15 jaar geleden, voor jou heb mogen werken. Je bent een dokter, onderzoeker, geleerde "pur sang". Dat bloed en met bloed het renine angiotensine systeem blijft kruipen waar het verstand niet bij kan, ligt niet aan jouw ijver en grijze stof. Sunt certe fines ...

Zeer velen zijn betrokken geweest bij de publicaties waarop dit proefschrift is gebaseerd. Patiënten, verplegenden, doktersassistenten, analytisch en administratief personeel, chemici, artsen, een schier onafzienbare rij min of meer cooperatieve mensen die tezamen het no-nonsense "milieu interieur" van het Rotterdamse Dijkzigt vormen. In deze omgeving is het goed toeven. Ik blijf erbij dat het onmatig is om de "output" van dit niet vervuilde milieu voor de eigen glorie aan te wenden, maar ala, het is nu gebeurd en de betrokkenen worden bedankt. Voor mijn homeostase in de Dijkzigt gemeenschap was symbiose met Arie Man in 't Veld en Frans Derkx, companen van het eerste uur, van grote betekenis. Hun namen sieren terecht menig bladzij van dit werkje. In publicaties niet genoemd maar onmisbaar bleek de hoofdzuster van 3N, Zr. A.M. Gantevoort. De stabiliserende bijdrage die zij op de achtergrond gedurende vele jaren heeft geleverd verdient alle lof.

Tot slot, lang wachten met promoveren heeft ook zijn voordelen, het thuisfront evolueert. Onder het kritisch oog van mijn mooie vrouw Wil, typte de in Dijkzigt geboren jongste zoon Paul dit dankwoord met acht meer vingers dan ik dat kan in de computer. Dat Sandra en Michael zich daar nog even mee bemoeiden spreekt voor zich.

CURRICULUM VITAE

Gerrit Jan Wenting was born in Bleiswijk, the Netherlands, on the 27th of august, 1943.

He received his undergraduate education at the 's Gravenhaags Christelijk Gymnasium (B division), and his professional training at the Medical School of the University of Amsterdam ("artsexamen" 1971).

After clinical clerkships in pathology and surgery he worked several years as general practioner. In 1974 he started a five-year residency in internal medicine at the University Hospital Rotterdam Dijkzigt (head of the department: prof.dr. J. Gerbrandy). Recording in the register of specialists of the Royal Netherlands Medical Association followed in 1979. Since that time, he is member of the staff of the Department of Internal Medicine I (head of the department: prof.dr. M.A.D.H. Schalekamp), University Hospital Dijkzigt, Erasmus University, Rotterdam.

His appointment to "chef de clinique" of the division of nephrology followed in 1986.

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