CARDIOVASCULAR AND RENAL EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITION IN MAN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. M.W. VAN HOF
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 4 SEPTEMBER 1985 DES NAMIDDAGS
TE 3.45 UUR

DOOR

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geboren te Leiden

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Financial support was received from the Dutch Kidney Foundation.

Sed non frustra.

CONTENTS

CHAPTER 1

Introduction

Since the classic experiments of Tigerstedt and Bergman (1) in 1898, in which a pressor substance, renin, was discovered in rabbit kidney extracts, and since the discovery in 1934 by Goldblatt and co-workers (2) that renal artery constriction caused hypertension in the dog, evidence for involvement of renin in the pathogenesis of various forms of clinical and experimental hypertension has been collected by many investigators (3-8). Renin itself has no effect on arterial pressure but acts through the formation of the vasopressor peptide, angiotensin II. In many respects this renin-angiotensin system resembles the kallikrein-kinin system, by which the vasodilator peptides, kallikrein and bradykinin, are formed. Indeed, in recent years, the kallikrein-kinin system has also been implicated in the regulation of blood pressure (9, 10).
Features of the two systems that are relevant to the pathogenesis of hypertension and to pharmacological modulation of their actions will be described in this chapter.

1.1. Renin-angiotensin system

Renin substrate, angiotensinogen, in plasma is produced by the liver. It is cleaved by a proteolytic enzyme, renin, liberating a decapeptide, angiotensin I (Fig. 1). Renin is produced in the juxtaglomerular cells of the kidney. Substances with renin-like activity have been described in uterus, placenta, brain, adrenal gland, submaxillary gland and in large arteries and veins. Recent experiments suggest that renin is produced as an inactive precursor form that is activated before and, possibly, also after its release into the circulation (11, 12). Angiotensin I, which has no biological activity, is subsequently cleaved by a dipeptidyl-carboxypeptidase, angiotensin-converting enzyme, to form the octapeptide, angiotensin II. Plasma levels of converting enzyme are relatively low but very high concentrations of the enzyme have been demonstrated in the vascular endothelial cells of the lung. More recently angiotensin-converting enzyme activity has also been demonstrated in brain, heart, kidney and lymph (13). Angiotensin II, which is formed by removal of the C-terminal histidyl-leucine group of angiotensin I, is a very potent vasoconstrictor. It also stimulates the adrenal cortex to secrete aldosterone (3) and it facilitates adrenergic neurotransmission (14). Angiotensin II can subsequently be converted into the heptapeptide, angiotensin III, which, in some species, stimulates the adrenal cortex to produce aldosterone and has also been shown to have angiotensin II-like effects on the renal circulation (15). Both angioten-

sin II and angiotensin III are finally degraded by angiotensinases into biologically inactive fragments.

1.2. Kallikrein-kinin system

Kinins are liberated from specific alpha$_2$-globulins, kininogens, by proteolytic enzymes called kallikreins (9, 10). Kininogens are present in two forms, high molecular weight kininogen and low molecular weight kininogen. Kallikreins have been divided into two classes, those derived from plasma and those produced by glands. Glandular kallikrein forms kallidin (lys-bradykinin), a decapeptide, from both high and low molecular weight kininogen. Plasma kallikrein forms bradykinin, a nonapeptide, from high molecular weight kininogen. The kinins are very potent vasodilators. Bradykinin has also been shown to be a potent stimulant of phospholipase A_2; it augments the availability of arachidonic acid for prostaglandin synthesis (16). Bradykinin and kallidin are inactivated by circulating and local kininases. Two kininases have been isolated from plasma, kininase I and II. Kininase II appears to be more prominent in tissue and has been shown to be identical to angiotensin-converting enzyme (13) (Fig. 2).

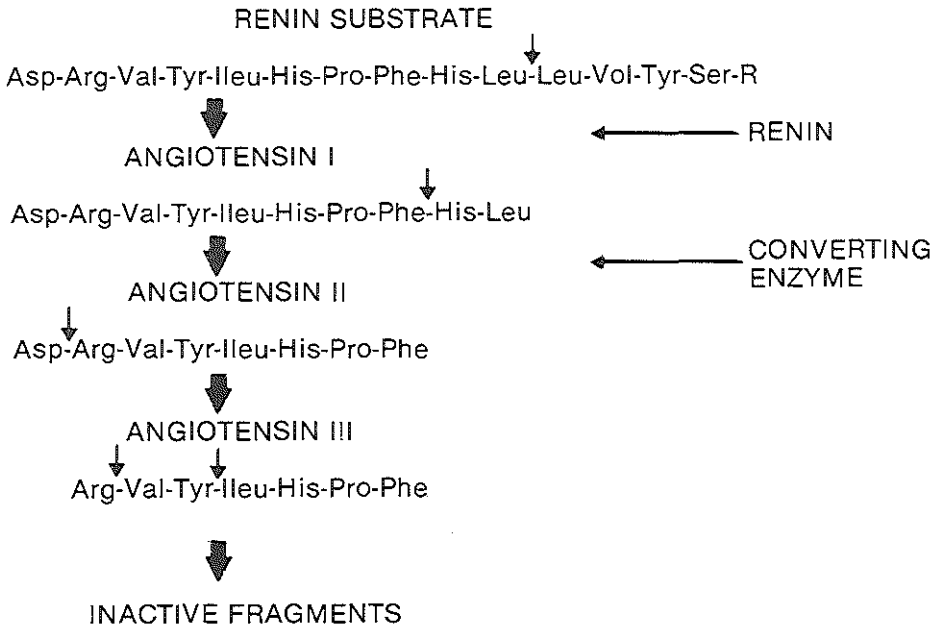

Figure 1. Renin-angiotensin system

1.3. Pharmacological modulation of the renin-angiotensin and kallikrein-kinin systems

Pharmacological modulation of the renin-angiotensin system is now possible at various steps in the system both as a research tool to establish the role of renin and angiotensin in different forms of hypertension and as a therapy in various clinical conditions (7,8). Although the concentration of renin substrate in plasma can be altered by drugs, there are little data on the physiological effects of such changes. The level of active renin in plasma can be influenced in various ways. Renin can be inhibited by substances, like pepstatin, but these are rather toxic and cannot be used in humans. Currently less toxic renin inhibitors are being tested (17 a-c). The method most commonly used to lower plasma active renin is by beta-adrenoceptor blocking agents, such as propranolol (3,8). These drugs, however, also have a profound effect on the sympathetic nervous system (19) and it is therefore difficult to determine which part of their blood pressure lowering effect is due to inhibition of the renin-angiotensin system. Another way to block the renin-angiotensin system is by the use of competitive inhibitors of its most important biologically active end-product, angiotensin II. Various analogues of angiotensin II have been studied, of which sar^1-ala^8-angiotensin II (Saralasin) is most widely used (20). However, its partial agonistic effect has limited its use as a research tool. These analogues are only active when given intravenously and they are short-acting, thereby limiting their use in clinical practice. More recently a new approach to the renin-angiotensin system has become available by the discovery of specific inhibitors of angiotensin-converting enzyme. These were isolated from the venom of a viper, Bothrops Jararaca (21). Of these compounds the nonapeptide Teprotide (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) has been studied most extensively. The drug is a very effective inhibitor of angiotensin-converting enzyme both in vitro and in vivo. It lowers blood pressure in hypertensive patients (22). The question can be raised whether this fall in blood pressure is really caused by blockade of the renin-angiotensin system. Theoretically, inhibition of angiotensin-converting enzyme, which is identical to kininase II, in the intact organism may not only lead to diminished angiotensin II production but also to bradykinin accumulation (23). Teprotide has to be administered parenterally. The finding that this agent lowers blood pressure in most hypertensive patients has provided the impetus for the development of orally active inhibitors of angiotensin-converting enzyme. Unlike most other antihypertensive drugs these inhibitors were the result of a rational drug design based on postulated molecular similarities between angiotensin-converting enzyme and other characterized carboxypeptidase enzymes (24). In 1973 it was reported that D-2-benzylsuccinic acid was an inhibitor of pancreatic carboxypeptidase A, presumably because of the interaction of the inhibitor molecule with three binding sites in the enzyme. This observation along with the apparent similarities between carboxypeptidase A and angiotensin-converting enzyme and the knowledge that all known venom angiotensin-converting enzyme inhibitors contain two proline residues in their C-terminal portion have led to the synthesis of a series of compounds that are potent and specific inhibitors of angiotensin-converting enzyme both in vitro and in vivo. Of these 2-D-3-mercapto 2-methylpropanoyl-L-proline (SQ 14225 or capto-

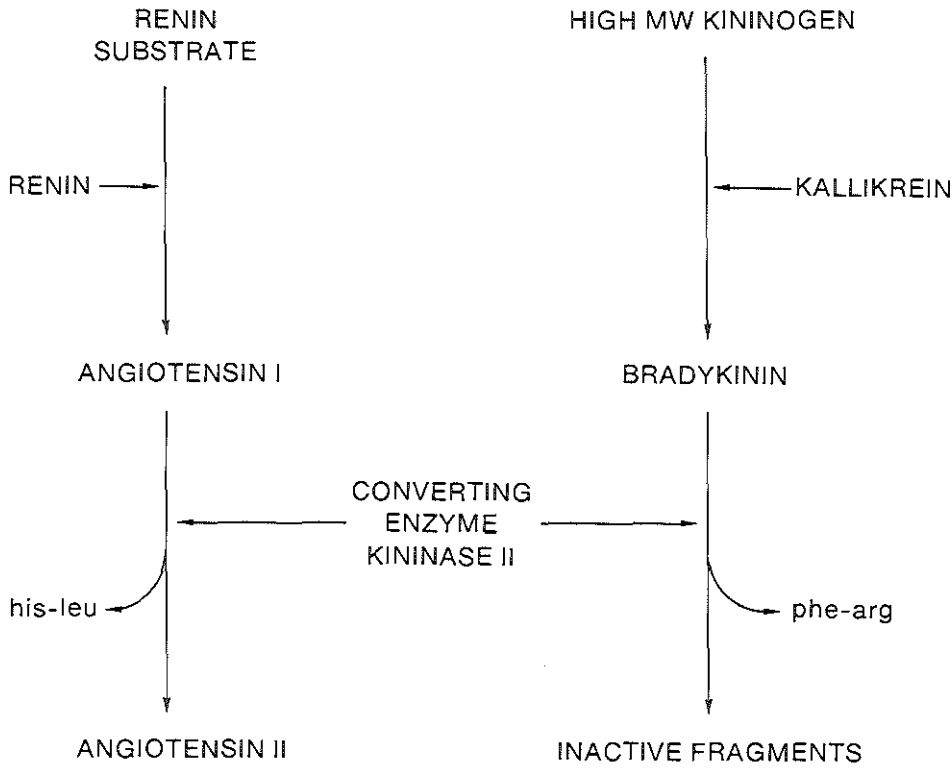

Figure 2. Renin-angiotensin and kallikrein-kinin systems. The central role of converting enzyme.

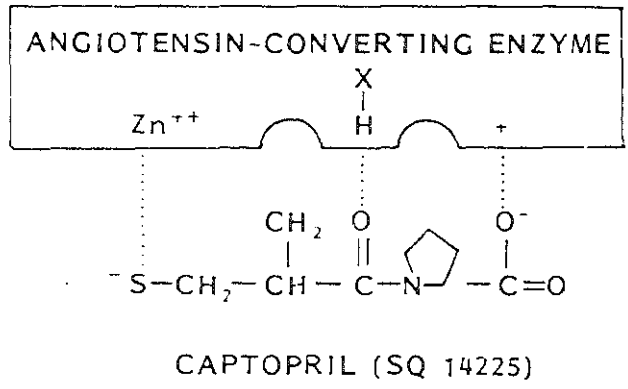

Figure 3. Proposed binding of captopril to the active site of angiotensin-converting enzyme.

pril) was selected as the optimal compound for clinical trials (Fig. 3).
Shortly after the introduction of captopril preliminary studies on the efficacy of the drug showed rather conflicting results. Some investigators demonstrated a very strong correlation between the acute fall of blood pressure, 60-90 minutes after the first oral dose of captopril, and the pretreatment levels of active plasma renin (25,26), whereas others were not able to find such correlation (27). Conflicting results have also been reported with respect to the chronic effect of captopril (25,26,28,29). Some authors have further reported that the chronic effect on blood pressure could still be demonstrated despite the fact that their measurements of converting enzyme in plasma seemed to indicate that the enzyme was not blocked during part of the day (30). In a small uncontrolled study in patients with various forms of hypertension Cody et al (31) showed that captopril acted as an arteriolar and venous vasodilator but from this study no conclusions can be drawn as to the influence of the pretreatment activity of the renin-angiotensin system on these haemodynamic responses. Although it has been shown that aldosterone falls after captopril, conflicting data have been reported on fluid balance during the first week of treatment with captopril (32). There is also little information on the more long-term effects of captopril on the renal circulation and body fluid volumes. Some authors have demonstrated an improvement of renal function during acute treatment with captopril (33) but deterioration of renal function has also been reported (34).

1.4. Aim of the study

The aim of the study presented in this thesis was to clarify firstly whether or not pretreatment plasma renin is an important predictor of the blood pressure response to captopril during both acute and chronic treatment and to establish in more detail the haemodynamic profile of actions of this drug in various forms of hypertension. For this purpose the effects of captopril treatment were investigated in patients with a wide range of plasma levels of renin i.e. normotensive anephric patients in whom active renin in plasma was almost zero, patients with essential hypertension with low to normal renin and patients with renovascular hypertension with normal to high renin and finally in normotensive patients with heart failure with renin levels that were often extremely high. A second goal of the present study was to provide some insight into the relation between the degree of inhibition of converting enzyme with captopril and the antihypertensive effect of this drug. Finally, some data were collected on the acute and chronic effects of captopril on renal function in essential and renovascular hypertension.
Chapter 2 gives a description of the methods we have used. Chapter 3 deals with the effects of captopril on haemodynamic parameters and body fluid volumes in mild to moderate essential and renovascular hypertension and in severe, so-called drugresistant, hypertension. Chapter 4 presents further data on the haemodynamic effects of captopril in essential and renovascular hypertension as well as in cardiac failure. The results are related to the pretreatment levels of plasma renin. Chapter 5 describes the effect of captopril on blood pressure in nephrectomized patients. In chapter 6 a

comparison is made between the acute effects of captopril on blood presure in renovascular and essential hypertension and in anephric normotensive patients. Chapter 7 deals with the measurement of converting enzyme in plasma in patients on captopril. The results of this measurement are related to the blood pressure response to this drug. Chapter 8 describes the effects of captopril on renal function in patients with renovascular hypertension. Chapter 9 describes the renal effects of captopril in patients with essential hypertension. In chapter 10 the conclusions to be drawn from our studies are discussed.

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CHAPTER 2

Methods

2.1. Arterial pressure and heart rate

Arterial pressure in the acute studies was measured intra-arterially through a catheter in either the radial or brachial artery. The catheter was connected with a pressure transducer (P23ID Gould-Statham) and a monitor with digital display (Hewlett-Packard 78205 C). In the chronic studies the London School of Hygiene sphygmomanometer (MK4) was used in order to minimize observer bias (1). Three consecutive pressure readings were averaged. Disappearence of Korotkoff sounds (phase V) was taken as diastolic pressure. Mean arterial pressure was calculated from cuff pressure readings as diastolic pressure + 1/3 x pulse pressure. Heart rate was calculated from a continuous ECG-recording.

2.2 Cardiac output

The methods for measuring cardiac output used in our studies are based on the principle of indicator dilution as described by Steward and Hamilton (2,3). The dye dilution technique is generally accepted as a standard procedure. This technique, however, is not suitable for repeated measurements within a short period of time because there is a limit to the amount of dye that can be injected. Moreover, this method is not suitable for repeated measurements over periods of weeks or months because it requires arterial cannulation.

Thermodilution method. In acute studies cardiac output was determined by triple-lumen thermal dilution Swan-Ganz catheter (model 93A-131-7F, Edwards Laboratories Inc.). This catheter was introduced under local anaesthesia by a modified Seldinger technique via an antecubital vein. The catheter tip was placed in a major segment of the pulmonary arterial bed, so that pulmonary arterial pressure and pulmonary wedge pressure could be recorded. Right atrial pressure was also measured at frequent intervals. Cardiac output was determined from three consecutive thermodilution curves. Ten ml of glucose solution (0.28 mol/l) at 0°C was used. The indicator solution was injected manually over less than three seconds. Incomplete or delayed injections were discarded. Dilution curves were recorded and cardiac output was calculated by a computer (Model no 9520, Edwards Laboratories Inc, California, USA).

Isotope dilution method. In the chronic studies cardiac output was measured by the use of an isotope dilution method (4-11). ^{99m}Technetium pertechnetate from a ^{99}Mo -^{99m}Tc generator (New England Nuclear) was used as an indicator. The ^{99m}Technetium pertechnetate was bound to human serum albumin (20% in 0.15 mol/l NaCl) by electrolytic complexation according to Benjamin et al (12), as modified by Dworkin and Guttowski (13).
Cardiac output was measured with 100-200 μCi (3.7-7.4 MBq) ^{99m}Tc-albumin in 0.2 ml 0.15 mol/l NaCl. The isotope was injected via a small intravenous catheter with a dead space of 0.6 ml. The tracer bolus was flushed into the circulation with 10 ml 0.15 mol/l NaCl. The volume of tracer injected was determined by weighting the syringe before and after injection. The radioactivity of the preparation injected was measured in a well-type scintillation counter.

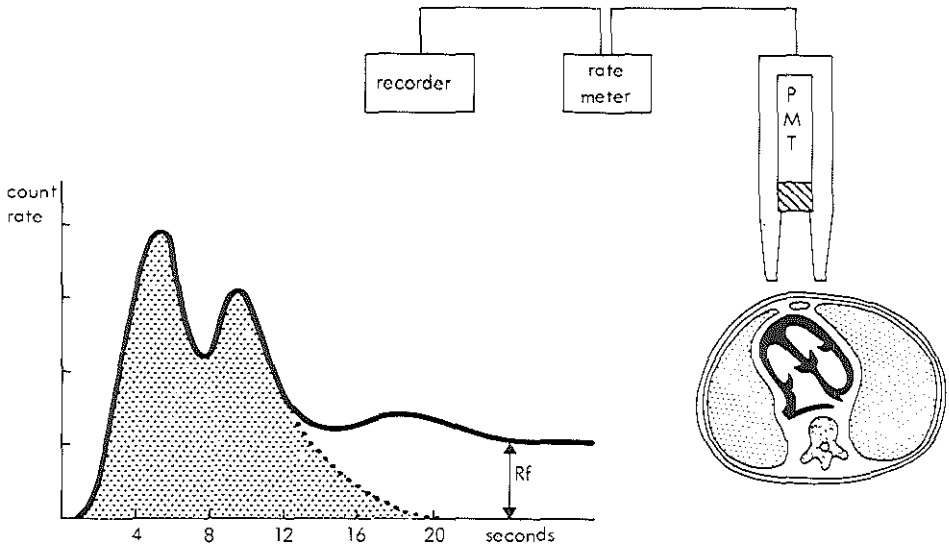

Figure 1. Recording of a radiocardiogram. PMT: photomultiplier tube. Shaded area represents first circulation of the isotope. Rf: reference value of blood radioactivity in the equilibrium phase as measured over the heart.

The detection device consisted of a thallium-activated sodium-iodine crystal of 5 x 5 cm, mounted on a photomultiplier tube (Philips-XL-7007/10). Collimation was effected by means of a conical lead collimator, wall thickness 2 cm, inner aperture 4.5 cm, outer aperture 6.7 cm, depth 8,1 cm (Philips-XL-6011/01). The detector was used in conjunction with a power supply and rate-meter (Philips-XL-1100), with a time constant of 0.4 seconds. A conventional pen recorder (Philips-PM 8010) with a chart speed of 25 cm per minute was used (full scale deflection 25 cm in 0.8 seconds). Recordings were made during 60 seconds after injection of the tracer, and again after five min and 10 min, when blood samples were taken for determination of plasma

radioactivity. The detector was placed over the fourth left intercostal space, with its centre over the left sternal margin. In this way curve recordings were obtained in all patients with accentuation of the right ventricle peak of the radiocardiogram. In this way the contribution of the area under the extrapolated part of the curve to the total area was reduced, while the length of the left ventricular downslope was still sufficient for accurate extrapolation.
Cardiac output (CO) was calculated from the equation:

$$CO\ (L/min) = \frac{\text{dose injected (cpm) x paper speed (cm/min) x Rf (cm) x } 10^{-3}}{\text{plasma radioactivity (cpm/ml) x area (cm}^2\text{) x (1-Hct)}}$$

Rf represents the deflection of the pen recorder in centimetres as a reference value for blood radioactivity in the equilibrium phase after five and 10 min. The plasma radioactivity after five and 10 min was measured in a well-type scintillation counter and converted into blood radioactivity by correcting for haematocrit (Hct). The area under the curve during the first phase was determined with a planimeter. To extrapolate the left ventricular downslope, it was transformed on semilogarithmic paper with linear extrapolation. The extrapolated values were than replotted on the original tracing. The arithmatic mean of the cardiac output values derived from the five and 10 min registration was calculated.

2.3. Validation of the isotope-dilution cardiac output measurement

Distribution volume of ^{99m}Tc-albumin. For the isotope method of cardiac output measurement to be valid the tracer should remain in the circulation during the first passage through the heart. This could be proved by comparing the in vivo distribution volume of ^{99m}Tc-albumin with the distribution volume of ^{131}I-albumin (5 μCi, 0.2 MBq) in 15 patients and of ^{113}In-transferrin (200 μCi, 7.4 MBq) in 25 patients (14-16). Oedematous patients were not included. The distribution volumes of ^{131}I-albumin and ^{113}In-transferrin are known to correspond with plasma volume. Blood samples were taken from the recumbent patients 0, 5, 10, 20, 30 and 40 minutes after injection of the tracer. Plasma radioactivity was determined in a well-type scintillation counter. Decrease of radioactivity with time was extrapolated to time zero for calculation of distribution volume. Loss of tracer from the circulation was less than 10 percent during the 40 min time of plasma volume measurement. Figure 2 represents the values of the ^{99m}Tc-albumin measurements versus ^{131}I-albumin and ^{113}In-transferrin measurements (n=15, r=0.94 and n=25, r=0.97 respectively). No systematic difference was found between the distribution of the two tracers. Free radioactivity, as determined by trichloroacetic acid precipitation of the ^{99m}Tc-albumin preparation (17), was 2.0±0.15% (mean±SEM, n=54).

Comparison with dye dilution. The correlation between the radioactive method and the indocyanine green dilution method was studied in 30 patients (n=57). In these patients ^{99m}Tc-albumin was flushed into the circulation simultaneously with 1 ml solvent containing 5 mg Cardiogreen. Cardiac output was measured using a Gilford

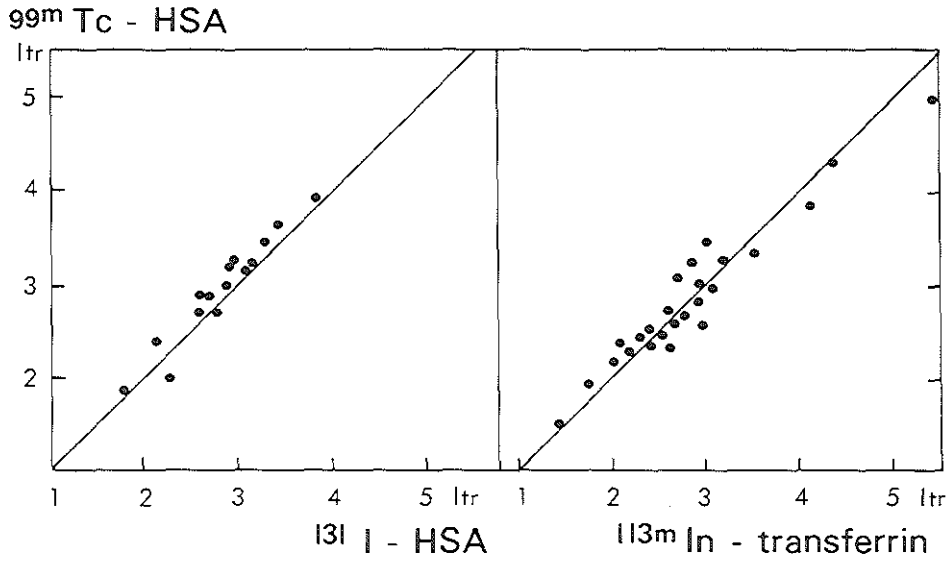

Figure 2. Correlation of distribution volumes of ^{99m}Tc-HSA versus ^{131}I-HSA (n=15, r=0.94) and ^{99m}Tc-HSA versus ^{113m}In-Transferrin (n=25, r=0.97). HSA=human serum albumin.

cardiac output system (model 140, Gilford Instrument Laboratories Inc, Oberlin, Ohio, USA), which is equipped with a dynamic calibration device and a small digital computer. Time concentration curves were recorded from blood drawn from a brachial artery. In 11 patients both indicators were injected simultaneously directly into the right atrium. In the remaining patients the tracers were injected into an antecubital vein.

Figure 3 compares the results of cardiac output measurements by the isotope and dye dilution methods after central injection (n=11, r=0.99). After injection into an antecubital vein in 30 patients (n=57) an r-value of 0.92 was obtained (Fig. 4).

Values measured by isotope dilution were systematically higher than dye dilution values. Factors contributing to this systematic difference are the wide-angle type of collimator and the type of isotope. The gamma radiation of ^{99m}Tc has a low energy, thereby raising the relative contribution of extracardial radioactivity in the equilibrium phase so that Rf is overestimated. The mean of the individual dye cardiac output/isotope cardiac output ratios was 0.75 and this factor was used for correction of isotope cardiac output values.

In 38 duplicate measurements (Fig. 5) the coefficient of variation was 6%. There was no statistical difference between the first and second determination ($p>0.05$, Student's t-test).

The influence of extreme positioning of the detector over the right and left side of the heart is shown in Fig. 6. Even in these extreme positions a reasonable correlation was found (n=18, r=0.82). Our results indicate that small variations in the position of the

detector over the heart during follow-up studies in individual patients are of minor importance.

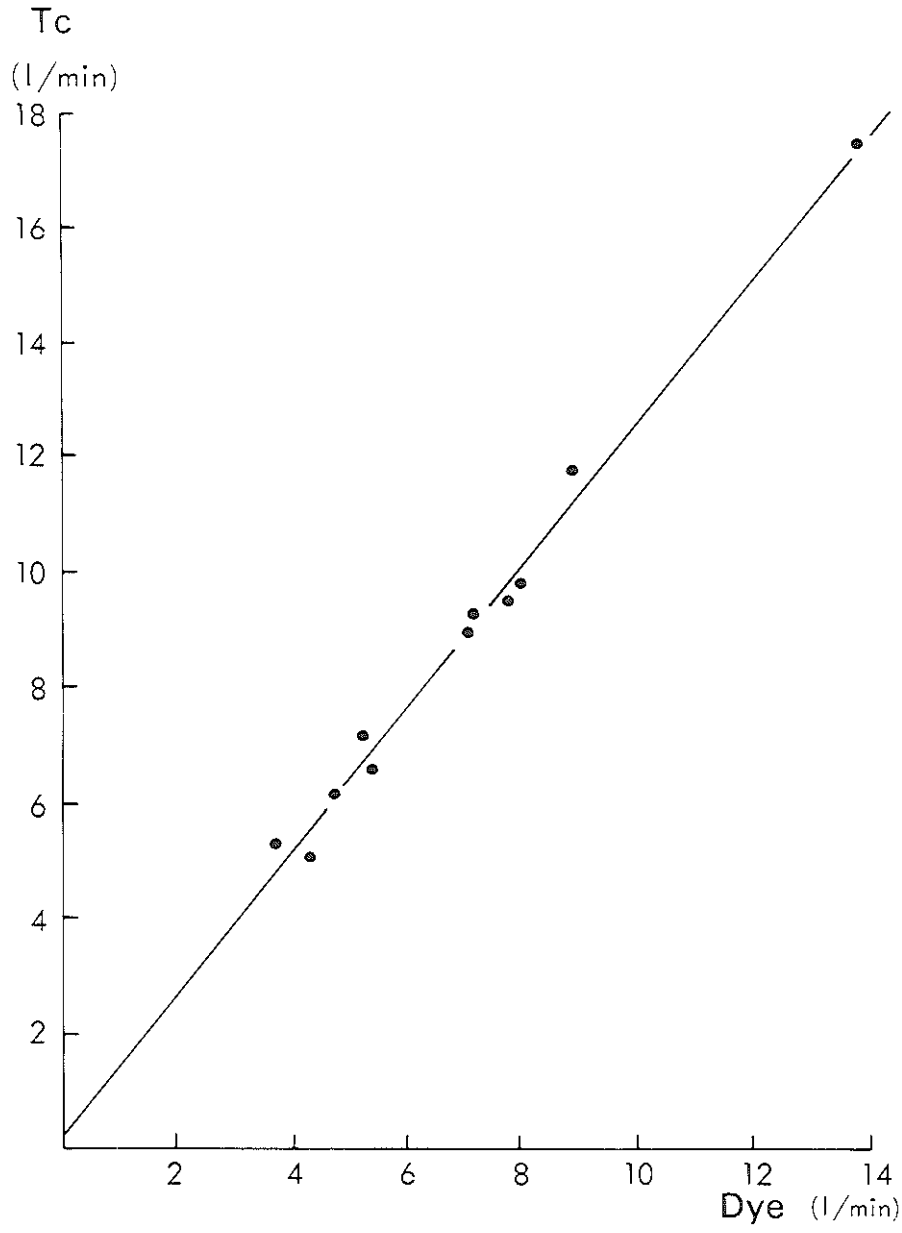

Figure 3. Correlation between cardiac output values obtained by isotope dilution (Tc) and dye dilution (Dye), after central injection of the indicators (n=11, r=0.99)

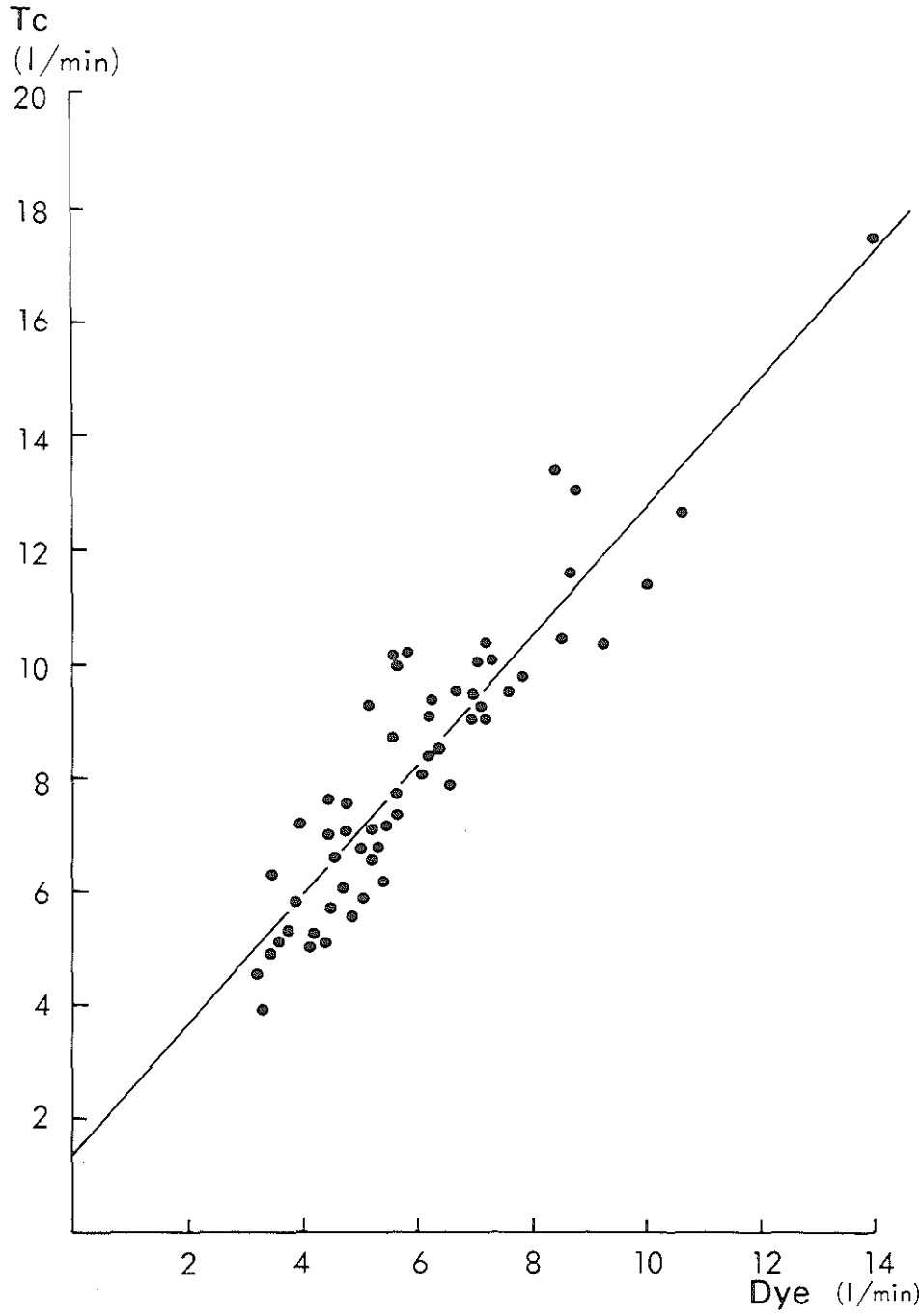

Figure 4. Correlation between cardiac output values obtained by isotope dilution (Tc) and dye dilution (Dye), after peripheral injection of the indicators (n=57, r=0.92)

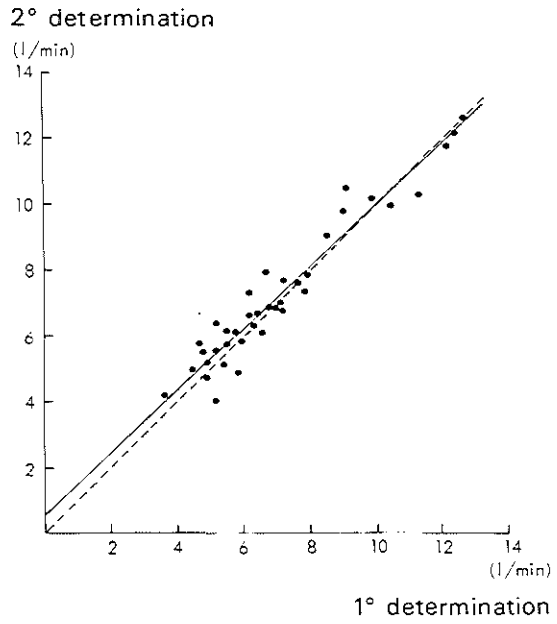

Figure 5. Duplicate measurements of cardiac output by isotope dilution (n=38, r=0.96). Dotted line represents line of identity. Drawn line is line of regression.

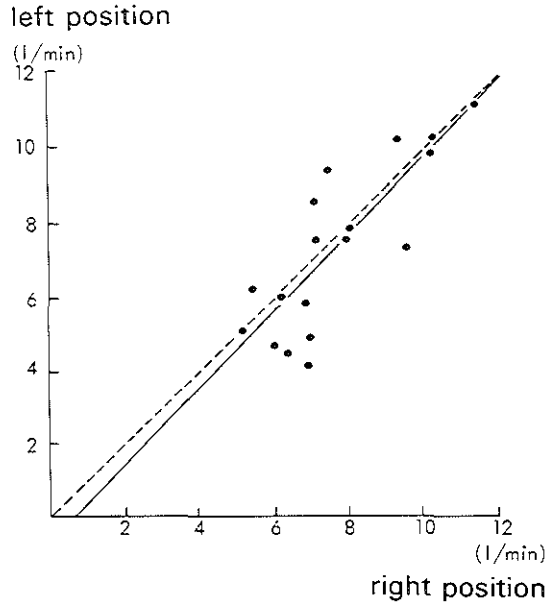

Figure 6. Cardiac output measurements by isotope dilution with the detector in extreme left and right positions over the heart (n=18, r=0.82). Dotted line represents line of identity.

2.4. Total renal function

Renal plasma flow and glomerular filtration rate were measured by clearance studies. When a substance is excreted exclusively by means of glomerular ultrafiltration, the clearance of this substance is equal to glomerular filtration rate. When a substance is completely and exclusively removed from plasma during one single passage through the kidney, the clearance of this substance is equal to renal plasma flow. The clearance of a substance, X, can be calculated by the standard formula:

$$C_X = \frac{U_X \cdot V_U}{P_X}$$

in which:
C_X = clearance of X expressed as ml/min
U_X = concentration of X in urine (mmol/min)
V_U = volume of urine (ml/min)
P_X = plasma concentration of X (mmol/min)

The major problem with this type of measurement is the requirement of correct urine collection. However, when a substance, which is neither formed nor metabolized in the body and which is excreted solely by the kidney, is infused at a constant rate sufficient to keep plasma levels constant, $U_X \cdot V_U$ can be substituted by the amount of substance infused per minute (18-20). The formula then becomes:

$$C_X = \frac{I_X \cdot V_I}{P_X}$$

in which:
I_X = concentration of X in the infusion fluid (mmol/min)
V_I = infusion rate (ml/min)

To reach a constant plasma concentration it is necessary that the substance is uniformly distributed in plasma within a short period of time. This can be achieved by administering a priming dose, which is then followed by a constant infusion of a lower dose of the substance. ^{131}I-sodium iodohippurate (hippuran) was used as a marker for measuring renal plasma flow and ^{125}I-thalamate was used as a marker for measuring glomerular filtration rate. Combined infusion of the two substances was used for measuring renal plasma flow and glomerular filtration rate at the same time (21,22). The sustaining solution contained 200 μCi (7.4 MBq) ^{131}I-hippuran and 50 μCi (1.8 MBq) ^{125}I-thalamate (Radiochemical centre, Amersham, UK) in 100 ml 0.15 mol/l NaCl. After at least two hours of supine rest a plasma blank sample was drawn from an indwelling teflon cannula in an antecubital vein. Then, a priming dose of 0.15-0.20 ml per kg body weight of the sustaining solution was infused by the use of an infusion pump at a rate of 1 ml/min. The infusion rate was then switched to 0.1 ml/min. In this way constant plasma levels of ^{131}I-hippuran and ^{125}I-thalamate were obtained

60-90 minutes after the start of the infusion (22). Blood samples were drawn at 60, 90, 120, 150, 180 and 210 minutes. Separation of plasma was performed immediately after collection of the blood samples. In 2 ml plasma and diluted (1/100) sustaining solution the activities of ^{131}I-hippuran and ^{125}I-thalamate were determined in a well-type gamma scintillation counter and the measured ^{125}I-values were corrected for the contribution of ^{131}I-hippuran in the ^{125}I-window by measuring a pure ^{131}I-hippuran sample. There was no detectable contribution of ^{125}I in the ^{131}I-window. Clearances were calculated from the 90-, 120-, 150-,180- and 210-min samples. The values were averaged. Because renal extraction of ^{131}I-hippuran was not complete during one single passage through the kidney, the value for renal plasma flow we have found was falsely low and was therefore considered to reflect the so-called effective renal plasma flow. In a series of 25 patients with uncomplicated essential hypertension this extraction ratio appeared to be 75.1 $\pm$ 1.0%. and by correcting for this, true renal plasma flow can be calculated. Renal blood flow is calculated by correcting for haematocrit (22).

2.5. Split renal function

For measuring the renal extraction ratio of ^{131}I-hippuran and of ^{125}I-thalamate blood samples were taken simultaneously from the aorta and the renal vein on each side. Single-kidney extraction ratio (extraction efficiency) was calculated as 100 (A-V)/A, where A = activity in abdominal aorta and V = activity in renal vein.
The single-kidney uptake of ^{99m}Tc-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA) was determined by scintillation camera renography. Approximately 5-10 mCi (185-370 MBq) ^{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 3-min 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 (23-24).

2.6. Extracellular fluid volume and plasma volume

Sulphate space was measured as an estimate of extracellular fluid volume (25,26) by intravenous injection of 3-4 ml of a solution of $Na_2{}^{35}SO_4$ in 0.15 mol/l NaCl (2.5-3.0 μCi/ml, 0.09-0.11 MBq/ml). The tracer was flushed into the circulation with 10 ml, 0.15 mol/l NaCl. The volume of tracer injected was determined by weighting the syringe before and after injection. At 30, 60, 80, 100, 120, and 140 minutes, blood samples were drawn. Plasma samples of 1 ml were counted in a liquid scintillation counter. After correction for quencing and background by the use of a plasma blank and an internal standard the results were plotted against time on semilogarithmic paper. For extrapolation to time zero the samples from 60-140 minutes were used and

sulphate space (extracellular fluid volume, ECV) was calculated according to the formula:

$$ECV\ (L) = \frac{\text{dose injected (cpm)} \times 10^3}{\text{plasma counts at time zero (cpm/ml)}}$$

No correction was made for the Donnan equilibrium between plasma and interstitial fluid, 'dry' plasma constituents and sulphate loss in the urine.
Plasma volume was estimated by the use of ^{99m}Tc-labeled human serum albumin at the time of cardiac output measurement, as described before.

2.7. Chemical assays

Angiotensin-converting enzyme. Angiotensin-converting enzyme in plasma was measured by a spectrophotometric assay based on the methods of Cushman and Cheung (27) and Le Treut et al (28). Peripheral venous blood was collected in tubes containing lithium heparin as the anticoagulant. Plasma was prepared by centrifugation at 4°C, immediately after blood sampling. A 50-μl portion of plasma was added to 200 μl of a freshly prepared solution of the substrate hippuryl-L-histidyl-L-leucine (Sigma Chemical Co, St.Louis, USA) in borate/NaCl buffer. The substrate solution contained the following components: hippuryl-L-histidyl-L-leucine (6.25 mmol/l), sodium borate (0.125 mol/l) and NaCl (0.375 mol/l); pH was adjusted to 8.3. The substrate/plasma mixture was incubated at 37°C in a shaking water bath for one hour. The reaction was stopped with 250 μl of HCl (1 mol/l). A blank was prepared by adding the acid before the incubation. Hippuric acid that was formed during incubation was extracted with 1.5 ml of ethylacetate by mixing. After centrifugation 1 ml of the organic layer was transferred to a new tube. The ethylacetate was evaporated and 2 ml of a NaCl solution (1 mol/l) was added, followed, after mixing, by 2 ml of light petroleum (boiling point 40-60°C). The samples were then mixed and after centrifugation the light petroleum layer was removed by aspiration. The absorbance at 228 nm was read in a 1-cm quartz cuvette. The absorbance of the blank was substracted from the absorbance of the corresponding test sample. Results are expressed in milliunits per millilitres of plasma, one unit being the quantity of enzyme that produces one micromole of hippuric acid per minute. The production of hippuric acid from hippuryl-L-histidyl-L-leucine was linear with time for at least one hour upto levels of 80 m-units/ml. Samples were assayed in duplicate. Both intra- and inter-assay coefficients of variation were less than 5%.

Active renin. The concentration of enzymatically active renin in plasma was measured as described by Derkx et al (29,30). Blood was collected in chilled plastic tubes containing disodium ethylenediaminetetra-acetate (EDTA) in a final concentration of 5 mmol/l. Blood was centrifuged at 4°C immediately after collection. Plasma was kept frozen at -20°C before use. Aliquots (0.10-0.25 ml) were added to 0.5 ml of renin substrate, and the volume was adjusted to 1.0 ml with phosphate buffer pH 7.5. Renin substrate was prepared from plasma of nephrectomized sheep. The final concentration of renin substrate in the incubation mixture was 2.5 μmol/l Ile^5-angiotensin I equiva-

lents, which corresponds with about 10 times K_M. After addition of 10 μl of 0.34 mol/l 8-hydroxyquinoline, 5 μl of 0.28 mol/l phenylmethylsulphonyl-fluoride in ethanol and 10 μl of aprotinin (10,000 kallikrein-inhibiting units/ml) the mixtures were incubated for three hours at 37°C. Parallel incubations at 4°C served as blanks. After incubation 1 ml of NaCl (0.15 mol/l) was added and the mixture was heated for ten minutes in a boiling water bath and centrifuged. The concentration of angiotensin I in the supernatant was measured by radioimmunoassay, using ^{125}I-labeled Ile^5-angiotensin I and rabbit anti-Ile^5-angiotensin I anti-serum. Semipurified human renal renin (MRC-standard 68/356) was used as an external standard (31). Renin concentration is expressed as μ-units of this standard per ml of plasma (μU/ml). The inter-assay variability was evaluated by weekly measurements of active renin in standard normal plasma. The mean value of active renin was 27±3 μU/ml for 36 assays (coefficient of variation 11%). Normal values in 17 healthy male subjects, who were recumbent for at least one hour before sampling and had a daily sodium intake of 50 mmol ranged from 14-43 μU/ml.

Noradrenaline. For the determination of plasma noradrenaline the radioenzymatic method described by Henry et al (32) was used. The method is based on the principle that noradrenaline in the presence of a radiolabeled methyldonor (S-adenosyl-L-(methyl-^{3}H) methionin, ^{3}H-SAM) is converted by the enzyme phenylethanolamine-N-methyl transferase (PNMT) into radiolabeled adrenaline. Ten ml of blood was collected into chilled tubes containing heparin and 15 mg gluthatione to prevent noradrenaline breakdown. Immediately after collection the tubes were put on ice and centrifuged at 4°C for 10 minutes. Immediately thereafter plasma (4.5 ml) was removed and deproteinized by adding 0.5 ml of 3 mol/l trichloroacetic acid under vigorous stirring. After centrifugation at 4°C for 15 minutes the supernatant was removed and stored at -20°C until assayed. The assay was performed by incubating 100 μl portions of the deproteinized plasma at 37°C with 5 μCi of ^{3}H-SAM (Radiochemical centre, Amersham, UK; 10-15 Ci/mmol) and with PNMT, which was prepared according to the directions of Molinoff et al (33). After one hour the incubation was stopped by adding Tris-phosphate buffer of pH 8.6 and the adrenaline formed was absorbed on acid-washed alumina as described by Anton and Sayre (34). Repeated washings with distilled water and precipitation with phosphotungstic acid removed most of the excess ^{3}H-SAM. The radioactive adrenaline was taken up in toluene containing 2% of diethylhexylphosphoric acid and counted for 10 minutes in a scintillation counter after addition of scintillation liquid (8 g of butyl-PBD (2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole). All measurements were made in duplicate, both with and without an internal standard (500 pg of noradrenaline). Blanks were prepared by substituting 0.3 mol/l trichloroacetic acid for deproteinized plasma. In each assay batch samples of a plasma pool were included. Inter- and intra-assay variability coefficients were less than 10%. Normal values are 100-450 pg/ml in the recumbent patient.

Other determinations. Blood haematocrit was measured by the microcentrifuge method using oxalate as the anticoagulant (35). Sodium and potassium in plasma and

urine were measured by a flame photometric method. Plasma was prepared by using lithium heparin as the anticoagulant. Creatinine was measured in serum and urine according to Jaffés alkaline picrate reaction. The measurements were made by the central laboratory for clinical chemistry at the university hospital.

2.8. Calculation of radiation dose

The mean absorbed doses resulting from the isotopes used in the in vivo investigations described in this thesis were calculated from the tabulated 'S-values' (absorbed dose per unit cumulated activity) of the various isotopes (36).

Isotope	Activity (MBq)	Dose (mGy/MBq)	Mean absorbed dose (mSv)
^{35}S (sulphate)	0.25	0.10	0.025
^{99m}Tc-(albumin)	7.40	0.08	0.592
^{125}I-(thalamate)	0.56	0.04	0.022
^{131}I-(hippuran)	2.22	0.23	0.511

Comparing these figures with those of the normal environmental radiation (2 mSv per year), the allowed yearly dose for general members of the public (5 mSv) and for radiological workers (50 mSv) and with X-ray investigations (0.5-8 mSv), we feel well justified to use these methods.

2.9. Statistics

Results are expressed as mean±standard error of the mean (mean±SEM). All values relevant to body size were converted to 1.73 m^2 body surface area. For comparison of results Student's t-test for paired and unpaired data was used. Correlations between parameters were assessed by linear regression analysis. Differences between results and correlation coefficients were considered to be significant at a p-value less than 0.05.

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CHAPTER 3

Haemodynamic profile of captopril treatment in various forms of hypertension

3.1. Summary

The effects of captopril 450 mg/day for four weeks on blood pressure, heart rate, cardiac output and extracellular fluid volume were compared in severe, often drug-resistant hypertension (n=23), mild to moderate hypertension associated with renal artery stenosis (n=10) and mild to moderate essential hypertension (n=20). Plasma renin in the three groups was 52±19, 58±17 and 20±4 μU/ml (mean±SEM), respectively. Blood pressure fell by 18±4%, 21±2% and 18±1%. The pressure drop was mainly due to a fall in peripheral vascular resistance. Addition of the diuretic hydrochlorothiazide (25-100 mg/day) caused a further fall in resistance. Despite the vasodilator effect of captopril, reflex cardiostimulation and reactive fluid retention were not observed. In severe hypertension, captopril alone was more effective in lowering blood pressure than combined diuretic-betablocker-vasodilator therapy. Moreover, cardiac output in these patients was higher and resistance was lower after captopril than during combined treatment. Thus, captopril was capable of normalising the abnormal haemodynamic state in patients with essential hypertension and in hypertension associated with renal artery stenosis. Despite marked differences in pre-treatment plasma renin, the effects of captopril on systemic haemodynamics were similar in all the patients.

3.2. Introduction

The orally active angiotensin I-converting enzyme inhibitor, captopril, belongs to a new class of antihypertensive agents, which may offer certain advantages over established drugs (1,2).

The efficacy of captopril in reducing blood pressure has been amply demonstrated in various forms of hypertension (3-8), but its effect, particularly during chronic treatment, has not been defined in terms of cardiac output and vascular resistance. Apart from a study by Atkinson et al (9), indicating that total exchangeable sodium had not changed after six weeks of captopril in eight patients with renal artery stenosis, there are few data on fluid balance during long-term captopril therapy. Information on these points is important, because it may help to delineate more clearly the place of this drug among the antihypertensive drugs now in common use.

We report here on a comparative study undertaken to collect such data in 1) severe,

often drug-resistant hypertension, 2) mild to moderate hypertension associated with renal artery stenosis, and 3) mild to moderate essential hypertension. In the group with severe hypertension, combined diuretic-betablocker-vasodilator treatment was compared with captopril alone and with captopril plus diuretic.

3.3. Methods

All measurements were made while the patients were recumbent for at least one hour. Blood pressure was measured with the London School of Hygiene sphygmomanometer (10) to minimise observer bias. Phase V was taken as diastolic pressure. Three consecutive readings were averaged. Cardiac output was determined by a non-invasive radioisotope dilution technique (11). Heart rate was calculated from a simultaneously recorded ECG. Immediately after the precordial time-radioactivity curve had been recorded, blood pressure was measured in triplicate, the average value of mean arterial pressure [diastolic pressure + 0.3 × (systolic pressure - diastolic pressure)] being used for calculating total peripheral resistance. Blood samples were then taken for measuring extracellular fluid volume (12) and active renin in plasma (13).
The results presented here were obtained while the subjects were followed as outpatients. The patients were advised to take a diet containing sodium 100 mmol/day. Adherence to this advice was not rigorously checked, but spot 24-h urine collections before and after four weeks of captopril in 30 patients gave values of 112 ± 8 and 108 ± 10 mmol sodium, respectively.
Measurements were made three to four hours after the morning dose of captopril or placebo. For blood pressure measurements the patients were seen weekly in the placebo-period and during the first eight weeks of captopril. Thereafter, they were seen at intervals of four weeks. Cardiac output, extracellular fluid volume and plasma renin were determined before captopril and after four weeks of captopril 450 mg/day.

3.4. Patients and study protocol

Fifty-three consecutive patients (Table 1), who had been referred to the hypertension clinic, were studied after they had given their informed consent to the procedures.

Severe hypertension. Twenty-three patients had a blood pressure of 200/130 mm Hg or higher at two or more visits to the outpatient clinic. Twelve patients had been untreated for at least two weeks while these pressures were recorded. Plasma renin was elevated in four of them. Eleven patients were on multiple drug therapy and remained hypertensive (160/100 mm Hg or higher) despite a 2-week course of so-called standard triple therapy, i.e. 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). A previous period of accelerated or malignant hypertension with bilateral retinal haemorrhages and exudates has been documented in 12 cases. In five patients hypertension was

TABLE I: BLOOD PRESSURE AND RENIN IN THE THREE PATIENT-GROUPS

Patient-group	No	Sex (male)	Age (yr)	Blood pressure (mm Hg)		Renin (μU/ml)
				Systolic	Diastolic	
Severe hypertension	23	19	51 ± 3	227 ± 11	142 ± 4	52 ± 19
Mild to moderate hypertension associated with renal artery stenosis	10	3	37 ± 4	181 ± 8	106 ± 4	58 ± 17
Mild to moderate essential hypertension	20	17	47 ± 2	170 ± 4	106 ± 2	20 ± 4

Blood pressure and renin values in severe hypertension are those before standard triple therapy (see text) and captopril were begun. The values in mild to moderate hypertension are those measured in the last week of placebo. Normal range of plasma renin is 15-40 μU/ml.

associated with renal artery stenosis, and in 12 with impaired renal function (serum creatinine 150-400 μmol/l). Patients were taken into hospital and, after previous medication had been stopped, captopril was begun in a dose of 25 mg orally, which was gradually increased to 450 mg/day, divided into three doses. The patients were then followed in the out-patient clinic. Blood pressure became normal on captopril alone in six patients. The remainder became normotensive after hydrochlorothiazide (25-100 mg/day) had been added.

Mild to moderate hypertension associated with renal artery stenosis. This group consisted of ten patients. Blood pressure after treatment had been stopped for at least three weeks was 150-200 mm Hg systolic and 90-120 mm Hg diastolic. They were admitted to hospital for renal arteriography and renal vein renin sampling, because of a unilateral abnormality on intravenous urography and/or radioisotope renography, which was suggestive of renal artery stenosis. Peripheral venous renin was elevated in seven patients. Renal arteriography was performed after the renal vein and artery samples had been collected, and it showed unilateral renal artery stenosis. The renal vein-to artery ratio of renin on the side of stenosis was 1.5-3.4. Patients were followed as out-patients for four weeks while on placebo. Captopril was then begun in a dose of 25 mg, which was increased over four weeks to 450 mg/day, divided into three doses. At the time of the study all patients had been on captopril for at least two months. By the time of preparation of this report six patients had undergone surgery, and in them three months later blood pressure was 160/90 mm Hg or less without medical treatment.

Mild to moderate essential hypertension. This group consisted of 20 patients. Routine investigations, including intravenous urography and radioisotope renography, had not revealed any cause for the hypertension. Blood pressure while the patients were untreated was 140-200 mm Hg systolic and 95-120 mm Hg diastolic. Plasma renin was elevated in two patients, it was normal in 12 and low in six. As in the previous group, captopril was begun after a 4-week placebo period, and the dose was increased

from 25 to 450 mg/day. At the time of this analysis, all patients had been on captopril alone for at least two months, and 12 patients for more than one year.
Data are presented as mean±SEM. Student's t-tests for paired and unpaired data were used for statistical analysis.

3.5. Results

After four weeks of captopril alone (450 mg/day), mean arterial pressure had fallen by 29±6 mm Hg (18±4%) in severe hypertension, by 29±3 mm Hg (21±2%) in mild to moderate hypertension associated with renal artery stenosis, and by 23±3 mm Hg (18±1%) in mild to moderate essential hypertension (Fig. 1,2). The pressure changes were not significantly different, despite the differences in plasma renin. Blood pressure became 150/90 mm Hg or less in six out of 23 patients with severe hypertension, in eight out of ten patients with mild to moderate hypertension associated with renal artery stenosis and in 16 out of 20 patients with mild to moderate essential hypertension. The hypotensive effect did not wear off with continued use of captopril over periods of more than one year.
In the patients with severe hypertension, the fall of blood pressure after four weeks of captopril monotherapy was paralleled by a fall in resistance. Blood pressure on captopril was significantly lower than during combined treatment with a diuretic, beta-adrenoceptor blocking agent and a vasodilator (Fig. 1). Moreover, cardiac output was higher and total peripheral resistance was lower on captopril than during triple therapy. Both pressure and resistance fell further when a diuretic was added to captopril (Fig 1).
In the patients with mild to moderate hypertension and renal artery stenosis cardiac output was unchanged after captopril, and in mild to moderate essential hypertension cardiac output was slightly lowered. In both groups with mild to moderate hypertension the fall in blood pressure was associated with a fall in resistance and, as in severe hypertension, the change in resistance was the main cause of the fall in pressure (Fig 2).
In contrast to many vasodilators, captopril did not cause reflex-cardiostimulation, and heart rate and stroke volume did not rise. Also unlike many vasodilators, captopril did not lead to fluid-retention, as judged from measurements of extracellular fluid volume. In the group with mild to moderate essential hypertension, a significant decrease in extracellular fluid volume was observed.
Plasma renin rose from 52±19 to 310±83 μU/ml after four weeks of captopril (450 mg/day) in severe hypertension; it rose from 58±17 to 410±72 μU/ml in mild to moderate hypertension associated with renal artery stenosis, and from 20±4 to 98±22 μU/ml in mild to moderate essential hypertension. Serum creatinine was unchanged after captopril alone. Some decline in renal function occurred in the patients who received both captopril and diuretic; creatinine rose by 11±2%.

Adverse reactions. Six patients developed a maculopapular skin rash. Two patients complained of loss of taste. These adverse reactions disappeared while the drug was

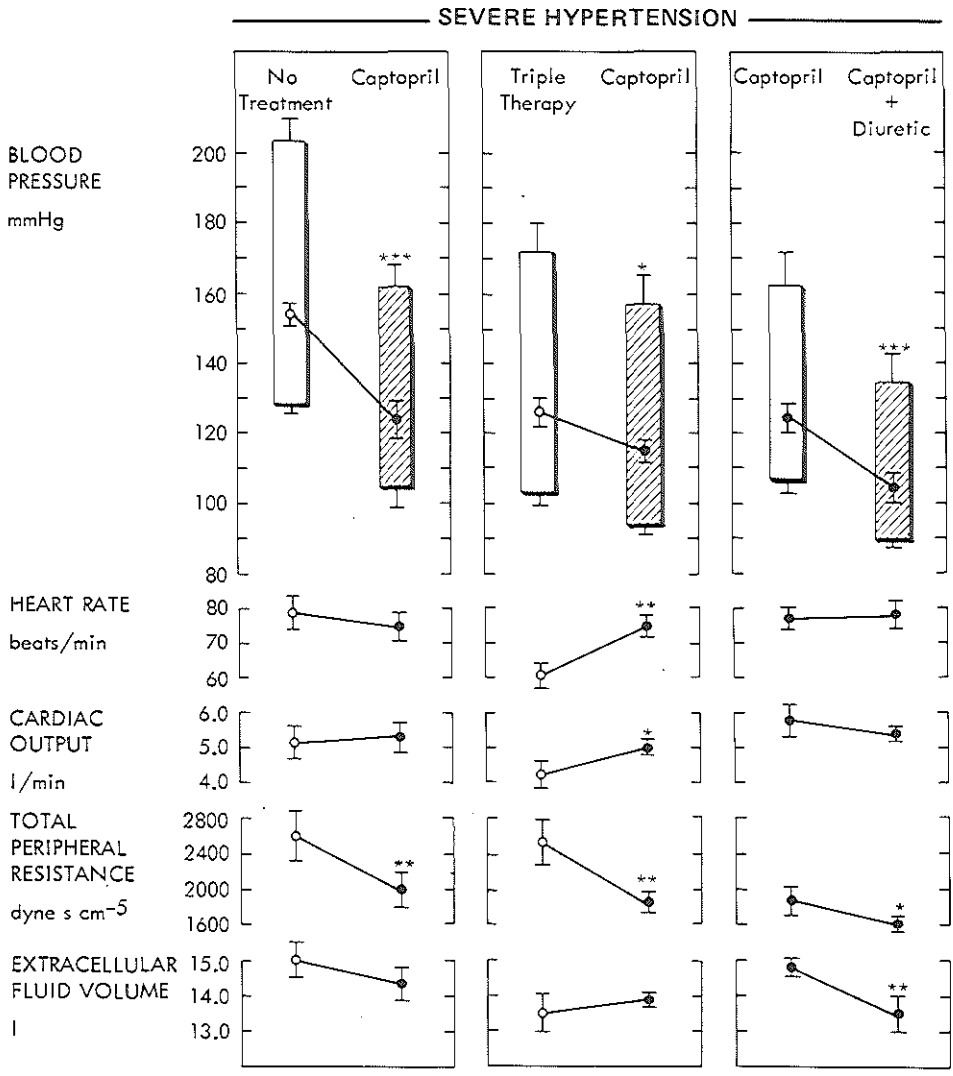

Figure 1. Effects of captopril 450 mg/day for four weeks in severe hypertension. Comparison with standard triple therapy (see text) and with captopril plus hydrochlorothiazide 25-100 mg/day for four weeks. Systolic, diastolic and mean arterial pressures are indicated. Numbers of patients: left panel, 12; middle panel, 11; right panel, 10. $*=p<0.05$, $**=p<0.01$, $***=p<0.001$.

continued. One patient with severe drug-resistant hypertension and renal parenchymal disease (endogenous creatinine clearence 15-20 ml/min), who was on captopril 450 mg/day and hydrochlorothiazide 100 mg/day for one year, developed oedema of the face. Both captopril and hydrochlorothiazide were withdrawn. The signs cleared within 24 hours and had disappeared after a week. Because blood pressure control

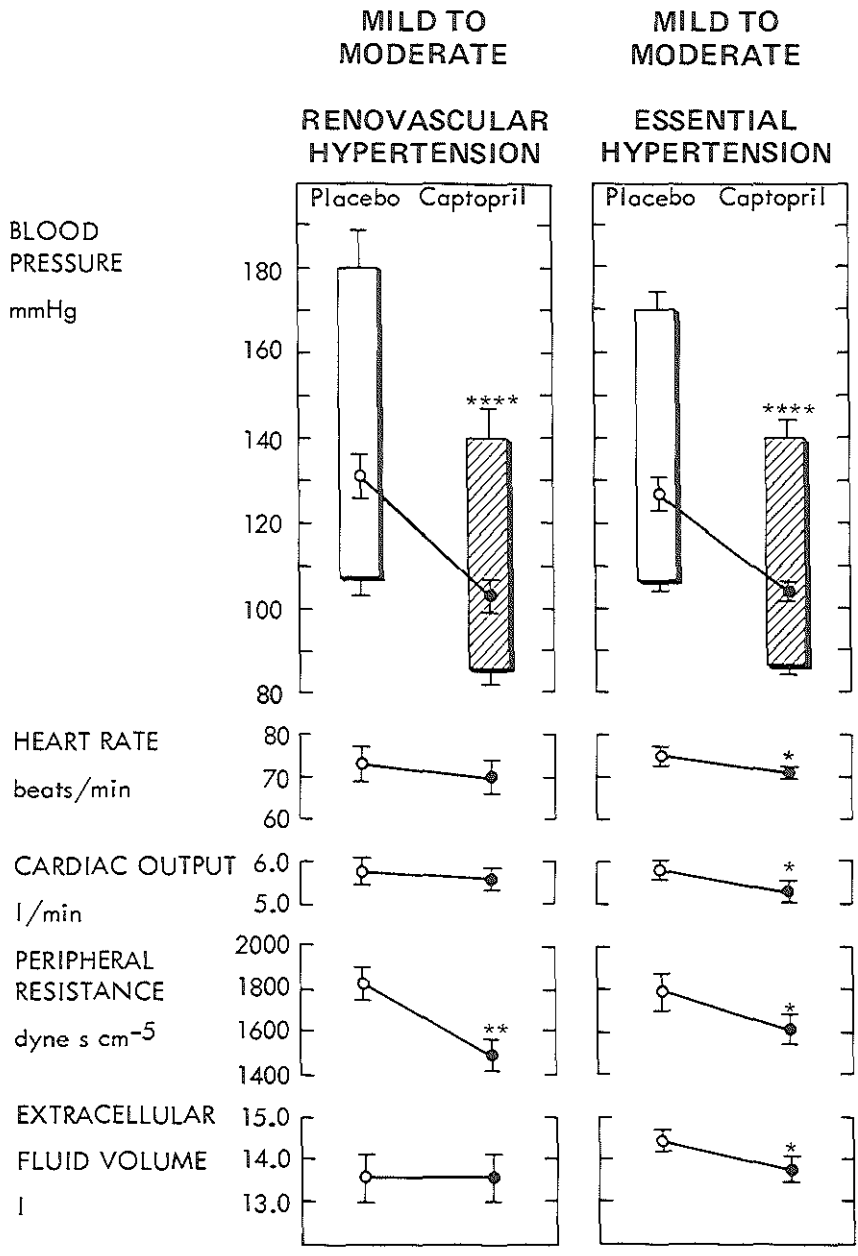

Figure 2. Effects of captopril 450 mg/day for four weeks in mild to moderate hypertension associated with renal artery stenosis (left panel), and in mild to moderate essential hypertension (right panel). Measurements during captopril therapy are compared with those in the fourth week of placebo treatment. Systolic, diastolic and mean arterial pressures are indicated. Numbers of patients: left panel, 10; right panel, 20. $*=p<0.05$, $**=p<0.01$, $***=p<0.001$.

was lost, captopril was given again, but in a dose of 100 mg/day and hydrochlorothiazide was added two weeks later. With this treatment satisfactory blood pressure control was achieved without any adverse effect.

3.6. Discussion

It has recently been advised to restrict the use of captopril, at least for the time being, to patients with severe hypertension and to cases with renovascular hypertension. This advice is sensible because captopril is very effective in these patients, even when established drugs have failed (14,15). The advice also seems logical because an attack on the renin-angiotensin system is thought to be a major component of the hypotensive effect of captopril; plasma renin is often elevated in accelerated or malignant hypertension and in hypertension associated with renal artery stenosis. This study confirms the efficacy of captopril in severe, often high-renin, hypertension. In addition the data show that the drug is no less effective in mild or moderate hypertension, no matter whether plasma renin is high or low. Other factors than inhibition of plasma-renin-mediated angiotensin II formation may be important for the hypotensive effect of converting-enzyme inhibition (16-20).

A 20% reduction in both systolic and diastolic pressures was observed with mild dietary sodium restriction and captopril as the only drug. This response is as good as or even better than those obtained with thiazide diuretics (21) or beta-adrenoceptor blocking agents (22,23). Captopril has an almost ideal haemodynamic profile. It lowers vascular resistance with little or no effect on cardiac output. The drug produces vasodilatation, but does not cause the usual side effects of vasodilatation, such as reflex tachycardia and fluid retention by the kidneys.

It is not clear why vasodilatation after captopril does not lead to baroreflex-mediated cardiostimulation. The absence of fluid-retention after captopril can be explained, at least in part, by reduced angiotensin II formation, since this peptide is known to promote renal tubular sodium reabsorption both directly and via aldosterone. A similar mechanism might underly the favourable haemodynamic response to the combination of captopril with a diuretic (14). Our patients showed a sustained reduction of extracellular fluid volume after hydrochlorothiazide had been added to captopril. This was associated with a significant fall in vascular resistance. Presumably, inhibition of angiotensin I conversion in these patients has prevented the compensatory rise in angiotensin II and aldosterone, which can limit the therapeutic effect of diuretics (24,25).

While there are no signs of increased adrenergic nervous activity counteracting the hypotensive effect of captopril, there is also no indication that the baroreflex-mediated response to upright posture is impaired by captopril, so that postural hypotension is seldom seen (26). From experiments in dogs there is even some evidence that the set point, but not the sensitivity, of the baroreflex is altered by converting-enzyme inhibition (27).

Some severe adverse reactions to captopril have been reported, including pemphigus-like skin eruptions, nephrotic syndrome, leucopenia and agranulocytosis. Such reac-

tions, however, have mostly been observed when hypertension was associated with renal disease, systemic lupus erythematosus or scleroderma, or when the patients were on immunosuppressive therapy. The incidence of such severe reactions in patients with uncomplicated hypertension seems less than 1% (28). Minor side effects, such as skin rash and taste disturbance, have been observed in about 14% of the hypertensive patients in a large series (28), which agrees with the incidence of 17% in the present study. Cutaneous reactions may be dose-related (29), and it is important that lower doses than originally recommended may be used without loss of therapeutic effect (19).

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CHAPTER 4

Haemodynamic effects of captopril in essential hypertension, renovascular hypertension and cardiac failure: correlations with circulating renin

4.1. Summary

The haemodynamic 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 two hours, and the effects of repeated doses, 450 mg/day in combination with mild dietary sodium restriction, for at least four 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. Serial 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, $r=0.47$, $p<0.01$, $n=44$). 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 favourable haemodynamic 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.

4.2. Introduction

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 differences between haemodynamic 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 six 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.

4.3. Patients and methods

Forty-four hypertensive and 14 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 (seven 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 three 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 two 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 (nine 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 four 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 two 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 two weeks.

Chronic heart failure. Fourteen patients (three 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).

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 four 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 one hour. Noninvasive measurements of cardiac output (vide infra) were begun when the patients were on placebo or standard triple therapy for at least two 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 haemodynamic 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 three weeks. Eleven patients on standard triple therapy were slowly titrated with increasing doses of captopril on an eight 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 haemodynamic 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.

Haemodynamics. All haemodynamic studies were performed while the patients were in the postabsorptive state and when they had been recumbent for at least one hour. The short-term effects of captopril were monitored invasively and the measurements were begun one 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 ^{99m}technetium 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 the midsternal line with the patient supine. Response curves were recorded for one to two minutes after rapid intravenous injection of the isotope. Additional recordings were made after five 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 four to 12 liters/min the coefficient of variation was calculated to be six 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 (8 to 12 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.
Data are presented as mean values±standard error of the mean. The t-tests for paired and unpaired data were used for comparison.

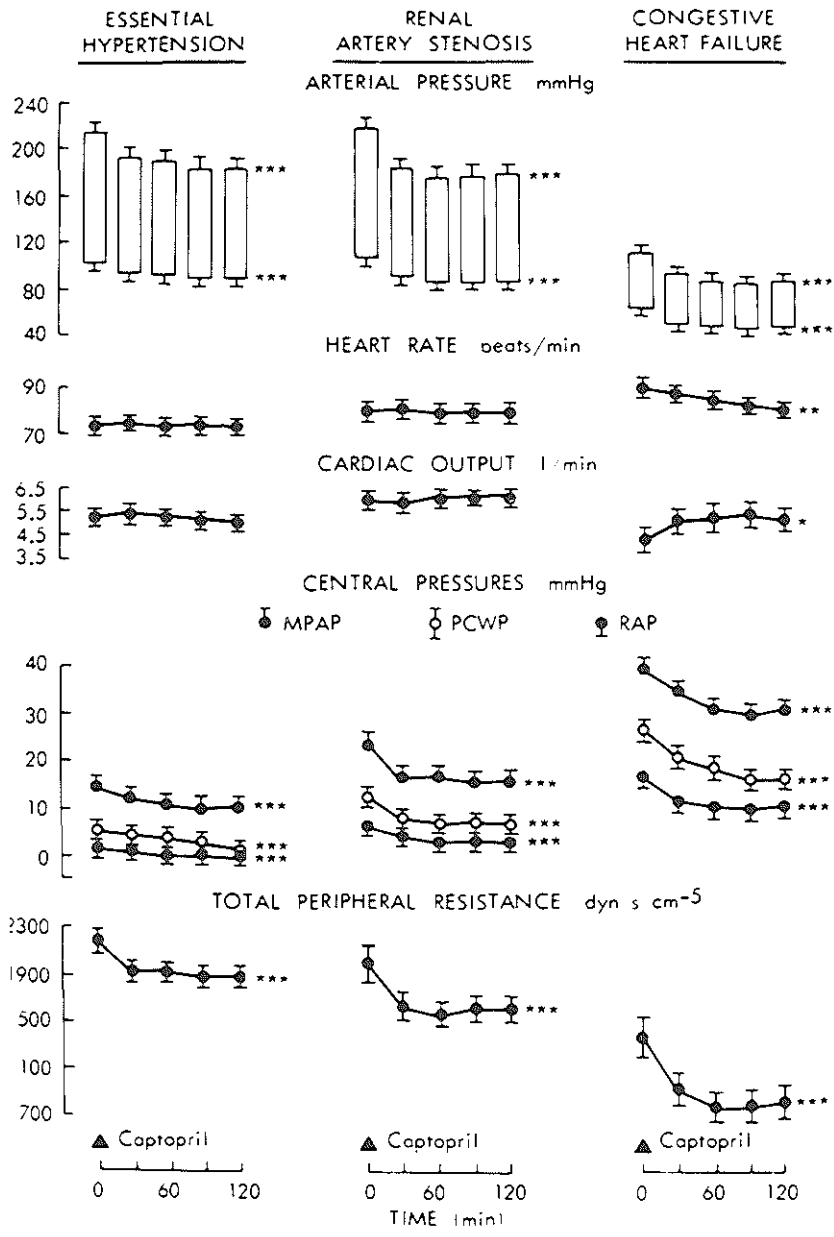


Figure 1. Short-term haemodynamic effects of captopril, 50 mg orally, in patients with essential hypertension (n=14), hypertension with renal artery stenosis (n=16) and chronic heart failure (n=14). Data on arterial pressure, heart rate and central pressures in heart failure were obtained in 14 patients; 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. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$.

4.4. Results

Short-term studies

Blood pressure and haemodynamic 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±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 2170±90 to 1880±90 dyn s cm^{-5} in essential hypertension, from 1980±160 to 1600±130 dyn s cm^{-5} in 'renovascular' hypertension and from 1360±210 to 770±130 dyn 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 2700±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 four 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/min before captopril and 69±2 after four weeks of captopril therapy. Cardiac output was 5.5±0.2 liters/min before the drug and 4.8±0.2 after four 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 2070±130 to 1830±92 dyn s cm^{-5} in the essential hypertension group and from 1940±87 to 1470±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

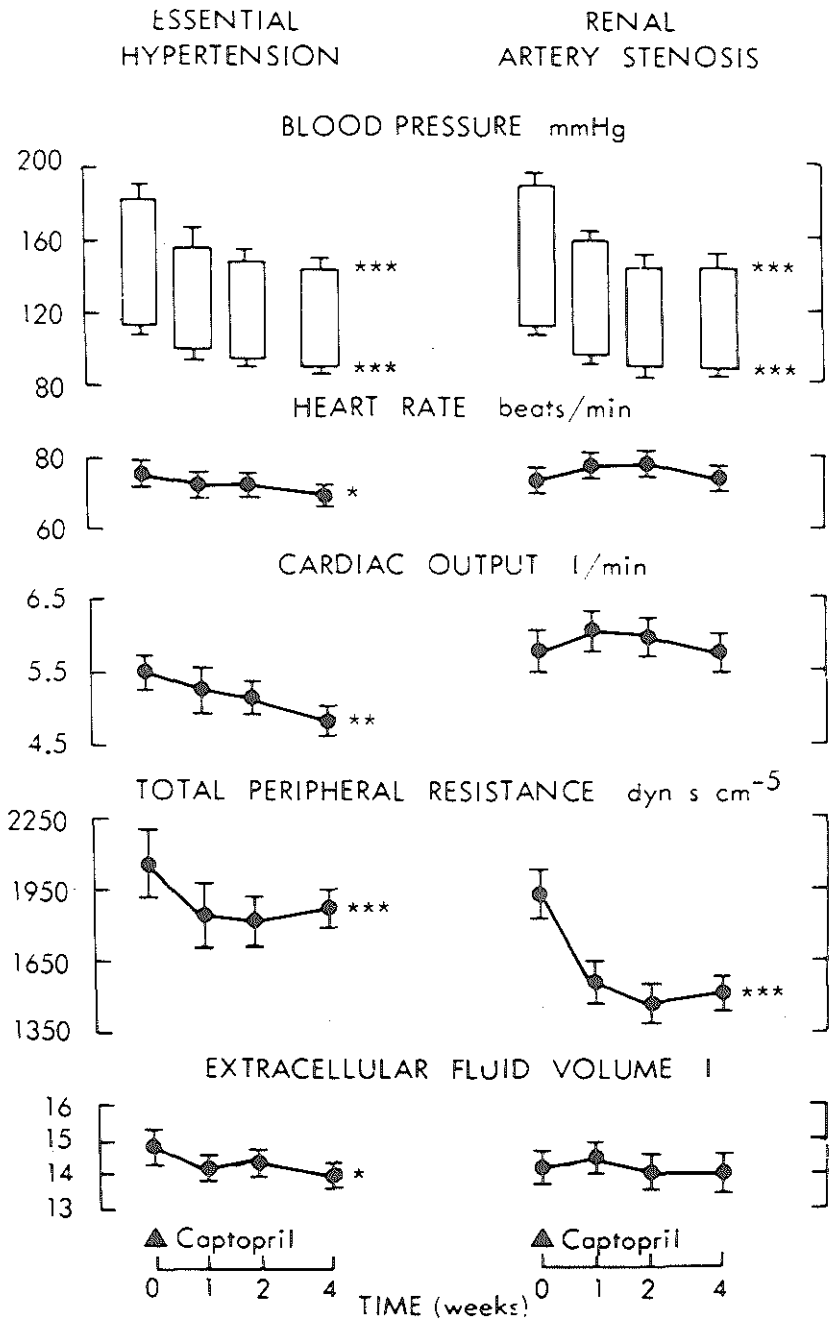


Figure 2. Long-term haemodynamic effects of captopril, 450 mg/day, in patients with essential hypertension (n=17) and hypertension with renalartery stenosis (n=14). Measurements during captopril therapy are compared with those in the last week of placebo treatment. For further details see legend to Figure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

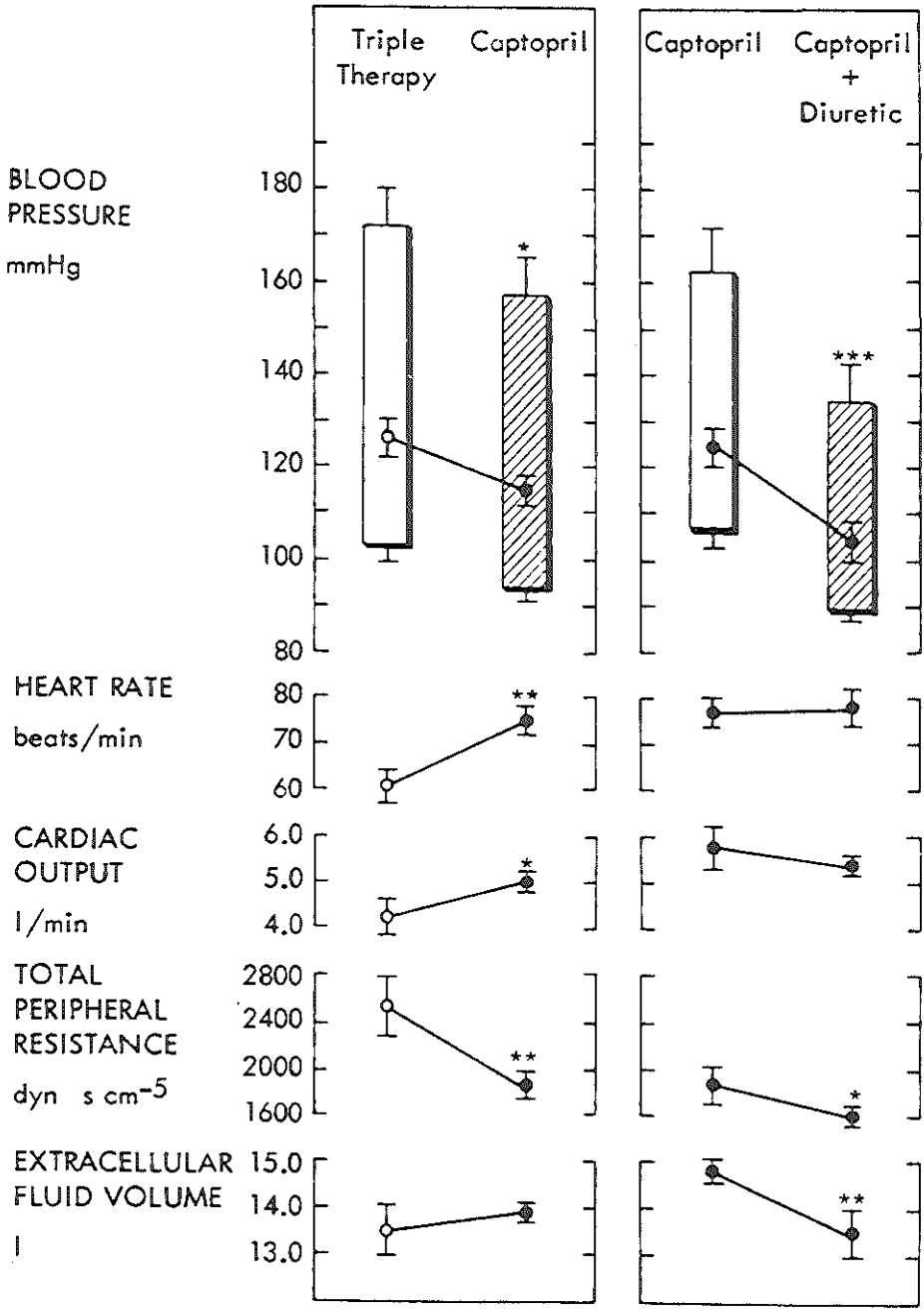


Figure 3. Long-term haemodynamic effects in the two hypertensive groups of captopril alone as compared with standard triple therapy (n=11) and with captopril combined with diuretic (n=10). For details see text and legend to Figure 1.

captopril and 14.0 liters after four weeks. In the 'renovascular' hypertension 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 100 ± 30 μU/ml after four weeks of captopril therapy, 450mg/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 haemodynamic effects of four weeks of captopril monotherapy, 450 mg/day, and four 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 haemodynamic profiles of four weeks of captopril alone, 450 mg/day, and four weeks of captopril plus hydrochlorothiazide, 25 to 100 mg/day, were compared in ten 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 haemodynamic responses and pretreatment plasma renin (Fig. 4). 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, $p<0.01$, $n=44$). 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 ($r=0.56$, $p<0.001$, $n=31$).

4.5. 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

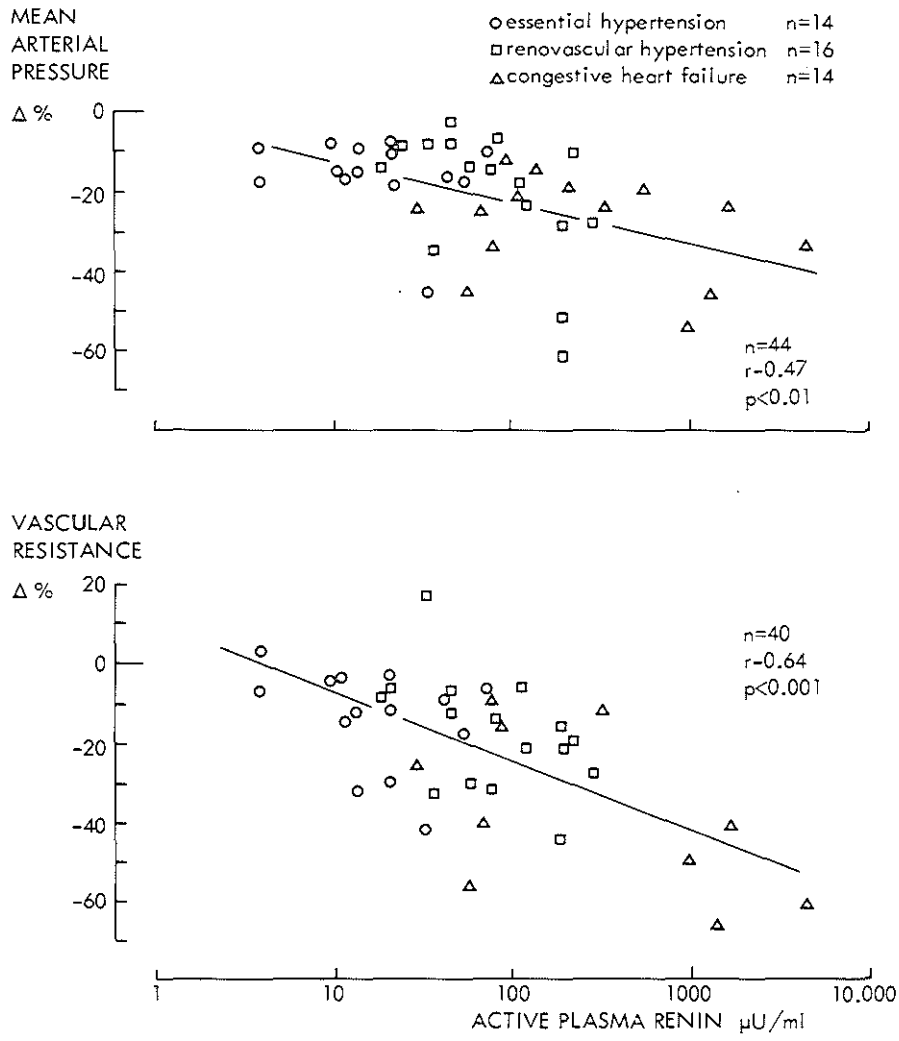
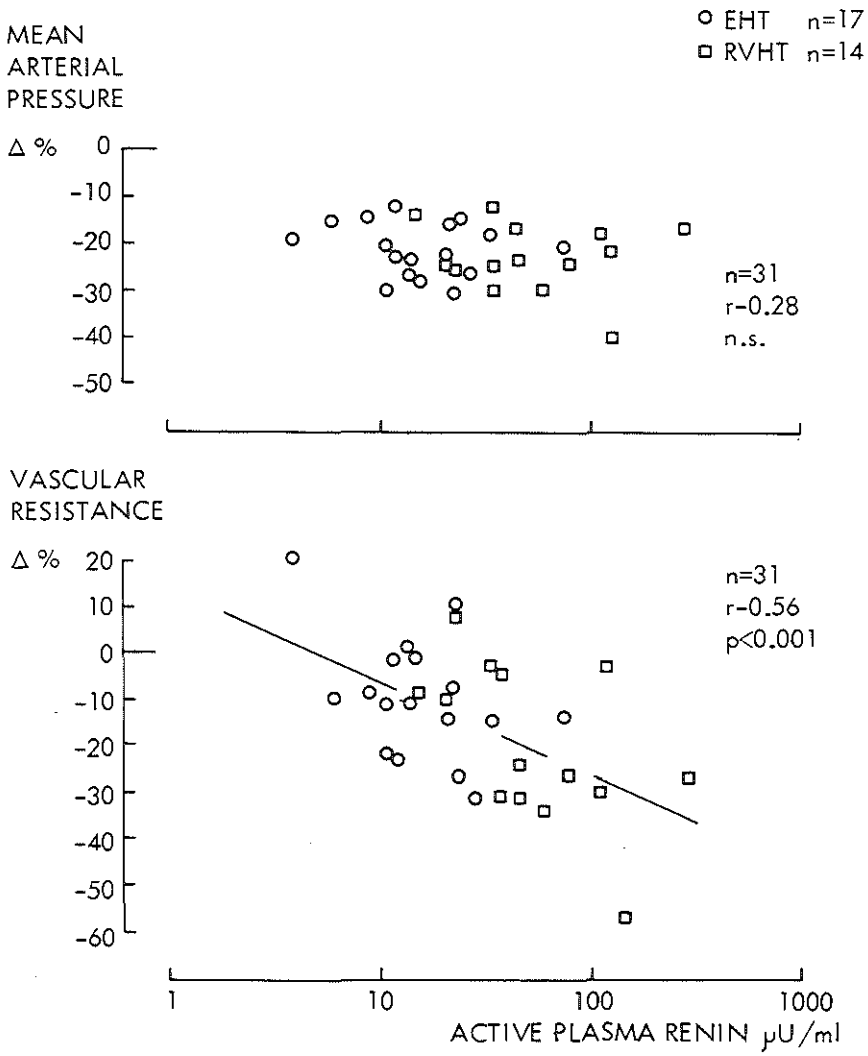


Figure 4. Linear regression analysis of short-term and long-term effects of captopril as related to plasma renin activity. Correlations of short-term percent changes in mean arterial pressure with log renin were significant in the group with hypertension and renal artery stenosis ($r=0.53$, $n=16$, $p<0.05$), 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 ($r=0.47$, $n=44$, $p<0.01$) 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

LONG TERM EFFECTS OF CAPTOPRIL
(0 vs. 4 weeks)



were significant in the hypertension groups together ($r=0.46$, $n=30$, $p<0.01$) 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; ○=essential hypertension; □=hypertension with renal artery stenosis; Δ=chronic heart failure.

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 haemodynamic 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 4600 μU/ml (normal less than 40). By increasing peripheral 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 ischaemia. Within 30 minutes after the intake of captopril the patients were less dyspneic. Indeed, haemodynamic 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 haemodynamic 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 favourable haemodynamic 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 favourable haemodynamic profile. The haemodynamic 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 haemodynamic 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 5

Effects of an angiotensin-converting enzyme inhibitor (captopril) on blood pressure in anephric subjects

5.1. Summary

Randomised, double-blind cross-over trials were performed in seven anephric patients to determine the effect of the orally active angiotensin-converting enzyme inhibitor captopril on blood pressure in fluid-depleted and fluid-replete patients. Patients were given captopril, 100 mg orally, or placebo one hour after haemodialysis, when they were fluid depleted. Their mean (±SEM) supine blood pressure fell from 127±12/71±6 mm Hg before captopril to 106±13/54±4 mm Hg 24 hours after the drug, while on placebo it rose from 123±11/73±5 mm Hg to 134±10/82±8 mm Hg. All patients developed orthostatic hypotension after captopril. In the fluid-replete state, two days after haemodialysis, captopril had no effect on blood pressure. The plasma concentration of active renin was extremely low and did not rise after fluid withdrawal or captopril. Thus the hypotensive effect of captopril did not appear to depend on circulating renin concentrations.
The concept of 'renin-dependent' hypertension, which is responsive to captopril, as opposed to 'volume-dependent' hypertension, which is not responsive to captopril, may therefore be invalid.

5.2. Introduction

Peptidyldipeptide hydrolase, which converts angiotensin I into the potent vasoconstrictor angiotensin II (angiotensin-converting enzyme) and degrades the vasodilator bradykinin (kininase II) (1), is inhibited by the orally active drug captopril (SQ14225) (2). Captopril's effectiveness as an antihypertensive agent has now been firmly established in both animals (1, 3-5) and man (6-8). The fall in arterial pressure after inhibition of the converting enzyme seems to be directly correlated with the pretreatment concentrations of circulating renin (9-11). It is therefore commonly believed that captopril lowers blood pressure mainly through its action on the renin-angiotensin system (6, 7). The greater fall in pressure after stimulation of the renal release of renin by sodium and water depletion seems to support this view (7, 9-12). Captopril therefore seems to be a simple and useful tool for searching out those patients whose hypertension depends on overactivity of the renin-angiotensin system and who would benefit particularly from drugs that suppress this system (6, 9, 10, 13).

Nevertheless, some data suggest that captopril given repeatedly over a period will lower blood pressure equally well in patients with low and normal renin concentrations (6-8). We describe here a study to determine the hypotensive effect of captopril in anephric subjects after they had been treated with ultrafiltration.

5.3. Patients and methods

Seven anephric subjects (four men) aged 30 to 58 years who had been anephric for one to nine years participated in the study. All gave their informed consent to the study. The effects of captopril and placebo on blood pressure were first compared in a randomised, double-blind cross-over study when the patients were relatively fluid depleted. The patients underwent dialysis three times a week on a Rhodial-75 single-patient unit with a RP-6 disposable polyacrylonitryl membrane kidney. The recirculating dialysate contained 140 mmol sodium per liter. The mean (±SEM) loss of body weight per dialysis session was 1.8±0.2 kg on captopril and 2.0±0.2 kg on placebo, the difference being statistically insignificant (see Table). Four patients were given captopril 100 mg orally, one hour after haemodialysis. The remaining three patients were given placebo, which was similar to the captopril tablet in taste, form, and colour. Two days later the patients who had been on captopril received placebo and vice versa, again one hour after haemodialysis. Blood pressure was measured at 15-minute intervals for two hours after the dose of captopril or placebo and again at 24 hours. It was recorded in duplicate by the same investigators using a London School of Hygiene sphygmomanometer (MK4, no 7125) to avoid observer bias and digit preference. Heart rate was computed from a continuous electocardiographic (ECG) tracing.

One week later the patients underwent a second trial with captopril, 100 mg orally, again in a double-blind fashion, two days after haemodialysis, when they were fluid replete. The effect of captopril on blood pressure was followed for two hours until the next dialysis session was started. Finally, captopril, 100 mg orally, was given on a third occasion one day after haemodialysis, and its effect was assessed after 24 hours, just before the next dialysis session was started.

Enzymatically active plasma renin was measured by radioimmunoassay of angiotensin I which was generated after incubation for three hours at 37°C and pH 7.5 with an excess of purified sheep renin substrate (14). Results were expressed as μU of an international renin standard (MRC 68/356). The normal value in our laboratory is 15-40 μU/ml. The converting enzyme activity of plasma was measured by the rate of production of hippuric acid from hippuryl-L-histidyl-L-leucine (15) and expressed as a percentage of the control value before captopril.

For statistical comparison of data we used Student's paired t-test. P values less than 0.05 were considered to indicate statistically significant differences.

TABLE I: EFFECT OF CAPTOPRIL 100 MG ORALLY OR PLACEBO ON ACTIVE PLASMA RENIN AND ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN THE FLUID-DEPLETED AND FLUID-REPLETE STATES.

		Active plasma renin μU/ml)	Converting enzyme activity (% of control value at 0 min)	Bodyweight (kg)
1 hour *after* haemodialysis:				
— placebo	0 min	1.8±0.5	100	56.7±1.6
	24 hours	1.5±0.3	99± 6	57.5±1.6
— Captopril	0 min	1.6±0.4	100	56.6±1.8
	30 min	1.6±0.5	49±11	
	60 min	1.2±0.5	37±14	
	120 min	1.1±0.3	28± 8	
	24 hours	2.1±0.4	30± 7	57.3±1.8
24 hours *after* haemodialysis:				
— Captopril	0 min	1.8±0.3	100	57.4±1.7
	24 hours	1.7±0.5	29± 8	58.5±1.6
48 hours *after* haemodialysis:				
— Captopril	0 min	1.7±0.3	100	58.4±1.6
	120 min	1.6±0.4	19± 9	

5.4. Results

In the fluid-depleted state one hour after haemodialysis body weight on captopril was not significantly different from that on placebo (see Table). Supine blood pressure on placebo, which was given one hour after dialysis, did not change over a period of two hours, and after 24 hours it had significantly increased ($p<0.01$; Fig. 1). In contrast, supine blood pressure fell with captopril and, 30-120 minutes after the drug had been given, blood pressure was significantly lower than on placebo. Two hours after placebo blood pressure did not change on standing for two minutes, but it fell after captopril ($p<0.05$; Fig. 2). Twentyfour hours after captopril had been given, all patients complained of dizziness and had overt orthostatic hypotension. Four of them nearly fainted on standing. There was no significant difference in supine heart rate between the captopril and placebo periods, and the increments of heart rate on standing were also the same (Fig. 2).

Figs. 3 and 4 compare the effects of captopril on supine and standing blood pressures in the fluid-replete state with those after fluid withdrawal. Supine and standing blood pressures were higher in the fluid-replete state two days after haemodialysis and blood pressure did not fall after captopril. The orthostatic fall in blood pressure induced by captopril in the fluid-depleted state was not observed after fluid repletion. The increments in heart rate on standing were similar before and after captopril in both fluid-depleted and fluid-replete states.

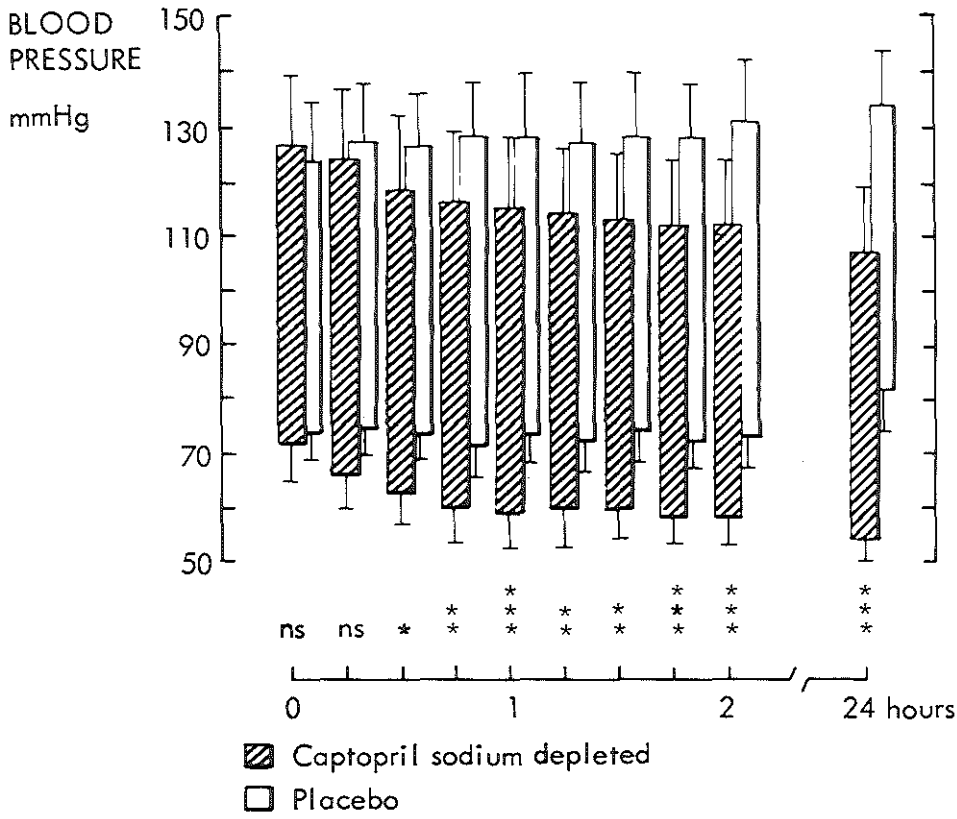

Figure 1. Effect of captopril versus placebo on blood pressure in seven fluid-depleted anephric subjects. NS=not significant. *=p<0.05, **=p<0.01, ***=p<0.001.

Active plasma renin concentrations were extremely low in all patients and did not respond to fluid withdrawal or to captopril (see Table). As judged from measurements of converting enzyme activity in plasma, captopril was effective for at least 24 hours in our patients.

5.5. Discussion

Our findings show that blood pressure can be greatly reduced in anephric subjects by captopril, an agent known to inhibit both the formation of angiotensin II and degradation of bradykinin. Since the main source of renin has been removed and plasma renin and angiotensin II concentrations are very low in these patients (16), it is unlikely that this effect depends on the circultating levels of these hormones. Blood pressure fell after captopril whenever the patients were not overloaded with fluid. A similar influence of fluid balance on the hypotensive response to angiotensin-converting

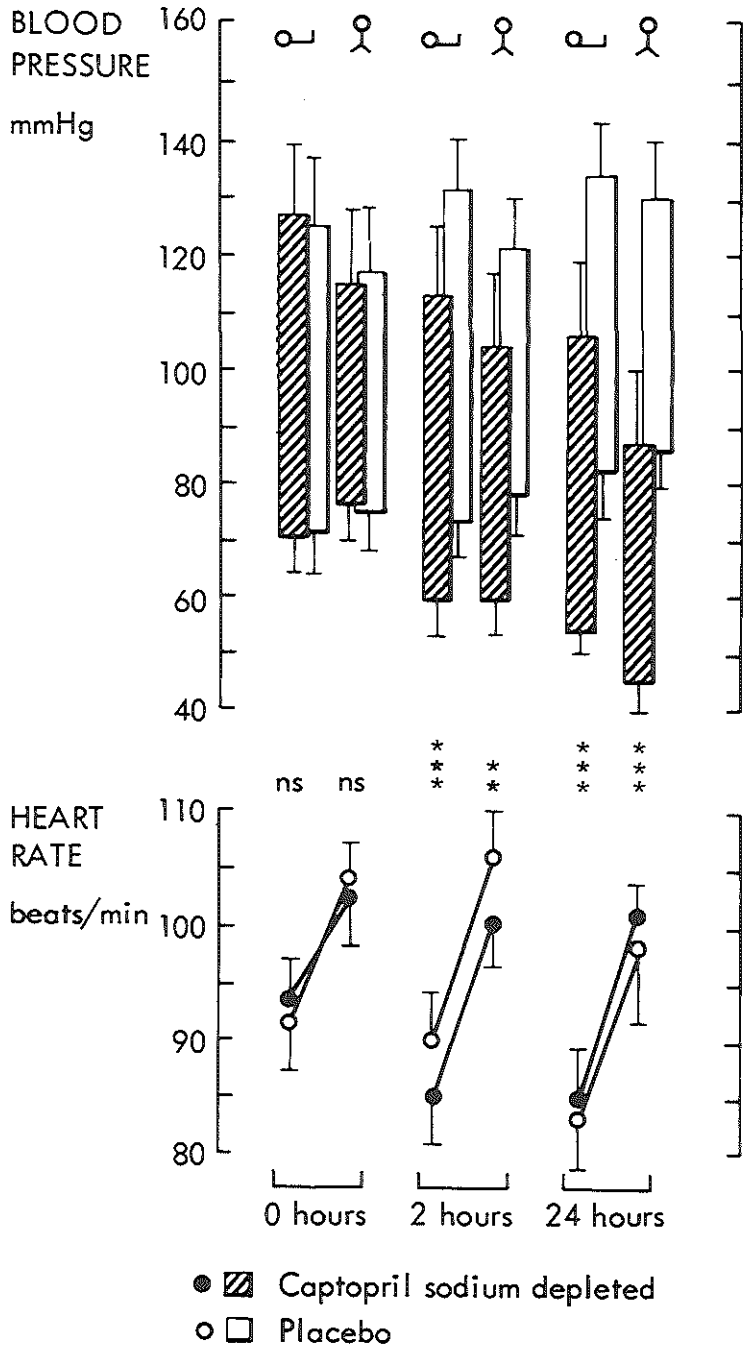

Figure 2. Effects of captopril versus placebo on supine and standing blood pressure and heart rate in seven fluid-depleted anephric subjects. See legend to Fig. 1 for statistical values.

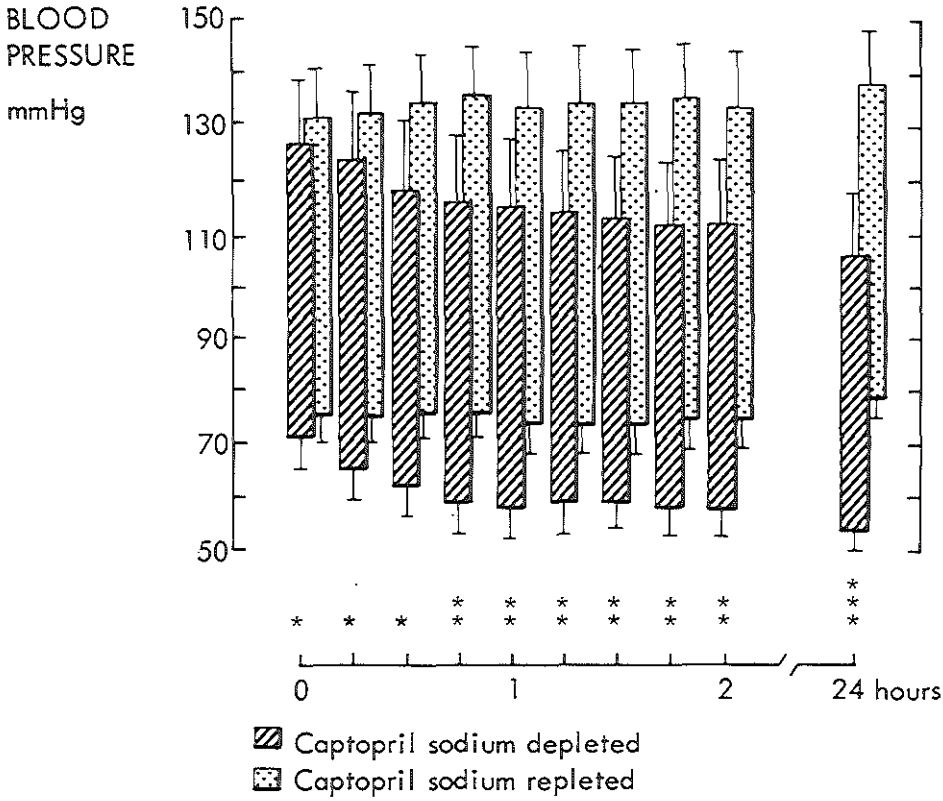

Figure 3. Effect of captopril in fluid-depleted and fluid-replete states on blood pressure in seven anephric subjects. See legend to Fig. 1 for statistical values.

enzyme inhibitors and angiotensin II antagonists has been observed in normotensive and hypertensive subjects who have not undergone nephrectomy (10-12, 17). This is commonly taken as evidence that blood pressure becomes 'renin dependent' when the release of renin is stimulated by sodium and water depletion. Our observations in anephric subjects, who had very low plasma renin concentrations which did not increase after fluid withdrawal, strongly suggest that factors unrelated to circulating renin are important for maintaining blood pressure after sodium and water depletion. The hypotensive effect of captopril is probably also not related to circulating bradykinin concentrations. Studies with the converting enzyme inhibitor teprotide (SQ 20 881) in man failed to show any effect on plasma bradykinin values (12, 18). A transient rise in plasma bradykinin after teprotide has been reported (19, 20), but the bradykinin values in that report were ten times higher than those in a more recent study which failed to show significant changes in plasma bradykinin in patients with essential hypertension treated with long-term captopril (21).

Angiotensin-converting enzyme is present in the vascular endothelium (22) and renin

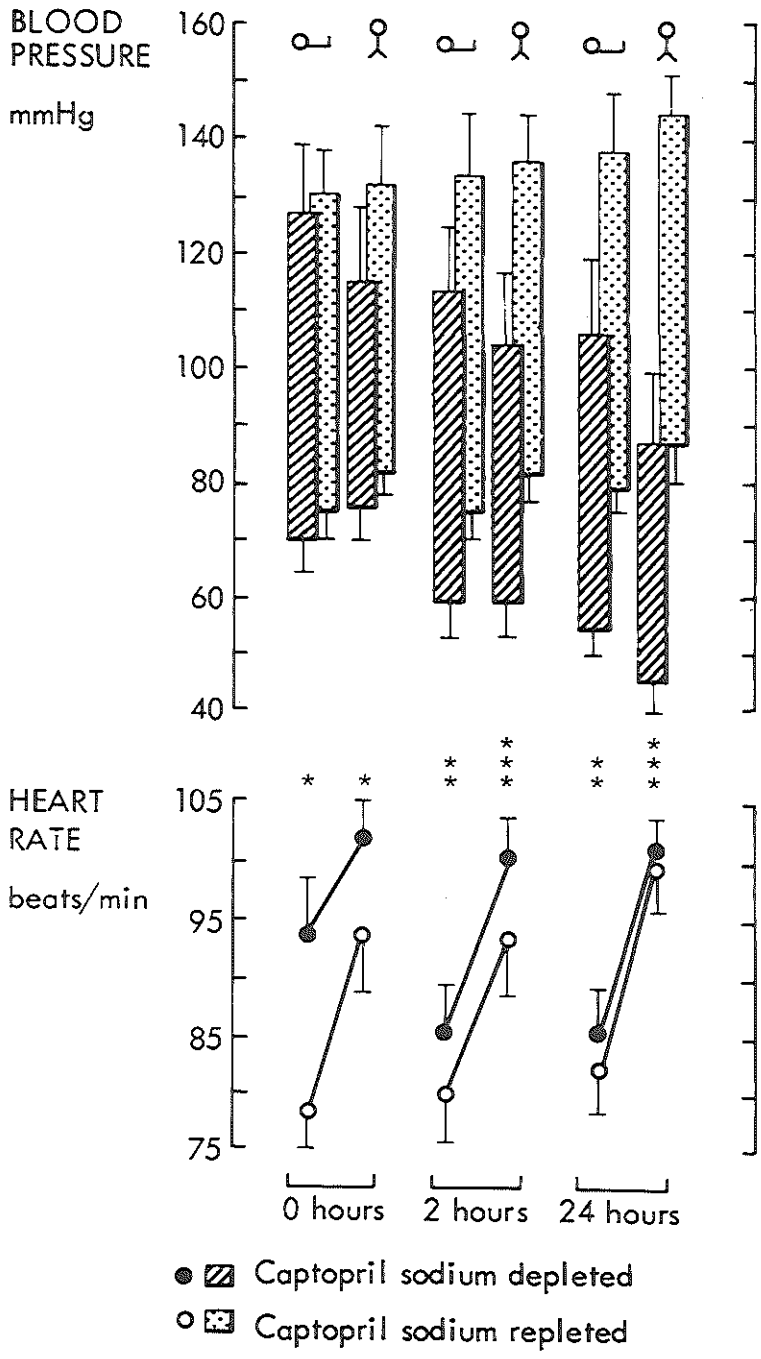

Figure 4. Effects of captopril in fluid-depleted and fluid-replete states on supine and standing blood pressure and heart rate in seven anephric subjects. See legend to Fig. 1 for statistical values.

has been extracted from the arterial wall (23, 24). A physiological role for vascular renin in maintaining blood pressure in the hypertensive anephric rat has been recently put forward by Thurston and Swales (25), who found that inhibition of converting enzyme resulted in a considerable fall in blood pressure one and two hours after nephrectomy, when plasma renin had already declined to insignificantly low values. Furthermore, plasma prekallikrein, which circulates as a complex with its substrate, high-molecular-weight kininogen, is rapidly absorbed together with factor XII on negatively charged surfaces, such as collagen and vascular basement membrane (26). This leads to activation of factor XII and the local formation of kallikrein and possibly also bradykinin. Captopril may thus act in the blood-vessel wall or in close contact with it, rather than in the circulating blood.
Haemodynamically, captopril acts as a vasodilator with an important effect on the veins (27, 28). Venous dilatation will tend to reduce venous return to the heart and consequently cardiac output. This might explain why captopril caused orthostatic hypotension in our anephric patients when they were fluid depleted. It may be relevant that the rare syndrome of familial hyperbradykininism is characterised clinically by severe orthostatic hypotension (29). The enhancement of captopril's hypotensive effect by sodium and water depletion, which has also been observed in subjects who have not undergone nephrectomy, might be an indication of the importance of blood volume rather than plasma renin for maintaining blood pressure. Hypotension will not develop as long as 'effective' blood volume and cardiac filling pressures are adequate. The alleged transition of so-called 'volume-dependent' hypertension into 'renin-dependent' hypertension after sodium and water depletion, for instance by diuretics, might therefore be an illusion rather than reality.
Whatever the underlying mechanism of captopril's hypotensive effect, our results lend further support to preliminary suggestions that this drug will be effective in the large group of hypertensive patients in whom plasma renin is not raised.

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CHAPTER 6

Captopril affects blood pressure equally in renovascular and essential hypertension and in the fluid-depleted anephric state

6.1. Summary

The haemodynamic effects of 100 mg of captopril in renovascular hypertension (n=11), essential hypertension (n=12) and the anephric state (n=7) were compared. Brachial artery pressure was measured in all patients, and changes in right atrial pressure, pulmonary artery pressure, pulmonary capillary wedge pressure and cardiac output were followed in renovascular and essential hypertension. Nephrectomized patients were studied before and after fluid withdrawal by ultrafiltration.
The pretreatment concentration of active renin in plasma was 100±24 μU/ml (mean ±SEM) in renovascular hypertension, and 24±4 μU/ml in essential hypertension. In nephrectomized patients pretreatment renin was 1.7±0.3 μU/ml, and renin was unresponsive to withdrawal of 1.8±0.2 liters of body fluid.
The effects of captopril were maximal after 60-90 min. Mean arterial pressure after 90 minutes was lowered by 19±4% in renovascular hypertension, by 17±4% in essential hypertension and by 16±3% in fluid-depleted nephrectomized patients. These changes were not significantly different despite the marked differences in renin. Captopril had no effect on arterial pressure in the fluid-replete anephric state.
The effects of captopril on cardiac filling pressures and cardiac output in renovascular and essential hypertension were also not different. It is concluded that the antihypertensive action of captopril may be largely independent of circulating renin.

6.2. Introduction

The orally active angiotensin-converting enzyme inhibitor, captopril (SQ 14 225), has now proved to be an effective antihypertensive agent. The drug is thought to act mainly or at least partly through interference with the conversion of angiotensin I into angiotensin II (1,2). It might therefore be expected that captopril would be especially effective in hypertensive patients with high levels of circulating renin.
Several reports, however, suggest that captopril is also effective when plasma renin or angiotensin II are normal or low (3-8). Atkinson et al (4), while demonstrating a close correlation between the fall in plasma angiotensin II and the fall in blood pressure after the initial dose of captopril, emphasized that this does not establish cause and effect; factors other than the change in angiotensin II might be involved. In an effort to assess the importance of plasma renin as a determinant of the cardiovascular responses

to captopril, we have compared the short-term haemodynamic effects of this drug in renovascular hypertension, essential hypertension and the anephric state.

6.3. Patients and methods

Eleven patients (mean age 47±SEM 5 years) with renovascular hypertension, who showed angiographic evidence of unilateral renal artery stenosis, 12 patients (age 50±3 years) with essential hypertension, and seven normotensive subjects (38±4 years) who had undergone bilateral nephrectomy, agreed to participate in the study.
The pretreatment concentration of active renin in plasma was 100±24 μU/ml (range 21-290 μU/ml) in renovascular hypertension, 24±4 μU/ml (range 4-68 μU/ml) in essential hypertension and 1.7±0.3 μU/ml (range 0.0-3.4 μU/ml) in nephrectomized patients. The normal range is 15-45 μU/ml (10).
The patients with renovascular and essential hypertension had a diet containing 100 mmol of sodium/day, and were on placebo for three weeks before captopril was given. Studies were performed in the recumbent patient. A catheter was introduced into the brachial artery for measuring arterial pressure and for blood sampling. A Swan-Ganz flow-directed triple-lumen catheter was introduced via an antecubital vein for recording right atrial pressure, pulmonary artery pressure and pulmonary capillary wedge pressure, and for measuring cardiac output by thermodilution. All patients received a 100 mg tablet of captopril.
The nephrectomized subjects underwent dialysis three times a week on a Rhodial-75 single-patient unit with a RP-6 disposable polyacrylonitryl membrane kidney. The recirculating dialysate contained 140 mmol of sodium/l. Captopril (100 mg) or placebo was given in a randomized cross-over study on two occasions: 1) after fluid withdrawal by ultrafiltration (weight loss 1.8±0.2 kg), one hour after the patients were disconnected from the kidney, and 2) two days after haemodialysis when the patients were fluid-replete. The concentration of active renin in plasma was unresponsive to fluid withdrawal. Blood pressure in these subjects was measured by the London School of Hygiene sphygmomanometer (11), and mean arterial pressure was calculated according to the formula: mean arterial pressure =diastolic pressure + 0.33 × pulse pressure.
The effects of captopril were maximal after 60-90 min. Data before and 90 minutes after captopril were analysed by paired t-test statistics.

6.4. Results

Arterial pressure. Mean arterial pressure fell from 141±6 to 114±6 mm Hg in renovascular hypertension, from 136±6 to 114±7 mm Hg in essential hypertension and from 90±7 to 75±6 mm Hg in the fluid-depleted nephrectomized patients. These changes were significant ($p<0.001$). Placebo in the fluid-depleted anephric state, and captopril in the fluid-replete anephric state, had no effect on arterial pressure.
Heart rate and cardiac index. In all groups of patients heart rate did not change significantly. Cardiac index in renovascular hypertension was 3.5±0.1 l/min before

captopril and 3.4±0.2 l/min after the drug. The difference was not statistically significant. Cardiac index in essential hypertension was 2.9±0.1 l/min both before and after captopril.

Cardiac filling pressures and pulmonary artery pressure. Right atrial pressure fell from 2.6±0.9 to 1.6±0.9 mm Hg ($p<0.05$) in renovascular hypertension, and from 0.7±0.8 to —0.8±0.9 mm Hg ($p<0.01$) in essential hypertension. Pulmonary artery pressure decreased from 16±2 to 13±2 mm Hg in renovascular hypertension ($p<0.01$) and from 12±1 to 10±1 mm Hg in essential hypertension ($p<0.01$). Pulmonary capillary wedge pressure fell from 7.5±1.1 to 5.2±1.2 mm Hg ($p<0.005$) in renovascular hypertension and from 4.4±0.9 to 1.0±1.0 mm Hg ($p<0.001$) in essential hypertension.

Plasma renin. Active renin in plasma rose both in renovascular and essential hypertension, to 890±110 and 116±24 μU/ml respectively. Renin did not change after captopril in the nephrectomized patients.

In Fig. 1 the percentage changes in mean arterial pressure 90 minutes after captopril as well as the pretreatment plasma levels of active renin are compared in the three groups of patients. It is clear that the effect of captopril on blood pressure was unrelated to pretreatment renin values.

6.5. Discussion

In the light of our present knowledge on captopril's mode of action, one would expect its effect on blood pressure to be proportional to the pretreatment level of plasma renin. Such a correlation has indeed been found by some authors (1, 6, 8, 12) but not by others (3, 5, 7). The correlation, if it was found, was rather weak or depended on inclusion of data obtained in patients with high plasma renin on low sodium diet or diuretics. In the present study the antihypertensive effect of captopril was similar in renovascular and essential hypertension despite the marked differences in pretreatment plasma renin.

Our haemodynamic studies confirm that captopril acts as a combined arterial and venous dilator (13-15), which in contrast to other vasodilators, does not lead to reflex tachycardia. Again, the effects of captopril on systemic and pulmonary haemodynamics were not different in our groups with renovascular and essential hypertension despite the difference in renin.

Diuretic treatment is kown to enhance captopril's anti-hypertensive effect (1, 6, 7), and in severe sodium depletion serious hypotensive responses have been observed (1, 9). This is often used as an argument in favour of the concept that captopril acts through inhibition of renin-mediated angiotensin II formation, because plasma renin is markedly increased by sodium and fluid depletion. It is, however, by no means certain that the increase in renin is the cause of the augmented blood pressure response to captopril. Actually, we found that captopril was capable of lowering blood pressure in anephric subjects only when they were fluid-depleted, whereas plasma renin after fluid depletion was as low as in the fluid-replete state. Blood pressure in the fluid-depleted anephric subjects was lowered by 16% 90 minutes after captopril, and this is

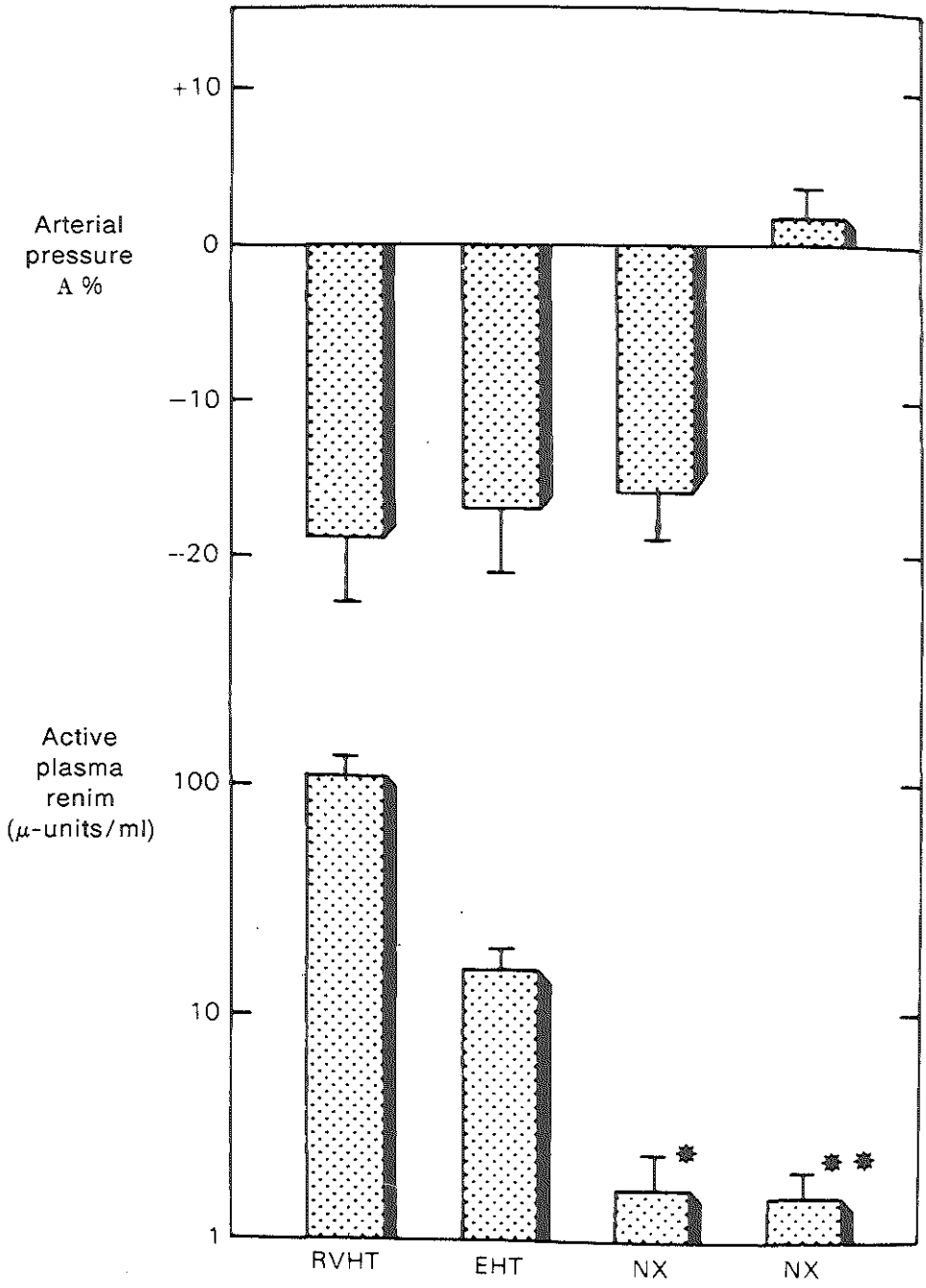

Figure 1. Pretreatment plasma renin (mean±SEM) and the percentage changes in mean arterial pressure 90 min after 100 mg of captopril in renovascular hypertension (RVHT), essential hypertension (EHT) and in the anephric state (N_x). *After ultrafiltration; **before ultrafiltration.

comparable with the effects we observed in renovascular and essential hypertension (Fig. 1).
These findings suggest that the antihypertensive action of captopril is largely independent of circulating renin.

6.6. References

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CHAPTER 7

Opposite effects of captopril on angiotensin I-converting enzyme 'activity' and 'concentration'; relation between enzyme inhibition and long-term blood pressure response

7.1. Summary

The relationship between the antihypertensive action of captopril and its inhibitory effect on angiotensin I (ANG I)-converting enzyme has been investigated. Converting enzyme was measured in plasma by its ability to generate hippuric acid from the synthetic substrate hippuryl-L-histidyl-L-leucine. Inhibition by captopril appeared transient on storage of the plasma samples at —20°C, so that measurements in such samples were not a valid index of the effect in vivo.

Rapid reversal of captopril's inhibitory effect on ANG I-converting enzyme in plasma was achieved by the addition of N-ethylmaleimide (0.1 mmol/l). In this way an estimate of converting enzyme 'concentration' was obtained both in stored and in freshly prepared samples. Measurements of converting enzyme 'activity' in freshly prepared samples in the absence of N-ethylmaleimide, were used as an index of inhibition in vivo.

In eight hypertensive subjects ANG I-converting enzyme 'concentration' did not change after a single oral 100 mg dose of captopril. Long-term treatment of 10 hypertensive subjects with captopril was associated with a gradual increase in converting enzyme 'concentration' from 29±2 to 47±3 μU/ml (mean±SEM) over a period of several weeks. In contrast, captopril caused a rapid fall of converting enzyme 'activity'. Abrupt withdrawal of captopril after long-term treatment caused a gradual decrease in ANG I-converting enzyme 'concentration' to the control value. In contrast, converting enzyme 'activity' rose rapidly and became equal to enzyme 'concentration' two days after the drug had been stopped.

The concentration of enzymatically active renin in plasma rose from 13±3 to 81±34 μU/ml during long-term captopril treatment (mean±SEM). Blood pressure fell from 168±4/108±3 to 140±3/90±3 mm Hg and had not returned to the control value in the first week after the drug had been stopped, despite the fact that circulating active renin and ANG I-converting enzyme 'activity' were elevated.

It is concluded that long-term inhibition of ANG I-converting enzyme by captopril is associated with an increased plasma concentration of this enzyme. The results also suggest that the level of converting enzyme 'activity' in plasma is not the only factor that determines the long-term effect of captopril on blood pressure.

7.2. Introduction

Captopril is now being evaluated as a promising drug for the treatment of various forms of hypertension. It is generally assumed that captopril's action on blood pressure is closely linked to its inhibitory effect on ANG I-converting enzyme (1, 2). More recently, however, some discrepancy between measured ANG I-converting enzyme in plasma and the response of blood pressure has been reported (3.). During the course of a study designed to collect data to clarify this point, we measured variable decrements of ANG I-converting enzyme in plasma of patients on captopril on storage of the samples at —20°C. This was not the case in plasma taken from subjects who were not on captopril. These findings suggested to us that inhibition of ANG I-converting enzyme by this drug was partly reversed after storage of plasma at —20°C, so that measurements of ANG I-converting enzyme in stored samples are not a valid index of inhibition *in vivo*.

We have, therefore, developed a method *in vitro* by which a reproducible and almost complete reversal of the inhibitory effect of captopril can be attained. This enabled us to measure ANG I-converting enzyme 'concentration' and to compare this with measurements of ANG I-converting enzyme 'activity'. The term 'concentration' is used here to designate the total concentration of circulating enzyme, i.e. the sum of inhibited and uninhibited enzyme. The term 'activity' refers to circulating uninhibited enzyme only. The results to be presented here indicate that ANG I-converting enzyme 'concentration' increases with long-term inhibition of the enzyme by captopril.

7.3. Methods and patients

Assay of ANG I-converting enzyme

Procedure. ANG I-converting enzyme in plasma was measured by a spectrophotometric assay based on the methods of Cushman and Cheung (4) and Le Treut et al (5). Peripheral venous blood was collected into tubes containing lithium heparin as anticoagulant. Plasma was prepared by centrifugation at 4°C, immediately after blood sampling. A 50 μl portion of plasma was added to 200 μl of a freshly prepared solution of the substrate hippuryl-L-histidyl-L-leucine (from Sigma Chemical Co.) in borate/NaCl buffer. The substrate solution contained the following components at the final concentrations indicated: hippuryl-L-histidyl-L-leucine (5 mmol/l), sodium borate (0.1 mol/l) and NaCl (0.3 mol/l); pH was adjusted to 8.3. The substrate/plasma mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped with 250 μl of HCl (1 mol/l). A blank was prepared by adding the acid before the incubation. Hippuric acid that was formed during incubation was extracted with 1.5 ml of ethylacetate by mixing on a vortex mixer (15 s). After centrifugation 1 ml of the organic layer was transferred to a new tube. The ethylacetate was evaporated and 2 ml of NaCl solution (1 mol/l) was added, followed, after mixing, by 2 ml of light petroleum (boiling point 40-60°C). The tubes were then vortexed (15 s) and after centrifugation the light petroleum layer was removed by aspiration. The absor-

bance at 228 nm was read in a 1 cm quartz cuvette. The absorbance of the blank was substracted from the absorbance of the corresponding test sample.
Results of ANG I-converting enzyme measurements are expressed in milli-units per millilitre of plasma (m-units/ml), 1 unit being the quantity of enzyme that produces 1 μmol of hippuric acid/min. The production of hippuric acid from hippuryl-L-histidyl-L-leucine was linear with respect to time for at least 1 hour and dilution experiments (Fig. 1) ascertained the linearity of the assay for ANG I-converting enzyme levels up to 80 m-units/ml. Samples were assayed in duplicate. Both intra- and inter-assay variabilities were <5%.
As described above, the incubation of plasma with substrate was carried out in sodium borate buffer of pH 8.3. In the original methods of Cushman and Cheung (4) and Le Treut et al (5) potassium phosphate buffer of the same pH was used. We found, however, that with the use of potassium phosphate at concentrations of 0.1 and 0.25 mol/l measured ANG I-converting enzyme was reduced by 35 and 65% respectively as compared with values in borate buffer.

Specificity and linearity of the assay. The cleavage of the synthetic substrate by plasma could be reduced to undetectable levels by the addition of the ANG I-converting enzyme inhibitor, captopril, to the incubation medium (see below). Thus peptidases that are not inhibited by captopril are not detected by the assay, thereby indicating that the reaction is mainly, if not only, dependent on ANG I-converting enzyme. Several workers ondertook the purification of ANG I-converting enzyme from tissue and plasma and found corresponding estimates of purity with the natural substrate ANG I and with synthetic substrates (6,7). This again is an indication of the specificity of the synthetic substrate. Moreover, a close linear correlation was found between the rate of cleavage of the synthetic substrate and the concentration of partly purified enzyme.
To confirm the validity of the assay procedure various dilutions of plasma from a hypertensive patient were added to (a) NaCl solution (0.15 mol/l), (b) plasma from the same patient five days after a 12-week course of captopril treatment (450 mg/day, orally) had been completed, and (c) plasma from a patient with sarcoidosis. ANG I-converting enzyme was measured as described before. The straight lines shown in Fig. 1 strongly suggest that a higher rate of cleavage of the synthetic substrate corresponds to a greater amount of enzyme.

Distinction between ANG I-converting enzyme 'concentration' and ANG I-converting enzyme 'activity' by the use of N-ethylmaleimide.
When captopril is not present in plasma, the rate of hippuric acid production from the synthetic substrate hippuryl-L-histidyl-L-leucine can be used as an estimate of the concentration of ANG I-converting enzyme. In the presence of captopril, however, the reaction velocity depends on that portion of the total quantity of enzyme that is not inhibited by captopril.
The inhibition of ANG I-converting enzyme by captopril, which contains a functional thiol group, can be reversed by adding N-ethylmaleimide to the incubation mixture of the assay. N-ethylmaleimide reacts with captopril to form a stable derivative, which

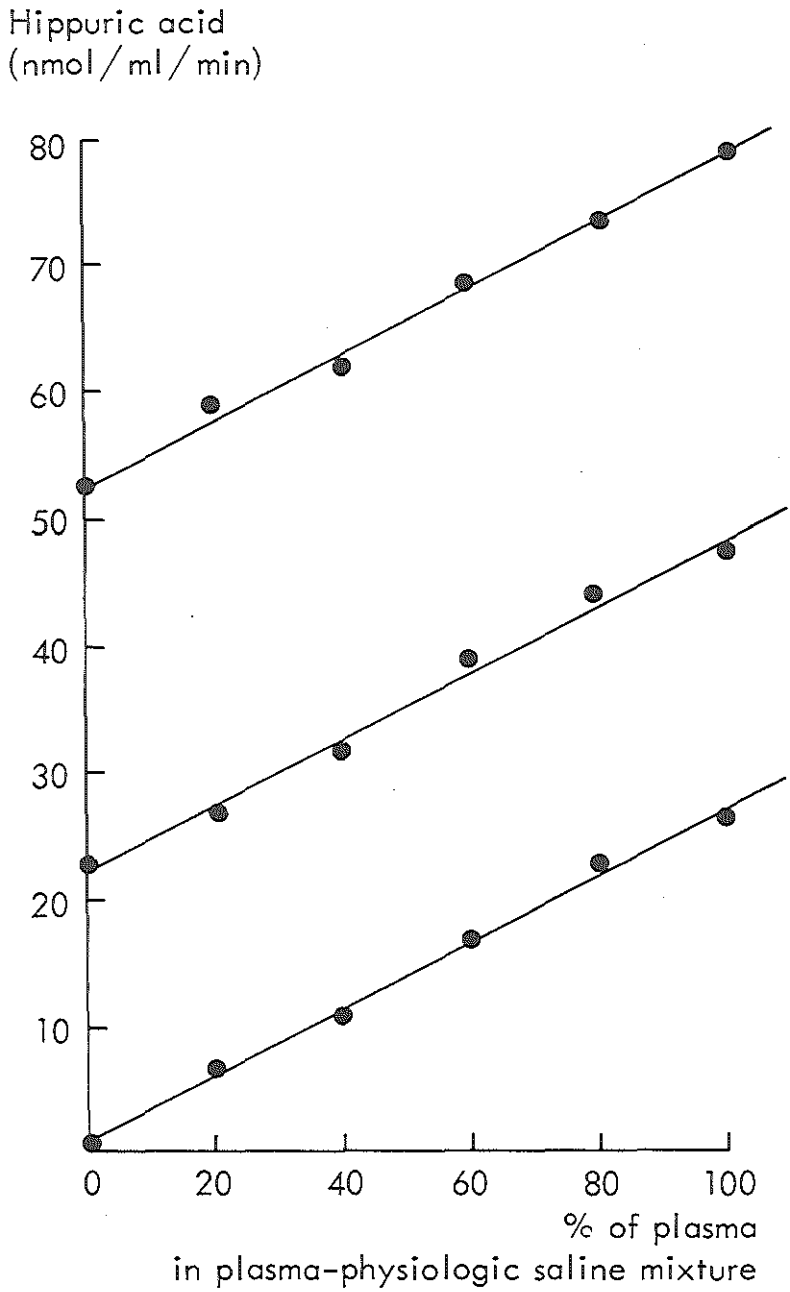

Figure 1. Linearity of ANG I-converting enzyme measurement in various dilutions of plasma. Diluted plasma (30 μl) from a hypertensive patient was added to 20 μl of (a) NaCl solution (0.15 mol/l), (b) plasma from the same patient 5 days after completion of a 12-week course of captopril treatment (450 mg/day, orally), and (c) plasma from a patient with sarcoidosis. Data are the means of five experiments.

does not inhibit ANG I-converting enzyme. This process is strongly dependent on the concentration of N-ethylmaleimide. Moreover, at high concentrations of this agent an increase in absorbance at 228 nm is observed after incubation at 37°C, no matter whether converting enzyme and synthetic substrate are present or not. Fig. 2 depicts a series of assays in which the influence of N-ethylmaleimide was assessed. The incubation was started by adding 50 μl of (a) normal plasma, (b) normal plasma containing captopril (10 μmol/l) or (c) NaCl solution (0.15 mol/l) to 200 μl of hippuryl-L-histidyl-L-leucine in borate buffer and 20μl of N-ethylmaleimide of various concentrations. An incubation of NaCl solution (0.15 mol/l) in borate buffer without synthetic substrate was also carried out (d). In these experiments the concentration of captopril in plasma was two times higher than the maximal plasma concentration of unchanged captopril in normal volunteer subjects after the oral intake of 100 mg of this drug (Squibb, Report no. 1924, 9/74). From these experiments it appears that N-ethylmaleimide in concentrations up to 0.1 mmol/l of incubate does not affect the assay of ANG I-converting enzyme and that at a concentration of 0.1 mmol/l complete reversal of captopril's inhibitory effect is achieved. From Lineweaver-Burk plots K_m of the reaction of ANG I-converting enzyme with hippuryl-L-histidyl-L-leucine was calculated to be 1.56 mmol/l, which agrees with previous reports (4,8). The addition of N-ethylmaleimide in a final concentration of 0.1 mmol/l had no significant effect on K_m or V_{max}. On the basis of these findings we have decided to use this concentration of N-ethylmaleimide for measuring the total concentration of converting enzyme, that is the sum of inhibited and uninhibited enzyme, in samples that contain captopril. A 20 μl portion of a solution containing N-ethylmaleimide (1.35 mmol/l) and NaCl (0.15 mol/l) was added to the tubes containing the synthetic substrate (200 μl) before the addition of plasma (50 μl). The addition of 20 μl of NaCl solution, without N-ethylmaleimide, had no effect on the measured enzyme activity.

In the subsequent analysis we shall refer to ANG I-converting enzyme measured in the absence of N-ethylmaleimide as ANG I-converting enzyme 'activity'. We shall refer to ANG I-converting enzyme measured in the presence of N-ethylmaleimide as ANG I-converting enzyme 'concentration', because in this way an estimate is obtained of the concentration of enzyme irrespective of inhibition of the enzyme by captopril. When plasma is free of captopril, the measurements of converting enzyme 'activity' and 'concentration' give identical results.

Assay of enzymatically active renin. Active renin in plasma was measured as described previously (9, 10). Plasma was prepared from blood containing disodium ethylenediaminetetra-acetate (EDTA, 5 mmol/l). Samples (2 ml) of EDTA-plasma were dialysed for 24 h at 4°C against sodium phosphate buffer (disodium hydrogen phosphate 86.7 mmol/l, sodium dihydrogen phosphate 12.2 mmol/l and NaCl 75.9 mmol/l), pH 7.5, containing EDTA (1 mmol/l) and 0.2% neomycin sulphate. Aliquots (0.1 or 0.2 ml) of the dialysed samples were diluted with the phosphate buffer pH 7.5, to a volume of 0.5 ml and 10 μl of 8-hydroxyquinoline (0.34 mol/l) and 5 μl of aprotinin (Trasylol, 10000 kallikrein-inhibition units/ml) were added. The mixture

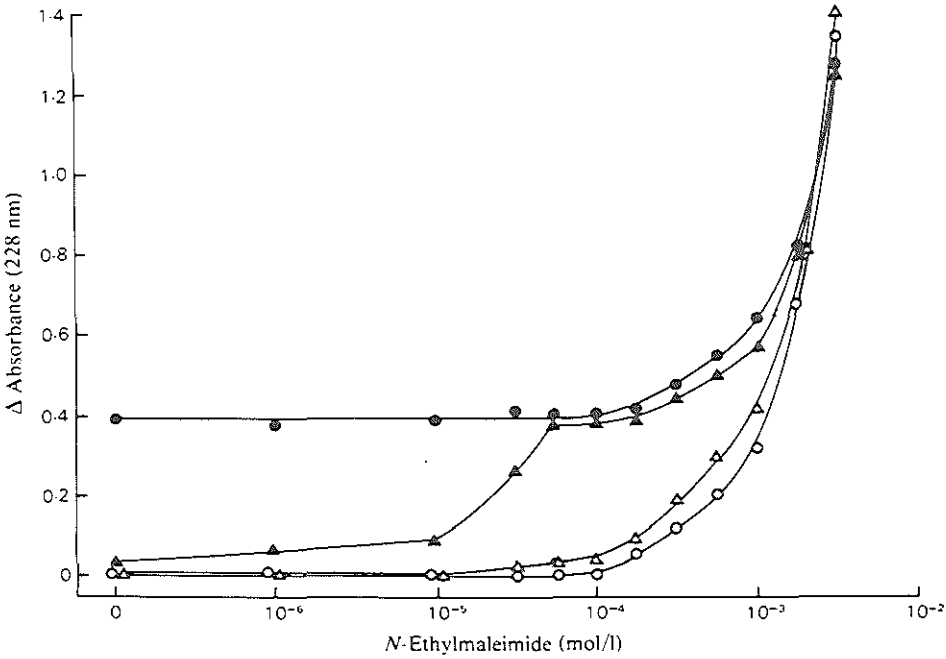

Figure 2. Influence of N-ethylmaleimide on ANG I-converting enzyme measurements: (a) 50 μl of normal plasma, 200 μl of hippuryl-L-histidyl-L-leucine in borate buffer and 20 μl of N-ethylmaleimide (•); (b) 50 μl of normal plasma containing captopril (10 μmol/l), 200 μl of hippuryl-L-histidyl-L-leucine in borate buffer and 20 μl of N-ethylmaleimide (▲); (c) 50 μl of NaCl (0.15 mol/l), 200 μl of hippuryl-L-histidyl-L-leucine in borate buffer and 20 μl of N-ethylmaleimide (o); (d) 50 μl of NaCl (0.15 mol/l), 200 μl of borate buffer and 20 μl of N-ethylmaleimide (Δ).

was incubated for three hours at 37°C with 0.5 ml of sheep renin-substrate in the phosphate buffer described above. The final concentration of substrate in the incubation mixture was 2.5 μmol/l, expressed as ANG I equivalents, which corresponds with 6.1 × K_m. After incubation the mixture was diluted with 1 ml of NaCl solution (0.15 mol/l), treated for 10 min in a boiling-water bath and centrifuged. The ANG I-concentration of the supernatant was determined by radioimmunoassay. A parallel incubation of the same quantities of dialysed plasma and substrate at 4°C served as a control. The value of the control was <10% of the value after incubation at 37°C. International Reference Standard renin was obtained from the National Insitute for Biological Standards and Control, London. This human kidney preparation (MRC standard 68/156) was used as an external standard (11). Renin concentration is expressed as micro-units of this standard per millilitre of plasma (μU/ml).

Measurement of blood pressure. The London School of Hygiene sphygmomanometer, model MK 4, was used to avoid observer bias and digit preference (12). Blood pressure was measured after at least ten min rest, phase V being taken as diastolic pressure.

Healthy subjects and patients. For studies *in vitro* blood was taken from 40 healthy hospital workers and from ten patients with untreated pulmonary sarcoidosis. The effects *in vivo* of captopril were studied in 18 hypertensive patients. Informed consent was obtained in each case. In the hypertensive patients blood pressure was at least 160 mm Hg systolic and 100 mm Hg diastolic, on two or more occasions when the patients were untreated. Routine examinations, including intravenous urography, did not reveal any cause of the hypertension in 14 patients. Abnormalities suggesting unilateral renal artery stenosis were found on intravenous urography in four hypertensive patients. In each of them the diagnosis was confirmed by arteriography. All drugs had been withdrawn for at least six weeks before the studies began.

Clinical studies. The effects of a single dose of captopril (100 mg, orally) were followed in four patients with essential hypertension and four patients in whom the hypertension was associated with renal artery stenosis. ANG I-converting enzyme 'activity' and 'concentration', renin and blood pressure were measured at regular intervals after captopril, as indicated in the Results section ('short-term study'). Ten patients with essential hypertension were put on long-term treatment with captopril. During a first period of treatment the dose of captopril was increased from 25 mg three times a day in week one, via 50 and 100 mg three times a day in weeks two and three, to 150 mg three times a day in week four. The last dose was continued for nine weeks. Captopril treatment was then discontinued and, after four weeks, a second course of captopril was given. During this period a fixed dose of captopril, 150 mg three times a day, was used for eight months. ANG I-converting enzyme 'activity' and 'concentration', renin and blood pressure were measured in both treatment periods and in the period where treatment was interrupted. Measurements were made at regular intervals as indicated in the Results section ('long-term study').
Data are presented as mean±SEM.

7.4 Results

Experiments in vitro

Inhibition of ANG I-converting enzyme in plasma by captopril in vitro and the effect of storage. Values of ANG I-converting enzyme in plasma of subjects who were not on captopril are shown in Table 1. Addition of captopril to this plasma led to inhibition of ANG I-converting enzyme. The inhibition was competitive and from Lineweaver-Burk plots K_i was calculated to be 1.9 nmol/l.
The inhibition was not permanent, but decreased on storage of the plasma at —20°C. One day of storage already had an appreciable effect (Table 1). Determinations of ANG I-converting enzyme 'activity' in plasma of subjects on captopril are therefore to be performed within a few hours after blood sampling.

TABLE I: EFFECTS OF STORAGE AND N-ETHYLMALEIMIDE (0.1 MMOL/1) ON ANG I-CONVERTING ENZYME IN PLASMA.

Source of plasma	Handling of plasma before assay	ANG I-converting enzyme (m-units/ml) measured in the absence of N-ethylmaleimide	ANG I-converting enzyme (m-units/ml) measured in the presence of N-ethylmaleimide
Patients with sarcoidosis, n=10	Plasma was stored at —20 °C for 2-6 months	54.1±8.6	57.9±9.5
Patients with essential hypertension, n=14	Plasma was stored at —20 °C for 2-6 months	33.3±3.2	30.6±2.1
Normal subjects, n=10	1) Plasma was stored at —20 °C for 2-6 months	32.1±2.4	31.3±2.6
	2) Plasma was stored at —20 °C for 2-6 months. After thawing captopril (0.05 μmol/1) was added immediately prior to assay	18.3±4.2	27.1±1.8
	3) Plasma was stored at —20 °C for 2-6 months. After thawing captopril (10 μmol/1) was added immediately prior to assay	0	28.1±2.0
	4) Captopril (10 μmol/1) was added to freshly prepared plasma. The samples were then stored at —20 °C for 2 weeks	14.4±4.5	28.4±1.5
Normal subjects, n=5	1) Plasma was stored on melting ice for <1 h:		
	- before 25 mg captopril	27.4±2.5	25.9±1.6
	- 1 h after 25 mg captopril	3.1±1.0	27.3±2.1
	2) Plasma was stored on melting ice for 4 h:		
	- before 25 mg captopril	23.7±1.2	24.4±2.1
	- 1 h after 25 mg captopril	6.4±2.5	24.0±1.9
	3) Plasma was stored at —20 °C for 1 day:		
	- before 25 mg captopril	23.2±3.2	26.0±1.4
	- 1 h after 25 mg captopril	9.8±2.1	26.1±1.3
	4) Plasma was stored at —20 °C for 6 months:		
	- before 25 mg captopril	27.6±2.3	25.8±1.8
	- 1 h after 25 mg captopril	25.7±2.0	26.4±1.8

Experiments in vivo

Short-term study. As shown in Fig. 3, ANG I-converting enzyme 'activity' measured in freshly prepared plasma without N-ethylmaleimide fell dramatically. In contrast, ANG I-converting enzyme 'concentration' measured in plasma with N-ethylmaleimide remained unchanged. After two hours renin had increased from 58±17 to 577±127 μU/ml and blood pressure had fallen from 215±10/115±5 to 171±17/93±7 mm Hg. The effects on ANG I-converting enzyme 'activity', renin and blood pressure were maximal at that time. Thereafter, these parameters gradually returned to the control values.

Long-term study. During the long-term treatment with captopril the measurements of ANG I-converting enzyme 'activity' and 'concentration', renin and blood pressure were made two hours after the last dose of captopril. ANG I-converting enzyme 'activity' as measured in freshly prepared plasma was 30±2 m-units/ml before treatment with captopril and 3±2 m-units/ml when the patients were on captopril for 12 weeks. It rose sharply after discontinuation of captopril treatment and after two days without captopril it had reached a value of 42±3 m-units/ml, which is higher than that before captopril. In the second course of captopril treatment ANG I-converting enzyme 'activity' was 6±3 m-units/ml, which is not different from the value during the first course. ANG I-converting enzyme 'concentration' as measured in the presence of N-ethylmaleimide, rose markedly after captopril. It was 29±2 m-units/ml before captopril and 47±3 when the patient was on captopril for 12 weeks. The effect of captopril intake on ANG I-converting enzyme 'activity' was observed within 30 min. The effect on ANG I-converting enzyme 'concentration', however, evolved over a period of two weeks. Also the return of ANG I-converting enzyme 'concentration' to normal after captopril had been withdrawn took several weeks. At the end of the first treatment period, which lasted for 12 weeks, ANG I-converting enzyme 'concentration' had the same high level as at the end of the second period, which lasted for eight months (Fig. 4). Thus the increase in ANG I-converting enzyme 'concentration' appears to level off with prolonged treatment, thereby reaching a new elevated plateau. Two days after discontinuation of captopril treatment the difference between ANG I-converting enzyme 'activity' and 'concentration' had disappeared. The antihypertensive effect of captopril, however, was still evident at that time despite elevated plasma levels of ANG I-converting enzyme 'activity' and active renin.

7.5. Discussion

Values for ANG I-converting enzyme in normal plasma observed here are somewhat higher than those reported by some authors using the same substrate, i.e.: 22.8 (13) 22.5 (14) and 20.2 m-units/ml (5). Our values are, however, similar to those reported by Friedland and Silverstein: 32.3 m-units/ml (8). The finding that ANG I-converting enzyme was increased in pulmonary sarcoidosis is in agreement with the experience of Lieberman (13) and many others (15).

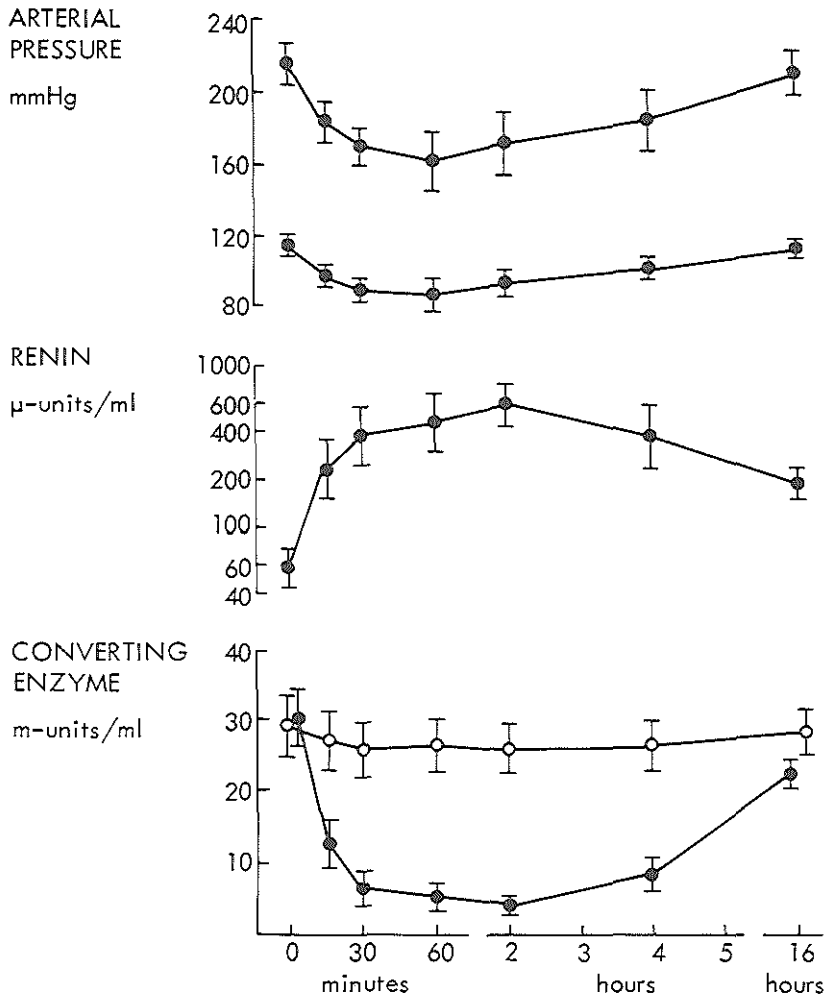

Figure 3. Effects of a single 100 mg dose of captopril on arterial pressure, plasma renin and ANG I-converting enzyme 'concentration' (o) and 'activity' (•). Results are presented as means±SEM.

The results of the present study show that measurements of ANG I-converting enzyme in plasma of patients on captopril can not be used as an index of the inhibition of circulating converting enzyme *in-vivo*, when the samples have been stored at —20°C. The inhibition is partly reversed on storage and the degree of deinhibition is variable. A similar observation has been made by Roulston and MacGregor (16). The effect of storage on ANG I-converting enzyme inhibition is possibly related to changes in the reactive thiol group of captopril, probably by oxidation to disulphides (17). As the thiol group is important for captopril's inhibitory effect (18), we speculated that N-ethylmaleimide, by its reaction with this thiol group, would inactivate the drug.

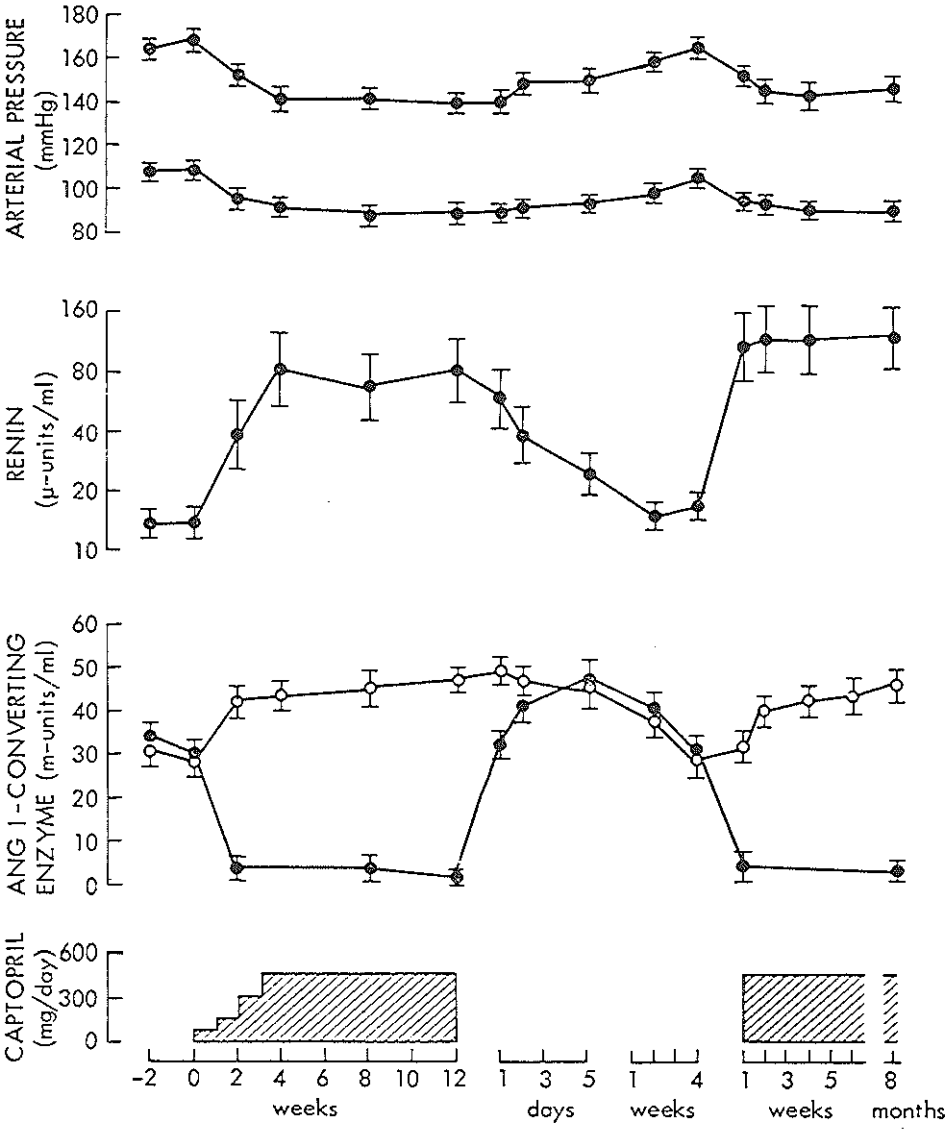

Figure 4. Effects of two courses of long-term captopril treatment on arterial pressure, plasma renin and ANG I-converting enzyme 'concentration' (o) and 'activity' (•). Results are presented as means±SEM.

Indeed the inhibition of ANG I-converting enzyme by captopril was completely reversed by N-ethylmaleimide. Measurements of converting enzyme in the presence of N-ethylmaleimide can therefore be used as an estimate of the total concentration of ANG I-converting enzyme, i.e. the sum of inhibited and uninhibited enzyme. Such measurements showed a gradual rise in converting enzyme 'concentration' after repea-

ted doses of captopril over a period of weeks. After withdrawal of captopril, ANG I-converting enzyme 'concentration' in plasma returned to normal, again over a period of weeks.
The slow response of converting enzyme 'concentration' contrasted with the rapid changes in converting enzyme 'activity' as measured in the absence of N-ethylmaleimide in freshly prepared plasma. The mechanism underlying the increase in circulating ANG I-converting enzyme 'concentration' by captopril is not known, but it is tempting to consider this an adaptive response to enzyme inhibition.
Waeber et al (3) have reported that the administration of 200 mg of captopril twice daily had an antihypertensive effect, despite their finding that ANG I-converting enzyme in plasma was normal for some part of the day. However, their measurements of converting enzyme were probably made in stored samples, and, as we have pointed out, such measurements underestimate the inhibition of this enzyme in plasma *in vivo*. Nevertheless their conclusion that the effect of captopril on blood pressure and ANG I-converting enzyme do not run in parallel is supported by our data. No rebound hypertension was observed in our study after abrupt withdrawal of long-term captopril treatment. On the contrary, blood pressure remained low for some time despite the fact that both circulating active renin and ANG I-converting enzyme 'activity' were elevated. It appears, therefore, that the level of converting enzyme 'activity' in the circulation is not the only determinant of the long-term effect of captopril on blood pressure.

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CHAPTER 8

Split renal function after captopril in unilateral renal artery stenosis

8.1. Summary

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 funtion on one side during captopril treatment may be diagnostic.

8.2. 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.

8.3. 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, beta-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 minutes 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

TABLE I: CLINICAL DATA ON PATIENTS WITH UNILATERAL RENAL ARTERY STENOSIS.

Case No	Age years	Sex m/f	Cause of renal artery stenosis A/F*	Plasma renin μU/ml+	Renal vein-to-artery renin ratio affected	contralateral
1	47	m	A	233	5.97	0.92
2	57	m	A	61	3.29	1.02
3	56	m	A	37	2.42	0.94
4	64	m	A	38	1.58	1.12
5	47	m	A	37	1.71	1.17
6	31	f	F	23	2.65	0.88
7	57	m	A	480	1.39	0.91
8	68	f	A	178	5.81	0.89
9	57	m	A	353	2.09	0.81
10	58	m	A	75	3.08	1.03
11	65	m	A	208	2.20	1.40
12	25	f	F	34	1.53	1.04
13	68	m	A	40	1.36	1.55
14	50	f	A	42	2.17	0.96

* A=atherosclerosis. F=Fibromuscular hyperplasia.
\+ Normal range: 5-45 μU/ml.

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.

8.4. 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 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

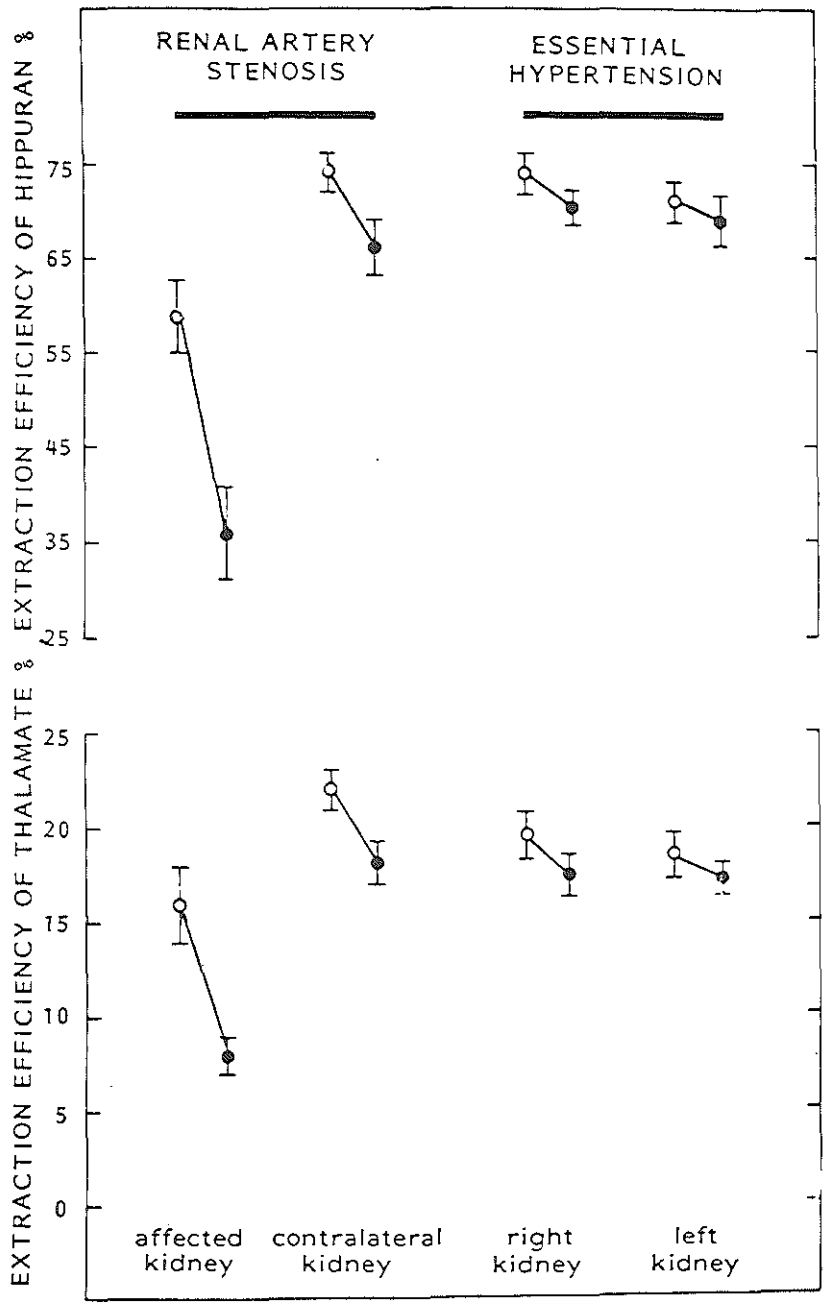

Figure 1. Effect of 50 mg captopril on renal extraction efficiencies of ^{131}I-sodium iodohippurate (E_H) and ^{125}I-thalamate (E_T) in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. In patients with renal artery stenosis changes in E_H and E_T were significant on both sides ($p<0.01$). Changes in essential hypertension were also significant ($p<0.05$).

TABLE 2: ACUTE EFFECTS OF CAPTOPRIL IN PATIENTS WITH UNILATERAL RENAL ARTERY STENOSIS. GROUP 1 v GROUP 2.

		GROUP 1 (n=7) (cases 1-7)		GROUP 2 (n=7) (cases 8-14)		p-Values for difference between group 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 hippuran (%)	- affected kidney	60±7	29± 7***	64±5	53± 9*	NS	<0.001
	- contralat kidney	75±3	71± 3	74±3	70± 5	NS	NS
Single-kidney extraction ratio thalamate (%)	- affected kidney	18±1	6± 1***	17±2	13± 3**	NS	<0.001
	- contralat kidney	24±2	22±3	22± 2	19± 3	NS	NS

values are means ± SEM
* =$p<0.05$ for difference from the pre-captopril value. ** =$p<0.01$. *** =$p<0.001$.

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 (Fig. 4 gives an example). By that time the plasma creatinine concentration 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.

8.5. 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

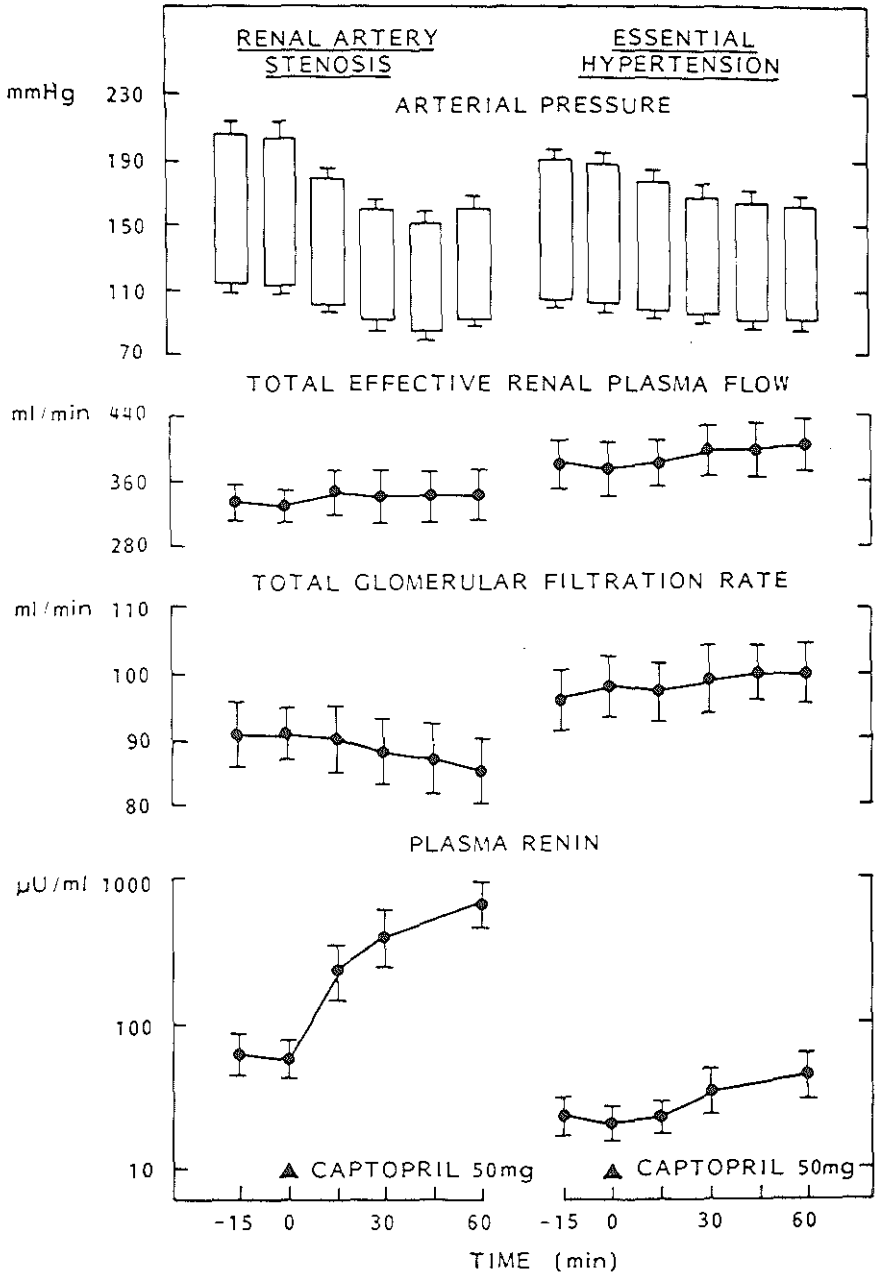

Figure 2. Effect of 50 mg captopril on total clearances of ^{131}I-sodium iodohippurate (effective renal plasma flow) and ^{125}I-thalamate (glomerular filtration rate) in 14 patients with uni-lateral renal artery stenosis and 17 patients with essential hypertension. Effect of captopril after 60 minutes was significant for systolic and diastolic intra-arterial pressure ($p<0.001$) and for renin ($p<0.01$).

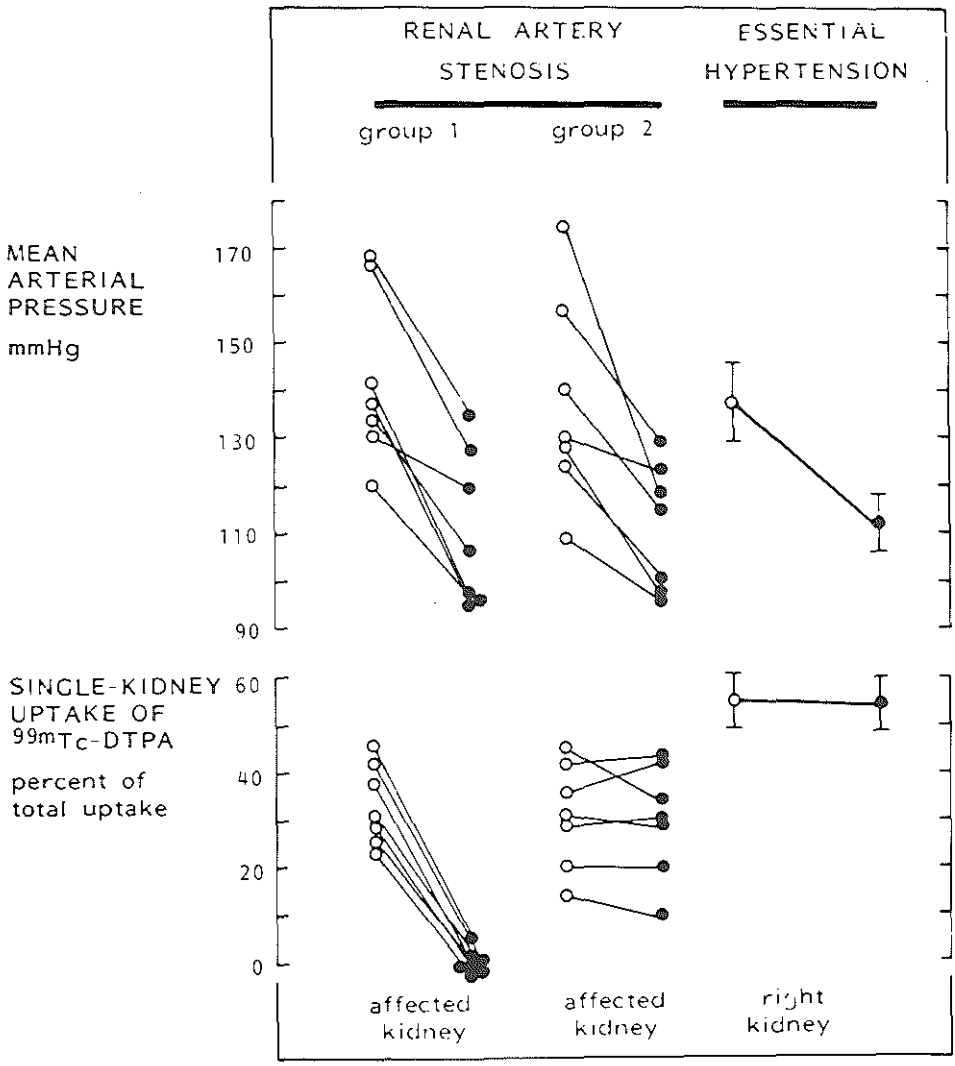

Figure 3. Effect of long-term captopril 150 mg daily on blood pressure and single kidney uptake of ^{99m}Tc-DTPA in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. Patiens with renal artery stenosis divided into two groups according to change in DTPA uptake (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 x 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.

TABLE 3: LONG-TERM EFFECTS OF CAPTOPRIL IN PATIENTS WITH UNILATERAL RENAL ARTERY STENOSIS. GROUP 1 v GROUP 2.

		GROUP 1 (n=7) (cases 1-7)		GROUP 2 (n=7) (cases 8-14)		p-Values for difference 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	percent of total uptake	34±3	<10	31±4	30± 4		

**=p<0.01 for difference from the pre-captopril value.
***=p<0.001.

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.

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.

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

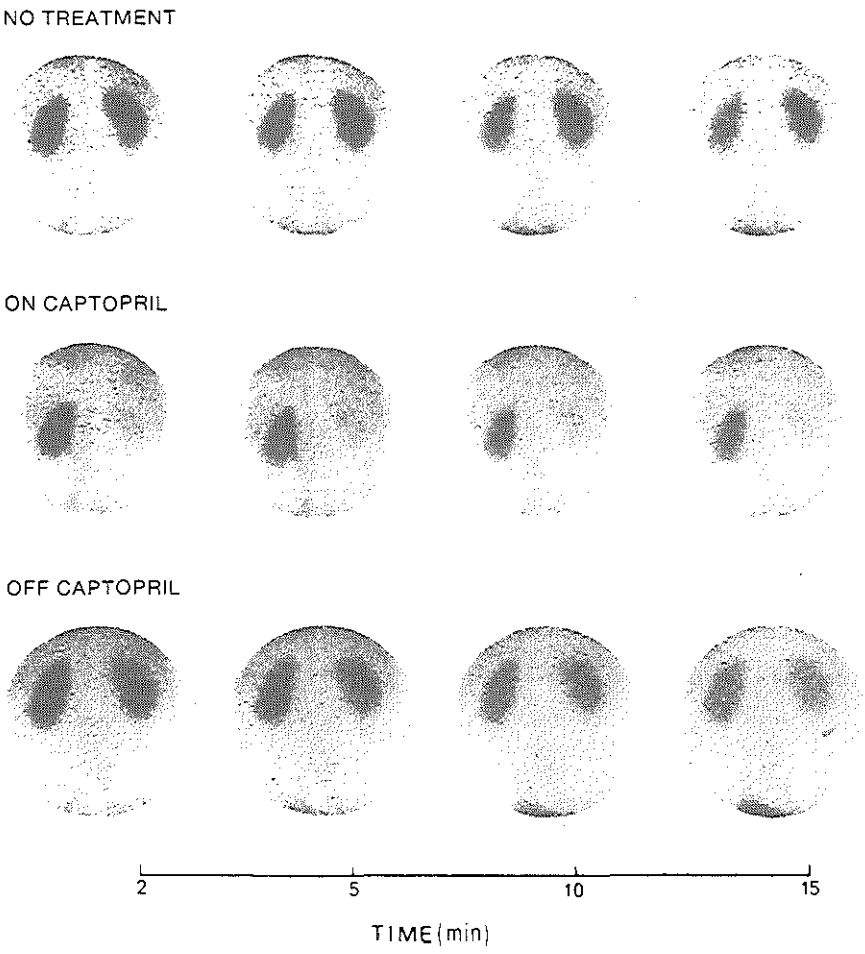

Figure 4. Sequential ^{99m}Tc-DTPA kidney scans in patient with unilateral renal artery stenosis (case 3; table I) before captopril, after four weeks of captopril 150 mg daily, and one week after stopping captopril. Time after radioisotope injection indicated.

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 postglomerular 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 problably 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.

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CHAPTER 9

Effects of one year captopril treatment on systemic and renal haemodynamics in essential hypertension

9.1. Summary

The effects of one-year treatment with the orally active angiotensin-converting enzyme inhibitor, captopril, on systemic and renal haemodynamics were compared with those of four-week and three-month treatment. The drug was administered at a dosage of 150 mg tid and the study was placebo-controlled and comprised nine male subjects with uncomplicated essential hypertension. Blood pressure fell from 162±5/105±1 mm Hg on placebo to 138±4/88±1 mm Hg after one year. Heart rate and cardiac output were unchanged. Effective renal plasma flow was 10 percent higher than on placebo and both total peripheral and renal vascular resistance were reduced by about 10 and 20 percent respectively. Glomerular filtration rate was 6% higher than on placebo and extracellular fluid volume was reduced by half a litre. These changes were not different from the effects observed after four weeks and three months, with the exception of glomerular filtration rate, which showed a progressive improvement over the one year period. Thus, the short-term cardiovascular effects of captopril monotherapy are sustained during long-term treatment. Captopril seems to have a weak diuretic action and causes a slight improvement of renal function. These two effects may contribute to the long-term effectiveness of this drug as an antihypertensive agent.

9.2. Introduction

In the previous chapters the effects of acute and four-week treatment with captopril have been discussed. During such treatment the drug lowers blood pressure mainly by arteriolar dilatation possibly combined with some venous dilatation. Captopril did not cause the reflex tachycardia and fluid retention that are often seen after treatment with other vasodilator drugs. It is uncertain whether these effects of captopril persist with long-term treatment. For instance it has been suggested that in patients with moderate hypertension the initial control of blood pressure was lost with long-term captopril treatment (1). This chapter describes the systemic and renal haemodynamic effects of one-year captopril monotherapy in patients with uncomplicated essential hypertension and normal plasma renin.

9.3. Patients and study protocol

Nine male patients (45±3 yr) with mild to moderate essential hypertension were treated as outpatients with captopril for one year. After an initial placebo period of eight weeks all patients started to take captopril 25 mg tid. The concentration of active renin in plasma at the end of the placebo period was 19±3 μU/ml (range 7-36 μU/ml). The normal range in subjects on unrestricted sodium intake is 5-45 μU/ml (n=30). After one week the dose of captopril was stepwise increased to 50 mg tid during the second week, 100 mg tid during the third week, and 150 mg tid during the fourth week. This dose was then continued during the one-year period of observation. All measurements were made two to four hours after the last morning dose, when the patients had been recumbent for at least one hour. Non-invasive measurements of blood pressure, heart rate, cardiac output, extracellular fluid volume, renal plasma flow and glomerular filtration rate were made as described in chapter two, at the end of the placebo period and after four weeks, three months and one year treatment with captopril. In addition, endogenous creatinine clearance was calculated on 24 hour urine collections. Sodium intake was not restricted in these patients but it was shown to be relatively constant throughout the observation period i.e. 145±13 mmol/day on placebo as compared to 156±14 mmol/day after one year on captopril.
In one of the patients antinuclear factor, which was negative on placebo, became positive after three months of treatment. It was negative again after one year, while captopril was continued. No other adverse reactions were seen in these patients. All data relevant to body size are corrected to 1.73 m^2 standard body surface area. Statistical analysis was performed by Student's paired t-test.

9.4. Results

Systemic haemodynamics. Blood pressure fell from 162±5 mm Hg systolic and 105±1 mm Hg diastolic on placebo to 138±4 mm Hg systolic and 90±2 mm Hg diastolic after four weeks ($p<0.001$) and to 138±4 mm Hg systolic and 88±1 mm Hg diastolic after one year of captopril treatment ($p<0.001$) (Fig. 1). Heart rate and cardiac output did not change. Calculated total peripheral resistance decreased from 1670±70 dyn s cm^{-5} on placebo to 1400±100 dyn s cm^{-5} after one month ($p<0.01$) and to 1480±70 dyn s cm^{-5} after one year ($p<0.05$). Extracellular fluid volume decreased from 14.6±0.3 l on placebo to 14.0±0.3 l after four weeks ($p<0.05$) and to 14.0±0.2 l after one year ($p<0.05$).

Renal haemodynamics. Effective renal plasma flow increased from 408±11 ml/min on placebo to 455±22 ml/min after four weeks ($p<0.05$) and to 445±20 ml/min after one year ($p<0.05$) (Fig. 2). Calculated renal vascular resistance decreased from 10.1±0.4 dyn s cm^{-5} on placebo to 7.8±0.4 dyn s cm^{-5} after four weeks ($p<0.001$) and to 7.8±0.3 dyn s cm^{-5} after one year ($p<0.001$). Filtration fraction decreased from 25.4±0.5% on placebo to 22.8±0.8% after four weeks ($p<0.01$) and returned to pretreatment level after one year, 25.3±0.6%. Glomerular filtration rate had not

changed after four weeks but increased from 103±3 ml/min on placebo to 109±3 ml/min after one year ($p<0.05$). Creatinine clearance increased from 111±7 ml/min on placebo to 120±7 ml/min after one year ($p<0.05$).

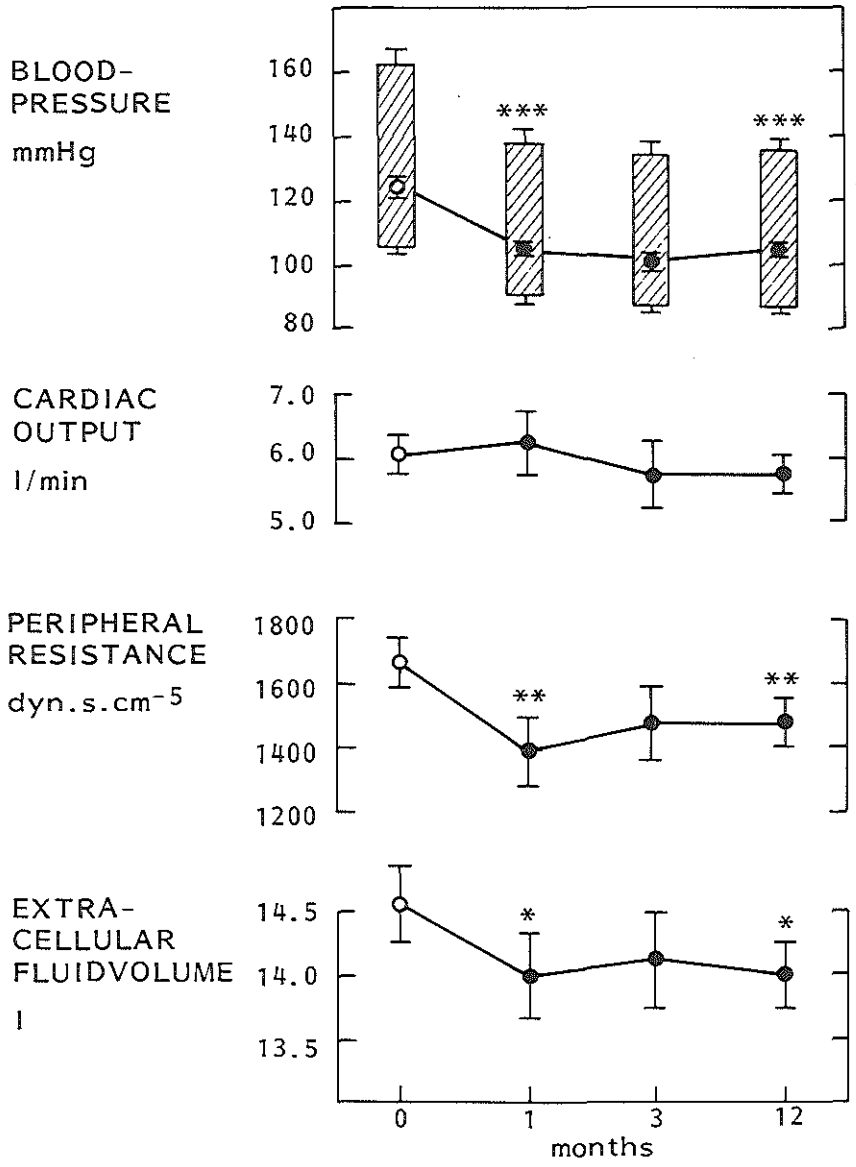

Figure 1. Systemic haemodynamics in essential hypertension. Effects of one year captopril (n=9). *=$p<0.05$; **=$p<0.01$; ***=$p<0.001$.

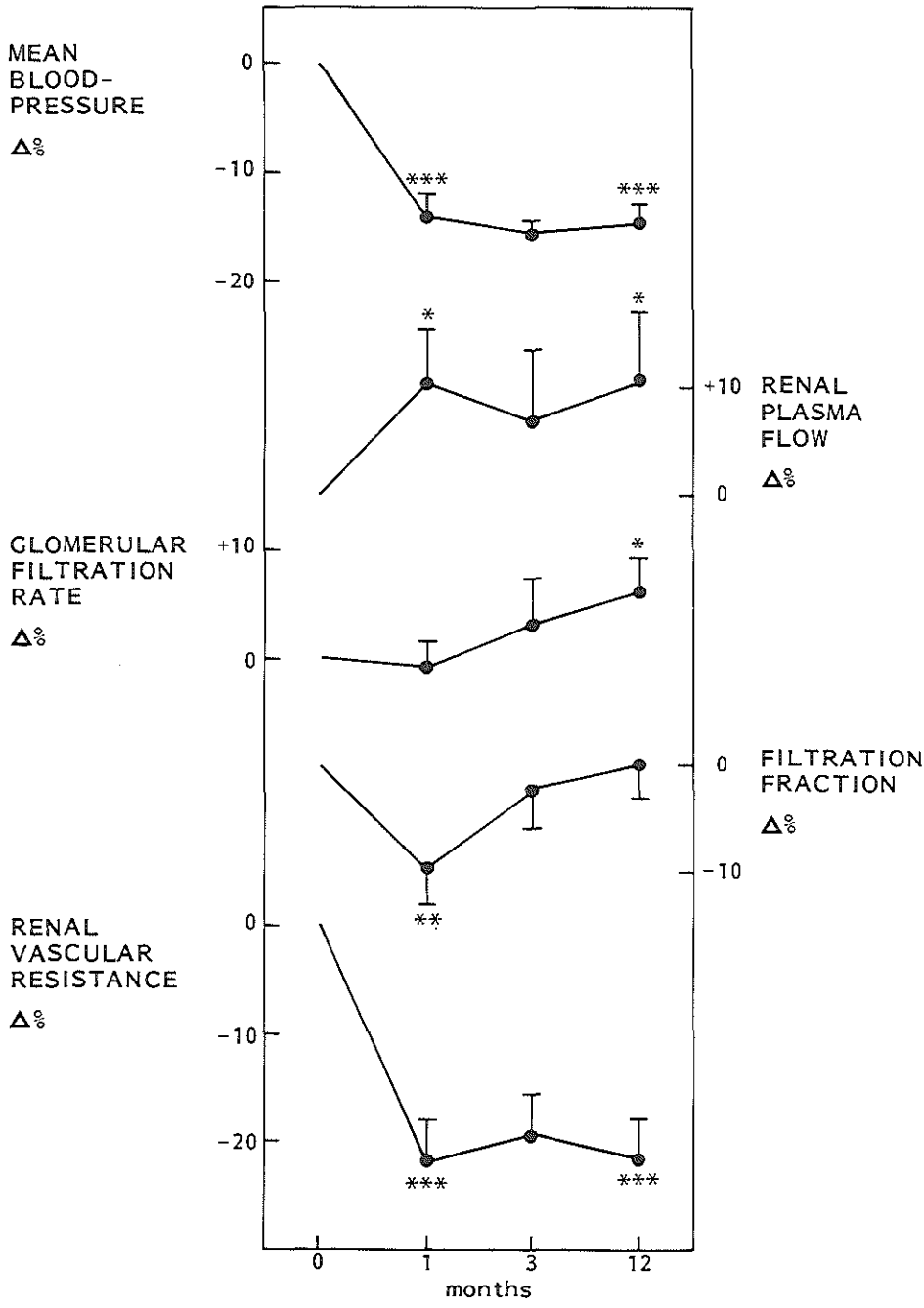

Figure 2. Effects of one year captopril on mean blood pressure and renal haemodynamics in patients with essential hypertension (n=9). *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$.

9.5. Discussion

Our data show that the effects of short-term treatment with captopril on systemic and renal haemodynamics are sustained over a period of one year. There was no evidence for the development of tolerance to the drug. As discussed in chapter eight, hippuran clearance was not significantly altered in patients with essential hypertension by a single gift of 50 mg captopril. However, the renal extraction of hippuran was less complete after this drug, indicating a shortening of the renal transit time of hippuran due to increased renal plasma flow. It seems likely, therefore that the small increments of hippuran clearance observed in the present study are an underestimation of the actual effect on renal plasma flow. A modest increase in renal plasma flow after a single gift of captopril to a similar group of patients has been reported in the literature (2).

In the present study we observed a small increase in glomerular filtration rate after one year of captopril treatment. As described in chapter eight, no significant effect on glomerular filtration rate was observed acutely after converting enzyme inhibition, although small increments have been reported in the literature (2, 3). Reduction of filtration fraction is a common finding in the short-term studies reported so far (4, 5), and is also observed in the present long-term study. This is compatible with the assumption that angiotensin II predominantly acts on the efferent glomerular arterioles. Inhibition of converting-enzyme will then lead to a reduction of the post-glomerular vascular resistance and hence to a reduction of the glomerular filtration pressure (6-8).

Captopril caused a small decrease in extracellular fluid volume suggesting a weak diuretic effect. This effect was sustained over the whole one year observation period.

In conclusion, this study demonstrates that the effects of one year treatment with captopril on blood pressure, cardiac output, renal plasma flow and extracellular fluid volume were not different from the effects on these parameters after four weeks of treatment. Glomerular filtration rate showed a progressive improvement over the one-year period. The reduction of extracellular fluid volume, which suggests a weak diuretic effect, together with the slight improvement of renal function may have contributed to the sustained antihypertensive effect of captopril.

9.6. References

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CHAPTER 10

Summary and conclusions

10.1. Acute effects of angiotensin-converting enzyme inhibition

Plasma converting enzyme activity is inhibited by more than 90% from 60 minutes upto four hours after a single oral dose of 100 mg of captopril. It gradually returns to pretreatment level within 24 hours. Plasma renin rises within 60 minutes after the first dose. It also gradually returns to pretreatment level within 24 hours. Blood pressure reduction is maximal 60-90 minutes after captopril both in patients with essential hypertension and in renovascular hypertension. A similar time course of blood pressure response is observed in patients with chronic heart failure and in fluid-depleted nephrectomized subjects.

The percent changes in blood pressure are not much different in these groups of patients despite large differences in the level of plasma renin. In patients with essential or renovascular hypertension the reduction of blood pressure is accompanied by a decrease in cardiac filling pressures and total peripheral resistance. Cardiac output is unchanged in hypertension. In patients with chronic heart failure a more marked decrease in cardiac filling pressures is observed, and cardiac output is increased. Heart rate is not changed in patients with essential or renovascular hypertension. In chronic heart failure, however, heart rate is decreased by captopril. Stroke volume is unchanged in essential or renovascular hypertension but it is increased in chronic heart failure. Plasma noradrenaline is not affected by captopril in essential or renovascular hypertension (1) but it is suppressed in chronic heart failure (2).

The profile of captopril's cardiovascular effects is similar to the effects of vasodilator drugs that mainly act on arterioles combined with some effect on venules and veins.

Correlations of the acute changes in blood pressure or total peripheral resistance with pretreatment levels of plasma renin can be demonstrated in patients with hypertension as well as in chronic heart failure. These correlations, however, are weak and are of little importance in clinical practice. Moreover, the correlation between the blood pressure response and pretreatment plasma renin is absent with chronic treatment.

The renal extraction of hippuran is slightly reduced after captopril in patients with essential hypertension but it is greatly reduced in renovascular hypertension on the side of the kidney affected by artery stenosis. Similar effects can be demonstrated for the renal extraction of thalamate. The reduced extraction of hippuran probably reflects a shortened plasma transit time through the kidney due to intrarenal vasodilatation. The reduced extraction of thalamate reflects a low filtration fraction, suggesting that the vasodilatation occurs, at least in part, at a postglomerular level. The renal

clearance of hippuran is not altered but because the renal extraction of hippuran is less complete after captopril, the renal plasma flow must be increased. Glomerular filtration rate is unchanged in most cases except in some patients with renal artery stenosis, where sharp reductions in glomerular filtration rate are observed on the affected side. We assume that in such cases the filtration pressure has dropped below a certain critical level. Unilateral loss of renal function may occur with minor changes of plasma creatinine. Although this effect appears to be reversible when captopril is withdrawn after a few weeks of treatment, it is possible that irrepairable damage will occur after long periods.

10.2. Chronic effects of angiotensin-converting enzyme inhibition

The concentration of angiotensin-converting enzyme in plasma is increased with chronic captopril treatment. Its activity, however, is decreased due to inhibition by captopril. The rise in plasma renin that is observed acutely after captopril is sustained during chronic treatment. Blood pressure decreases over a period of two to four weeks both in patients with essential hypertension and in patients with renovascular hypertension. The acute effect on total peripheral resistance is also sustained during chronic treatment. Cardiac output is unchanged in both forms of hypertension. Body fluid volumes are not significantly altered in renovascular hypertension but a small sustained decrease in extracellular fluid volume is observed in essential hypertension.
The vasodilator effect of captopril on the kidney is also demonstrable during chronic treatment.
When captopril is withdrawn after chronic treatment, the angiotensin-converting enzyme activity in plasma increases within two to five days to levels above pretreatment. At that time plasma renin is still elevated, but in spite of this, the blood pressure does not rebound and returns gradually to pretreatment values over a period of two weeks. In our study, the concentration of uninhibited plus inhibited angiotensin-converting enzyme in plasma and the degree of inhibition have been measured by a chemical assay technique. It has been shown that inhibition of enzyme activity measured in this way *in-vitro* is an accurate reflection of the inhibition of the conversion of circulating angiotensin I *in-vivo,* as determined by measuring the pressor response to intravenously infused angiotensin I. Our results are therefore an indication that blockade of the circulating renin-angiotensin system is not the only factor that determines the chronic effect of captopril on blood pressure.
Fluid withdrawal enhances the effect of captopril on blood pressure not only in hypertensive patients with normal or high circulating renin but also in nephrectomized patients with virtually no circulating renin. This again suggests that the antihypertensive effect of captopril does not solely depend on its action on the circulating renin-angiotensin system.

10.3. Interpretation of results with respect to captopril's mechanism of action

Actions that are independent of the circulating renin-angiotensin system. Most of circulating angiotensin II originates from angiotensin I-to-II conversion by angiotensin-converting enzyme 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 conversion at these two sites is not the only mechanism by which captopril exerts its antihypertensive action. Originally captopril was thought to act mainly through blockade of the circulating renin-angiotensin system. Our observations, however, indicate that the effect on circulating angiotensin II is more important for the acute blood pressure response to this drug than for its long-term effect.

A likely alternative explanation for captopril's antihypertensive effect is inhibition of angiotensin-converting enzyme at the tissue level, resulting in diminished local formation of angiotensin II. Such inhibition has been demonstrated in animals to occur in blood vessels, kidney, adrenals and brain (3). 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 cells (4,5). There is growing evidence that local angiotensin II formation within some critical tissues is involved in blood pressure regulation.

Angiotensin-converting enzyme inhibition or administration of the competitive angiotensin II antagonist, sar^1-ala^8-angiotensin II, have been demonstrated to lower blood pressure in spontaneously hypertensive rats after they have been bilaterally nephrectomized and their circulating renin has dropped to a very low level (6). Converting enzyme inhibition also lowers the blood pressure of the two-kidney one-clip renal hypertensive rat after removal of both kidneys (7).

It is interesting to note that the plasma concentration of enzymatically active renin was very low in our nephrectomized patients but that the plasma level of inactive renin (prorenin) was much higher. In some patients it is even within the normal range (8, 9). One may speculate that prorenin is taken up in the vascular wall and subsequently converted into renin. Whereas in the rat the plasma level of renin becomes almost zero within 60-90 minutes after bilateral nephrectomy, the level of renin decreases more slowly in the aorta (7). The decrease in blood pressure after converting enzyme inhibition in nephrectomized animals occurred while aortic renin was still present. It has further been reported that in the spontaneously hypertensive rat the level of systolic blood pressure is closely correlated with the level of renin in the aorta and not with plasma renin (10).

More direct evidence for the importance of a local vascular renin-angiotensin system for blood pressure regulation has recently been provided by experiments in which isolated rat hindquarters were perfused with the synthetic tetradecapeptide analog of angiotensinogen. At constant flow, perfusion pressure was increased by this analog, and this effect was reduced by captopril and by a specific inhibitor of renin (11).

A third explanation for captopril's blood pressure lowering effect in the presence of low plasma renin could be an effect that is not mediated by reduction of angiotensin II but by accumulation of bradykinin. Measurements of bradykinin in plasma after

captopril have given conflicting results but now most investigators agree that plasma bradykinin is not increased by converting enzyme inhibition (12). We have not performed bradykinin measurements in the present study. There are still formidable technical difficulties in accurately quantifying low levels of circulating bradykinin. Moreover, the kallikrein-kinin system is regarded as a local system, important in regulating organ blood flow and vascular resistance, rather than as a circulating hormonal system. Circulating levels, therefore, may not reflect increased local tissue kinin levels. Indeed, urinary kinins, which arise from intrarenal activity of the kallikrein-kinin system have been reported to increase after converting enzyme inhibition therapy of hypertensive patients (13). Furthermore, bradykinin receptors decrease after such treatment suggesting down regulation of these receptors by increased local kinin concentration.

Actions on target organs that are involved in blood pressure regulation. A decrease of angiotensin II in the circulation lowers blood pressure because of reduced occupancy of postsynaptic vascular angiotensin II-receptors that mediate vasoconstriction. Reduced occupancy of adrenal angiotensin II-receptors with subsequent suppression of aldosterone secretion does not contribute to captopril's acute effect on blood pressure but may add to its chronic effect. The fall in plasma aldosterone reported by others (14, 24) and the small but significant decrease in extracellular fluid volume we have observed in our patients with essential hypertension are consistent with such a mechanism. The decrease in circulating angiotensin II after captopril may interfere with neurogenic vasoconstriction by reducing the occupancy of presynaptic angiotensin II-receptors on sympathetic nerve terminals (15). Captopril is known to pass the blood-brain barrier and neurogenic vasoconstriction may therefore also be suppressed by intracerebral converting enzyme inhibition. Studies in isolated blood vessel preparations, in isolated rat kidneys, in pithed rats and in spontaneously hypertensive rats provided some evidence supporting bot a central effect on sympathetic neuronal activity and a peripheral, presynaptic, effect on sympathetic neurotransmission (16). In humans, it has been demonstrated that the pressor response to intravenous infusion of noradrenaline is reduced by a single oral gift of 50 mg of captopril (17). In our hypertensive patients resting supine levels of plasma noradrenaline and the reflex-mediated increments in heart rate and plasma noradrenaline were not reduced by captopril (1) but this does not exclude an effect of this drug on sympathetic neuronal activity or neurotransmission.

If bradykinin metabolism is affected by converting enzyme inhibitors, then this could stimulate the production of the vasodilator, natriuretic, prostaglandin, PGE_2 (18). Indeed, captopril does increase PGE_2-production in hypertensive man (19) and by isolated renomedullary interstitial cells (20). Accordingly, cyclo-oxygenase inhibitors, like indomethacin or similar drugs that inhibit prostaglandin synthesis, can antagonize the vascular effects of converting enzyme inhibition (21, 22).

Finally, the split renal function studies we have performed strongly suggest that angiotensin II, either circulating or locally formed within the kidney, has an important function in maintaining a normal glomerular filtration rate in the presence of renal artery stenosis. Interference with this mechanism by converting enzyme inhibitors

may induce renal insufficiency in some patients with renovascular hypertension. Thus, we are left with an unexpected dilemma. Converting enzyme inhibitor therapy has originally been advocated, and rightly so, for those forms of hypertension where circulating renin-angiotensin is high (23, 24). Now, it is realized that this therapy is equally effective and causes less adverse reactions in essential hypertension, when circulating renin-angiotensin is normal or low.

10.4. References

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HOOFDSTUK 11

Samenvatting en conclusies

11.1. Doel van de studie

Vele jaren heeft men getracht door farmacologische modulatie van het renine-angiotensine systeem, de betekenis van angiotensine II, het biologisch actieve eindproduct van dit systeem, voor de pathogenese van verschillende vormen van hypertensie aan te tonen. De hiervoor gebruikte middelen, zoals bèta-adrenoceptor antagonisten die het systeem remmen en diuretica die het systeem stimuleren, hebben echter het bezwaar dat zij in het intacte organisme behalve het renine-angiotensine systeem ook andere bloeddrukregelende systemen, zoals het sympathische zenuwstelsel, beïnvloeden. Een nieuwe wijze van benadering werd mogelijk door de synthese van competitieve angiotensine II antagonisten en door de ontwikkeling van captopril. Angiotensine II antagonisten zijn peptide-analogen van angiotensine II. Zij zijn alleen werkzaam na intraveneuze toediening en hebben bovendien het nadeel dat het partiële agonisten zijn. Captopril is een oraal werkzame specifieke remmer van het enzym dat het biologisch niet-actieve angiotensine I omzet in het bloeddrukverhogende angiotensine II. Remming van dit "converting enzyme" zal, zo mag men verwachten, vooral de bloeddruk verlagen bij die patiënten, bij wie het renine-angiotensine systeem is gestimuleerd, zoals bijvoorbeeld bij renovasculaire of maligne hypertensie. De eerste studies met captopril bij patiënten met hypertensie leverden echter wat dit betreft tegenstrijdige resultaten op. Ook werd een langdurig effect van dit middel op de bloeddruk waargenomen, terwijl bepalingen van het gehalte aan angiotensine-converting enzyme in plasma leken aan te geven dat de aanvankelijke remming van dit enzym reeds was opgeheven. Bij enkele patiënten werd aangetoond dat captopril werkt als een arteriële en veneuze vaatverwijder, maar onduidelijk bleef of de hoogte van de plasma reninespiegel vóór captopril enige voorspellende waarde had voor de te verwachten haemodynamische effecten. Over de haemodynamische effecten van chronisch captopril gebruik bij verschillende vormen van hypertensie is nog weinig bekend. Dit geldt ook voor de invloed van dit middel op de nierfunctie. Hoewel in kortdurende studies een verbetering van de nierfunctie werd aangetoond, is ook verslechtering van de nierfunctie na captopril beschreven.
Het hier beschreven onderzoek had tot doel na te gaan of de hoogte van het renine gehalte in plasma vóór behandeling met captopril gerelateerd is aan het effect van dit middel op de bloeddruk, zowel na de eerste dosis als bij chronische toediening, en vervolgens om de haemodynamische effecten van angiotensine-converting enzyme remming bij verschillende vormen van hypertensie nauwkeuriger te bepalen. Hiertoe

werden de effecten van captopril bij de behandeling van patiënten met zeer sterk uiteenlopende spiegels van plasma renine onderzocht: normotensieve anefrische patiënten, bij wie het plasma vrijwel geen enzymatisch actief renine bevatte, patiënten met essentiële hypertensie met lage tot normale plasmaspiegels van renine, patiënten met renovasculaire hypertensie met normale tot hoge reninespiegels en tenslotte normotensieve patiënten met decompensatio cordis bij wie de reninespiegels soms extreem hoog waren. Ook werd getracht enig inzicht te verkrijgen in de relatie tussen de mate van remming van angiotensine-converting enzyme en het antihypertensieve effect van captopril. Tevens werden de acute en chronische effecten van dit middel op de nierfunctie bestudeerd.

11.2 Acute effecten van angiotensine-converting enzyme remming

De converting enzyme acitiviteit in plasma is van 60 minuten tot vier uur na 100 mg captopril per os voor meer dan 90% geremd. Over een periode van 24 uur wordt geleidelijk de uitgangswaarde weer bereikt. Plasma renine stijgt binnen 60 minuten na de eerste dosis en keert ook geleidelijk binnen 24 uur terug naar de uitgangswaarde. De maximale bloeddrukdaling wordt 60 tot 90 minuten na toediening van captopril bereikt, zowel bij patiënten met essentiële hypertensie als bij patiënten met renovasculaire hypertensie. Vergelijkbare effecten op de bloeddruk worden gezien bij nierloze patiënten na ultrafiltratie-haemodialyse en bij patiënten met decompensatio cordis. Ondanks grote verschillen in plasma renine bij deze groepen patiënten is de procentuele bloeddrukdaling niet erg verschillend.
Bij patiënten met essentiële of renovasculaire hypertensie is er naast de bloeddrukdaling sprake van daling van de vullingsdrukken van het hart en neemt de perifere vaatweerstand af. Het hartminuutvolume verandert hier niet. Bij patiënten met decompensatio cordis wordt een sterkere daling van de vullingsdrukken van het hart waargenomen en stijgt het hartminuutvolume. De hartfrequentie verandert niet bij patiënten met hypertensie maar daalt bij patiënten met decompensatio cordis. Het slagvolume verandert niet bij hypertensie maar neemt toe bij decompensatio cordis. Plasma noradrenaline verandert niet na captopril bij essentiële of renovasculaire hypertensie, maar daalt bij patiënten met decompensatio cordis.
Het haemodynamische profiel van captopril lijkt sterk op dat van vaatverwijdende stoffen, die hun werking voornamelijk uitoefenen op de arteriolen en daarnaast ook enig effect hebben op venulen en venen.
Er kan een relatie worden aangetoond tussen de acute veranderingen in bloeddruk of perifere weerstand na captopril enerzijds en de hoogte van de reninespiegel in plasma vóór de behandeling anderzijds, niet alleen bij patiënten met hypertensie maar ook bij patiënten met decompensatio cordis. De correlaties zijn echter zwak en voor de praktijk daarom van weinig belang. Verder is tijdens chronische behandeling met captopril een dergelijke relatie tussen de verandering in bloeddruk en de reninespiegel vóór de behandeling niet aantoonbaar.
Bij patiënten met essentiële hypertensie neemt de fractionele extractie van hippuran door de nieren, uitgedrukt als het percentage van het aan de nier aangeboden hippuran

dat wordt uitgescheiden, na captopril enigszins af. Bij patiënten met renovasculaire hypertensie is er sprake van een veel sterker afname van deze fractionele renale extractie van hippuran, althans aan de kant waar de nierarteriestenose zich bevindt. Vergelijkbare effecten konden worden aangetoond voor de fractionele renale extractie van thalamaat. De afgenomen extractie van hippuran is mogelijk een uiting van een versnelde doorstroming van de nier als gevolg van intrarenale vasodilatatie. De afname van de thalamaatextractie is het gevolg van een daling van de filtratiefractie, wat de suggestie wekt dat de vasodilatatie, tenminste voor een deel, op postglomerulair niveau plaatsvindt. Omdat de totale renale klaring van hippuran niet verandert, terwijl de fractionele renale extractie van hippuran na captopril afneemt, moet de plasma doorstroming in beide nieren gezamenlijk zijn toegenomen. De glomerulaire filtratiesnelheid blijft in de meeste gevallen ongewijzigd, behalve bij enkele patiënten met een nierarteriestenose, bij wie na captopril een zeer sterke afname van de glomerulaire filtratiesnelheid aan de kant van de stenose optreedt. Dit ernstige nierfunctieverlies kon worden aangetoond met behulp van renografie waarbij radio-actief DTPA (diëthyl-triamino-penta-azijnzuur) werd gebruikt. Het is aannemelijk dat in een dergelijk geval de filtratiedruk tot onder een kritische grens is gedaald. Eenzijdig nierfunctieverlies kan optreden ook wanneer de bloeddruk in de grote circulatie weinig daalt. Het kreatinine gehalte van plasma stijgt vaak nauwelijks onder deze omstandigheden, omdat de gezonde nier de functie van de aangedane nier overneemt. Hoewel dit effect reversibel blijkt wanneer captopril na enkele weken wordt gestopt, lijkt het toch niet onmogelijk dat bij langdurige behandeling met een converting enzyme remmer onherstelbaar verlies van functie van de nier met een stenotische arterie kan optreden.

11.3 Chronische effecten van angiotensine-converting enzyme remming

Tijdens de behandeling met captopril neemt de *concentratie* van angiotensine-converting enzyme in plasma toe. De *activiteit* van het enzym is echter afgenomen als gevolg van de remming door captopril. De vrijwel onmiddellijk na de eerste dosis captopril optredende stijging van het plasma renine blijft tijdens langdurige toediening aanwezig. Zowel bij patiënten met essentiële hypertensie als bij patiënten met renovasculaire hypertensie daalt de bloeddruk geleidelijk tot dat een minimumwaarde na twee tot vier weken is bereikt. De na de eerste dosis waargenomen daling van de perifere weerstand blijft ook bij langdurige behandeling gehandhaafd. Het hartminuutvolume verandert bij beide vormen van hypertensie niet. Bij patiënten met renovasculaire hypertensie verandert het extracellulaire vloeistofvolume niet, maar bij essentiële hypertensie wordt blijvend een geringe afname hiervan gevonden.
Het vaatverwijdende effect van captopril in de nier blijft ook bij langdurige behandeling aantoonbaar.
Als captopril na langdurige behandeling wordt gestopt, neemt de *activiteit* van angiotensine-converting enzyme in plasma binnen twee tot vijf dagen toe tot ver boven het uitgangsniveau. Hoewel er op dat moment nog steeds sprake is van een verhoogd plasma renine treedt er toch geen rebound-hypertensie op. Na het abrupt staken van chronische behandeling met captopril stijgt de bloeddruk in twee weken

langzaam naar het uitgangspunt. De toegenomen *concentratie* van converting enzyme in plasma is waarschijnlijk een uiting van toegenomen synthese, mogelijk ter compensatie van de enzymremming.
In het hier beschreven onderzoek werd de som van de concentraties van geremd en ongeremd angiotensine-converting enzyme, alsmede de mate van remming van het enzym, gemeten met behulp van een chemische methode. Er is aangetoond dat de zo *in-vitro* gemeten mate van remming van het enzym een goede weergave is van de mate van remming van de omzetting van circulerend angiotensine I *in-vivo*, gemeten aan de bloeddrukstijging na intraveneus toegediend angiotensine I. Onze resultaten moeten dan ook betekenen dat remming van het circulerend renine-angiotensine systeem niet het enige mechanisme is waardoor captopril de bloeddruk verlaagt.
Het onttrekken van vocht versterkt het effect van captopril op de bloeddruk, niet alleen bij patiënten met hypertensie, bij wie plasma renine vóór behandeling normaal of hoog is, maar ook bij nierloze patiënten met vrijwel geen enzymatisch actief renine in het bloed. Ook dit is een reden om te veronderstellen dat de werking van captopril niet alleen afhangt van het effect op het circulerende renine-angiotensine systeem. Het is bekend dat vóórbehandeling met een diureticum de bloeddrukverlagende werking van captopril kan versterken. Het ligt voor de hand om te veronderstellen dat dit berust op de door het diureticum veroorzaakte stimulatie van het renine-angiotensine systeem. Het onderzoek bij nierloze patiënten wijst er echter op dat verkleining van het extracellulaire vloeistofvolume de werking van captopril potentieert, onafhankelijk van het renine-angiotensine systeem.

11.4 Interpretatie van de resultaten met betrekking tot het werkingsmechanisme van captopril

Effecten die optreden onafhankelijk van het circulerend renine-angiotensine systeem.
Het meeste angiotensine II in de circulatie wordt gegenereerd door omzetting van plasma angiotensine I door converting enzyme, gelocaliseerd in de celmembraan van endotheelcellen van capillairen vooral in de long. Ook in plasma zelf vindt deze omzetting plaats. Uit onze resultaten blijkt dat de bloeddrukverlagende werking van captopril niet alleen door remming van converting enzyme op deze plaatsen kan worden verklaard. Bij de introductie van captopril werd aangenomen dat de daling van circulerend angiotensine II na captopril het belangrijkste werkingsmechanisme van dit middel was. Onze gegevens wijzen er echter op dat de daling van circulerend angiotensine II belangrijker is voor het acute effect op de bloeddruk dan voor het chronische effect.
Een andere verklaring voor de bloeddrukverlagende werking van captopril is de remming van angiotensine-converting enzyme in de weefsels met als gevolg een afname van locale angiotensine II productie. Bij proefdieren is dit aangetoond voor de vaatwand, nieren, bijnieren en hersenen. Recent is beschreven dat alle componenten van het renine-angiotensine systeem worden gevonden in bepaalde celkweken. Ook is aangetoond dat bij spontaan-hypertensieve ratten, ook nadat de nieren zijn verwijderd en plasma renine zeer sterk is gedaald, toediening van een converting enzyme remmer

of van een competitieve angiotensine II antagonist de bloeddruk verlaagt. Daarnaast blijkt converting enzyme remming de bloeddruk te verlagen bij ratten met hypertensie ten gevolge van een eenzijdige nierarteriestenose, ook nadat beide nieren zijn verwijderd. In de rat daalt het gehalte aan renine in plasma binnen 60-90 minuten na nefrectomie, terwijl het gehalte aan renine in de aorta veel langzamer afneemt. Bij de genefrectomeerde ratten was op het moment van de bloeddrukdaling renine nog steeds aantoonbaar in de aorta. Ook is vastgesteld dat de hoogte van de bloeddruk in de spontaan-hypertensieve rat wel gecorreleerd is aan het reninegehalte van de aorta, maar niet aan de reninespiegel in plasma. In dit verband is het van belang dat de plasmaconcentratie van actief renine in onze nierloze patiënten zeer laag was, maar dat de spiegel van inactief renine (prorenine) veel hoger was, soms was deze zelfs in het normale bereik. Het is denkbaar dat prorenine in de vaatwand wordt opgenomen en vervolgens wordt omgezet in actief renine.
De gedachte, dat een lokaal renine-angiotensine systeem belangrijk is voor de bloeddrukregulatie, vindt verder steun in recente resultaten van experimenten met geïsoleerde geperfundeerde achterpoten van de rat, waarbij de weerstandsverhoging, die werd veroorzaakt door perfusie met een synthetisch angiotensinogeen analoog, kon worden tegengegaan door toevoeging van een remmer van angiotensine-converting enzyme of van een directe remmer van renine.
Een derde verklaring voor de bloeddrukverlagende werking van captopril in situaties waarin plasma renine laag is zou kunnen liggen in het feit dat deze niet door een afname van angiotensine II, maar door een ophoping van bradykinine wordt veroorzaakt. Gegevens over veranderingen in de bradykininespiegel in plasma zijn niet eensluidend, maar de meeste onderzoekers zijn het er over eens, dat deze na captopril niet toeneemt. Trouwens, het kallikreine-kinine systeem wordt over het algemeen meer als een locaal werkend dan als een circulerend hormoonsysteem beschouwd. Toename van de locale concentratie van kinines in bepaalde weefsels, behoeft dus niet te leiden tot stijging van kininespiegels in plasma. In dit verband is het van belang dat bij patiënten met hypertensie werd aangetoond dat de kininespiegels in de urine stegen na converting enzyme remming. Ook is het mogelijk dat een locale toename van bradykinine na converting enzyme remming leidt tot toename van het vaatverwijdende en natriuretische prostaglandine, PGE_2. Dit is zowel bij patiënten met hypertensie als in kweken van geïsoleerde niermergcellen aangetoond. In deze richting wijzen ook waarnemingen dat geneesmiddelen die de protaglandinesynthese remmen de cardiovasculaire effecten van converting enzyme remming ten dele tegen gaan.

Effecten op organen die bij de regeling van de bloeddruk zijn betrokken. Een afname van angiotensine II in plasma verlaagt de bloeddruk door een lagere bezettingsgraad van postsynaptische angiotensine II receptoren in de vaatwand en daardoor verminderde vasoconstrictie. Afname van plasma angiotensine II leidt ook tot een verminderde bezettingsgraad van angiotensine II receptoren in de bijnierschors en zo tot een afname van de secretie van aldosteron. Dit laatste draagt niet bij tot het acute effect van captopril op de bloeddruk, maar kan een rol spelen bij het chronische effect, een gedachte die gesteund wordt door de door sommige onderzoekers gevonden afname van plasma aldosteron en door onze waarneming dat bij patiënten met essentiële

hypertensie het extracellulaire vloeistofvolume afneemt onder chronische behandeling met captopril.
De afname van circulerend angiotensine II kan de neurogene vasoconstrictie beïnvloeden door een verminderde bezettingsgraad van presynaptische angiotensine II receptoren op sympathische zenuwuiteinden. Captopril passeert de bloed-hersen barrière en afname van neurogene vasoconstrictie kan dus ook door intracerebrale converting enzyme remming worden veroorzaakt. Uit studies met "gepende" ratten en met spontaan-hypertensieve ratten en uit proeven met geïsoleerde vaatpreparaten en rattenieren komen argumenten naar voren zowel voor een centraal effect op de activiteit van het sympathische zenuwstelsel als voor een perifeer, presynaptisch, effect op de sympathische prikkeloverdracht. Bij de mens is aangetoond dat een enkele gift van 50 mg captopril de bloeddrukverhoging na intraveneus toegediend noradrenaline vermindert. Bij onze hypertensiepatiënten daalde de plasmaspiegel van noradrenaline onder captopril niet en werden ook de reflexmatige stijgingen van de hartfrequentie en het plasma noradrenaline bij houdingsverandering niet beïnvloed. Dit sluit echter een effect van captopril op de activiteit van het sympathische systeem of op de sympathische prikkeloverdracht niet uit.
Uit het door ons verrichte gescheiden nierfunctie onderzoek blijkt dat converting enzyme remming leidt tot vaatverwijding in de nier, waarschijnlijk vooral door afname van de postglomerulaire weerstand. Tevens blijkt dat locaal in de nier gevormd angiotensine II of circulerend angiotensine II een belangrijke functie heeft voor het handhaven van een normale glomerulaire filtratiesnelheid, vooral in een nier met een stenotische arterie. Interruptie van dit mechanisme door captopril kan bij sommige patiënten met een nierarteriestenose tot nierinsufficiëntie leiden.
Er is dus sprake van een onverwacht dilemma. Oorspronkelijk zijn de angiotensine-converting enzyme remmers ontwikkeld voor de behandeling van die vormen van hypertensie, waarbij de plasmaspiegels van renine en angiotensine II verhoogd zijn. Het betreft hier vaak patiënten met een renale hypertensie en een reeds gestoorde nierfunctie of patiënten met een nierarteriestenose. Nu blijkt echter dat behandeling met een converting enzyme remmer niet minder effectief en zeker veiliger is bij patiënten met ongecompliceerde essentiële hypertensie met een normaal of zelfs laag renine in plasma.

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Naschrift

Het in dit proefschrift beschreven onderzoek toont dat onderdeel van het werk van de hypertensie werkgroep van de afdeling Inwendige Geneeskunde I, waarvoor ik tot mijn vertrek naar Enschede in eerste aanleg verantwoordelijk was. Onder de enthousiaste leiding van Maarten Schalekamp en in hechte samenwerking met Gert Jan Wenting, Arie Man in 't Veld, Frans Derkx, Frans Boomsma en Lies Tan-Tjiong was het mogelijk deze zeer uitgebreide en arbeidsintensieve studies te doen. Ook de steun van vele collegae van de afdeling, met name van Jeroen Vincent en Arend Jan Woittiez heeft hiertoe bijgedragen.
Zonder de hulp van Gusta, Gertrud, Carola en Paulien in en rondom de onderzoekcentrale zou dit werk niet mogelijk zijn geweest; met name door de wijze waarop zij de patiënten bij deze vaak zeer langdurige onderzoeken hebben opgevangen en begeleid hebben zij veel aan het resultaat van deze studies bijgedragen. Dankzij het werk van René, Jeannette, Marjan, Diny en Manorma kwamen de vele renine, aldosteron, noradrenaline en converting enzyme bepalingen tot stand.
Mijn speciale dank gaat uit naar collega I.M. Schicht, afdeling nefrologie Academisch Ziekenhuis Leiden, voor haar hulp bij het bestuderen van de anefrische patiënten en naar prof. dr. J. de Graeff, die ons de gelegenheid gaf dit op zijn afdeling te doen.
Dank ben ik ook verschuldigd aan de afdeling Radiologie (hoofd: prof. K. Hoornstra) en met name aan collega A.J. van Seijen voor het vele werk bij de niervene catheterisaties en aan de afdeling Nucleaire Geneeskunde, met name P.P.M. van der Kooij voor het vervaardigen en beoordelen van de nierscans en aan de verpleegkundigen van de afdeling Intensive Care (hoofd: prof. dr. C. Hilvering) en van de afdeling Coronary Care (hoofd: prof. P.G. Hugenholtz) voor hun hulp bij de Swan-Ganz catheterisatie studies.
Veel dank ben ik verschuldigd aan Marina Monté en Herma Ardesch voor al hun werk bij het vervaardigen van het manuscript. De fraaie illustraties werden verzorgd door Carla Swaab.
Ik ben Mr. J.C. van Rooijen en dr. J.F. Hill van E.R. Squibb and Sons Inc. erkentelijk voor het beschikbaar stellen van captopril.
De patiënten die zoveel onderzoek ondergingen ben ik erkentelijk voor hun medewerking en vertrouwen.
Ingeborg's gastvrijheid heeft aan de kwaliteit van het proefschrift wezenlijk bijgedragen. Zonder Marianne's loyaliteit en humor zou dit proefschrift waarschijnlijk nooit tot stand gekomen zijn.

Curriculum vitae

J.H.B. de Bruijn werd op 27 juli 1946 geboren te Leiden. In 1965 deed hij eindexamen Gymnasium-bèta aan het Leids Stedelijk Gymnasium. In dit zelfde jaar begon hij zijn studie in de Geneeskunde aan de Rijksuniversiteit te Leiden. In 1970 behaalde hij het doctoraal examen en in 1973 werd het arts-examen afgelegd. Tijdens zijn studie was hij van 1970 tot 1973 als student-assistent werkzaam bij de Stichting Eurotransplant te Leiden (hoofd: prof. dr. J.J. van Rood). Na het vervullen van de militaire dienstplicht welke werd doorgebracht op het Longobservatiecentrum van het Militair Hospitaal dr. A. Mathijsen (hoofd: dr. S.S.T. Feenstra), begon hij in 1974 zijn opleiding tot internist in het Rode Kruis Ziekenhuis te Den Haag (opleiders: dr. J. Roos, dr. W.S. Cost). In 1976 vervolgde hij de opleiding in de afdeling Interne Geneeskunde I van het Academisch Ziekenhuis Rotterdam "Dijkzigt" (hoofd: prof. dr. J. Gerbrandy).
Na zijn inschrijving in het Specialisten Register op 1 juni 1979 was hij werkzaam in deze afdeling als wetenschappelijk hoofdmedewerker in de functie van chef de policlinique. Sinds 1981 is hij werkzaam als internist in het Ziekenhuis de Stadsmaten te Enschede.

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