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STUDIES ON THE INHIBITORS OF ANGIOTENSIN

CONVERTING ENZYME

A thesis presented to the University of
Glasgow, Faculty of Medicine, for the
degree of Doctor of Medicine by

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SUMMARY

Several aspects of the pharmacological properties, therapeutic efficacy, side-effects and tolerability of captopril and two new converting enzyme inhibitors have been examined.

CAPTOPRIL

(a) Therapeutic efficacy

The long-term use of captopril has been examined in 70 patients with severe hypertension resistant to previous conventional antihypertensive therapy. Good blood pressure control was achieved in nearly all patients, with essential and secondary forms of hypertension, but most required large doses of loop diuretic. Diuretic-induced tachycardia occurred in one third of patients but there were few instances of electrolyte disturbance or deterioration in renal function.

(b) First dose hypotensive effect.

In this study, 8% of 65 severely hypertensive patients sustained an acute reduction of mean arterial pressure in excess of 50% within two hours of receiving captopril. Six patients developed symptoms of acute hypotension, including dizziness, stupor, dysphasia and hemiparesis. Analysis of several pre-treatment variables, including renal function, serum sodium and severity of blood pressure, did not permit consistent prediction of a

severe first dose effect in individual patients.

(c) Side-effects

In a study of 100 consecutive patients treated with captopril, a 20% incidence of toxic side-effects was encountered. Analysis shows that the serious side-effects of proteinuria and neuropathy occurred only in patients with poor renal function who received high doses of captopril. There was no evidence in this study that the minor side-effects of taste loss and skin rash were related to dose or renal function, and occurred occasionally even when captopril dosage was low.

(d) Tolerability and mood change.

Despite the high incidence of adverse reactions and side-effects, captopril was well accepted. Many patients commented on an enhanced sense of well-being while taking captopril when compared with their previous antihypertensive therapy. A possible euphoriant property of captopril was thus examined in a placebo-controlled trial in 8 patients with moderate hypertension. This showed no evidence of mood enhancement while taking captopril, indeed there was statistically significant lowering of mood when compared with placebo. It is likely that the well-being experienced while taking captopril reflects the absence of mood lowering side-effects of previous therapy.

ENALAPRIL (MK421) AND LYSINE ANALOGUE

(a) Pharmacological properties.

The immediate and long-term effects of these new inhibitors on blood pressure, serum electrolytes, renin-angiotensin system and sodium balance have been assessed and compared in a placebo-controlled study of 12 healthy subjects. In a dose of 10 mg once daily, MK421 and MK521 were shown to be potent inhibitors with a long duration of action. Both inhibitors fully suppressed converting enzyme activity and plasma angiotensin II 6 hours after administration but the effects of MK521 were more long-lasting.

In this study in which sodium intake was fixed, a significant natriuresis occurred during the administration of both drugs, although this effect had largely disappeared after 8 days. Both drugs were well-tolerated and there were no serious side-effects.

(b) Therapeutic efficacy and tolerability.

The therapeutic effects of enalapril have been assessed in a long-term study of 20 hypertensive patients with renal artery stenosis. Ten patients had previous therapy withdrawn and detailed measurements of changes in blood pressure, serum and body electrolytes, and components of the renin-angiotensin system were made. In doses of 10-40 mg once daily there was

sustained suppression of plasma angiotensin II and all patients achieved good blood pressure control. Nine patients had evidence of a significant natriuresis with a fall in total exchangeable sodium. All patients had a small but significant rise in serum creatinine but none suffered marked renal impairment.

Ten further patients (five with bilateral renal artery stenosis) were too ill to be studied to the above protocol. All were started on enalapril and previous therapy was then gradually removed when possible. Even in this group with many adverse features, excellent blood pressure control was achieved by all patients and only three required additional therapy. There were no cases of deterioration in renal function even in the patients with bilateral arterial disease.

Enalapril was well-tolerated by all patients and there were no serious side-effects.

(c) Effects on baroreflex function.

The effects of captopril and enalapril on α_1 -adrenoreceptor and baroreflex function have been compared in a study of 6 healthy volunteers. By comparing the pressor responses to infused phenylephrine, no effects on α_1 -receptor function were demonstrated. There was, however, evidence of significant baroreflex re-setting, though not impairment, which was equal with both drugs. This phenomenon probably explains the lack

of postural tachycardia during converting enzyme inhibition.

CONCLUSION

Converting enzyme inhibitors appear to be effective and well-tolerated antihypertensive agents. Captopril, the first orally active inhibitor, has several side-effects which limit its use, even when low doses are used. The new inhibitors, enalapril (MK421) and lysine analogue (MK521), have a longer duration of action and lack a toxic sulphhydryl group. They appear to be promising therapeutic agents but further testing is required to exclude significant toxicity.

CHAPTER 1

INTRODUCTION AND AIMS OF THESIS

INTRODUCTION

If a survey is made of casual blood pressures in a given population sample it is seen that both systolic and diastolic pressures are distributed in a unimodal fashion. This means that the frequency distribution of the different blood pressure values follows a smooth, roughly bell-shaped curve. It is evident that there is no clear demarcation between "hypertension" and "normal" blood pressure; any attempted definition of "hypertension" in terms of absolute blood pressure readings is thus necessarily arbitrary.

Nevertheless, numerous epidemiological studies, as well as the data of insurance companies, have emphasised that the risk of various cardiovascular complications is increased in direct proportion to the height of the casual arterial pressure. It is for this reason that we are concerned to identify those members of the population who have blood pressures towards the upper end of the distribution curve, and to bring these people with raised blood pressure under treatment.

The impressive fall in mortality rates for hypertensive diseases since the 1950's in many countries

must be largely attributed to the drug treatment of severe and moderate hypertension. In addition there is increasingly clear evidence that the treatment of mild, uncomplicated hypertension can prevent the appearance of a variety of hypertension-related cardiovascular complications. A good case can now be made for preventive antihypertensive therapy in adults with Vth phase diastolic pressures persistently above 95 mmHg. This realisation has produced a corresponding need for effective drugs, with few side-effects; agents such as guanethidine, reserpine, methyldopa and clonidine are less readily accepted in this context. Beta-adrenergic blocking agents, thiazide diuretics, prazosin and hydralazine are generally better tolerated, although not free from unwanted effects. Thus the addition to the therapeutic armoury of a new class of antihypertensive is to be welcomed.

In 1898 the Finnish physiologist Robert Tigerstedt initiated the research which has led to our present understanding of the renin-angiotensin-aldosterone system and its role in the regulation and maintenance of blood pressure and sodium homeostasis.

The use in recent years of inhibitors of the system has both increased our knowledge of the precise mechanisms of the system and has led to the development of a new concept in antihypertensive therapy - the inhibition of angiotensin I converting enzyme.

The development of captopril, the first orally active converting enzyme inhibitor represents a triumph of skilled and purposive drug research. In the last 5 years it has shown distinct promise as a therapeutic agent: its undoubted therapeutic value has however been associated with several side-effects and adverse reactions which have limited its use.

The search for better tolerated converting enzyme inhibitors has led to the recent production of several drugs of potential therapeutic value with many more in earlier stages of development. If these agents are shown to have the efficacy of captopril, without the spectrum of side-effects, then they might well challenge our present concepts of the drug management of mild hypertension, with implications for millions of patients. The therapeutic potential of converting enzyme inhibition has been established - the search proceeds for the inhibitor which is both potent and tolerable.

AIMS OF THESIS

In this thesis I shall present work on two new converting enzyme inhibitors, enalapril maleate (MK421) and its lysine analogue (MK521). Particular attention will be paid to the magnitude and duration of their effects on blood pressure, baroreceptor function and the renin-angiotensin system in normal man. Their therapeutic efficacy and tolerability will be assessed in a

study of 20 patients with hypertension and renal artery stenosis.

These new inhibitors must be assessed against a background of the enormous knowledge and experience gained from the earlier development of captopril. The development of captopril depended in turn on a detailed understanding of the functions and interactions of the renin-angiotensin system in health and disease.

Thus, a general discussion of the renin-angiotensin system and the evolution of drug inhibitors is followed by a detailed description of the development and therapeutic uses of captopril. The development of enalapril is also discussed where appropriate.

As will be seen, there are many aspects of the use of captopril which require clarification and this includes the major problem of tolerability. Some of these questions are examined in this thesis and are outlined in the introduction to each chapter.

Studies on enalapril and its lysine analogue are then presented, and although direct experimental comparisons are not made, some attempt will be made to see the new drugs in the light of experience gained with the old.

CHAPTER 2

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

I. THE RENIN-ANGIOTENSIN SYSTEM

In 1898 Tigerstedt and Bergman, exploring the known association between kidney disease and elevated blood pressure, observed a rise in blood pressure when saline extracts of fresh rabbits' kidneys were injected intravenously into other rabbits; they called the pressor extract renin⁽¹⁾. Bingel, Strauss and Claus in 1909 and 1910^(2,3) confirmed these findings with renal press juice which was inactivated by alcohol, acids and alkalis. Many workers then failed to find evidence of a pressor substance in normal kidneys and considerable doubt as to the existence of renin was raised; it was assumed that the pressor substances found by some investigators were the products of tissue autolysis, or of bacterial action^(4,5). Then in 1934, Goldblatt, in his classical experiments in dogs, produced sustained hypertension by renal artery constriction⁽⁶⁾. This, and the work of Pickering and Prinzmetal^(7,8), re-awakened interest in the role of renin in the physiological control of blood pressure in health and disease. It was quickly shown that application of an arterial clip to transplanted kidneys^(9,10) or local denervation⁽¹¹⁻¹³⁾, failed to prevent the development of hypertension. This further supported the idea of a pressor extract and then,

in 1939, Helmer and Page prepared a renin solution from pig kidney cortex that was free of depressor material. When this renin preparation, after dialysis, was added to the fluid being perfused through an amputated dog's tail, it caused no vasoconstriction^(14,15) and the factor in plasma which reacted with renin was called renin activator. It became apparent that renin was an enzyme and not a direct pressor substance.

It is now known that renin exists in the kidney, in the circulation and bound to vascular walls. It exists in inactive form with a molecular weight of around 55,000 and in active form with a molecular weight of around 40,000; conversion to active form is thought to occur by removal of a smaller inactivator molecule by a serine protease. Activation in vitro occurs following treatment with acid or trypsin or exposure to cold and depends upon factor XII, prekallikrein and possibly plasmin⁽¹⁶⁻²⁰⁾.

In 1939, the search for the active substance resulting from the action of renin in plasma in vitro culminated in the extraction by Page and Helmer^(14,21) of a potent vasoconstrictor and pressor substance which they called angiotonin. Independently, Braun-Menendez⁽²²⁾ discovered the same factor which he named hypertensin; for the substrate in the plasma he used the term hypertensinogen. In 1958 the problem of nomenclature was resolved by a joint communication in which they suggested the new name angiotensin⁽²³⁾. Currently the dual nomen-

clature , angiotensinogen and renin-substrate, is still being used for the substrate upon which renin reacts, an α_2 -globulin synthesised in the liver.

In 1954, Skeggs and coworkers⁽²⁴⁾ found that angiotensin existed in two forms that were separable by counter current distribution. The product obtained from incubations of renin with its plasma substrate in the presence of chloride was named angiotensin II, that obtained in the absence of chloride was called angiotensin I. By studies in artificially perfused kidney and on the isolated aortic strip⁽²⁵⁾ it was then shown that angiotensin I lacked direct vasoconstrictor action.

The enzyme responsible for conversion of the inactive decapeptide angiotensin I to the vasoconstrictor octapeptide angiotensin II was partially purified from horse plasma by Skeggs⁽²⁶⁾ and given the name angiotensin converting enzyme. The enzyme appeared to contain a tightly bound functional metal ion, since it was inhibited by metal chelating agents such as EDTA, but lost no activity upon dialysis.

Interest in converting enzyme waned until the late 1960's when synthetic angiotensin I became available. Ng and Vane^(27,28) showed that although significant conversion of angiotensin I to angiotensin II occurred in plasma, it occurred mainly in transit through the lungs. Quantitative and specific spectrophotometric

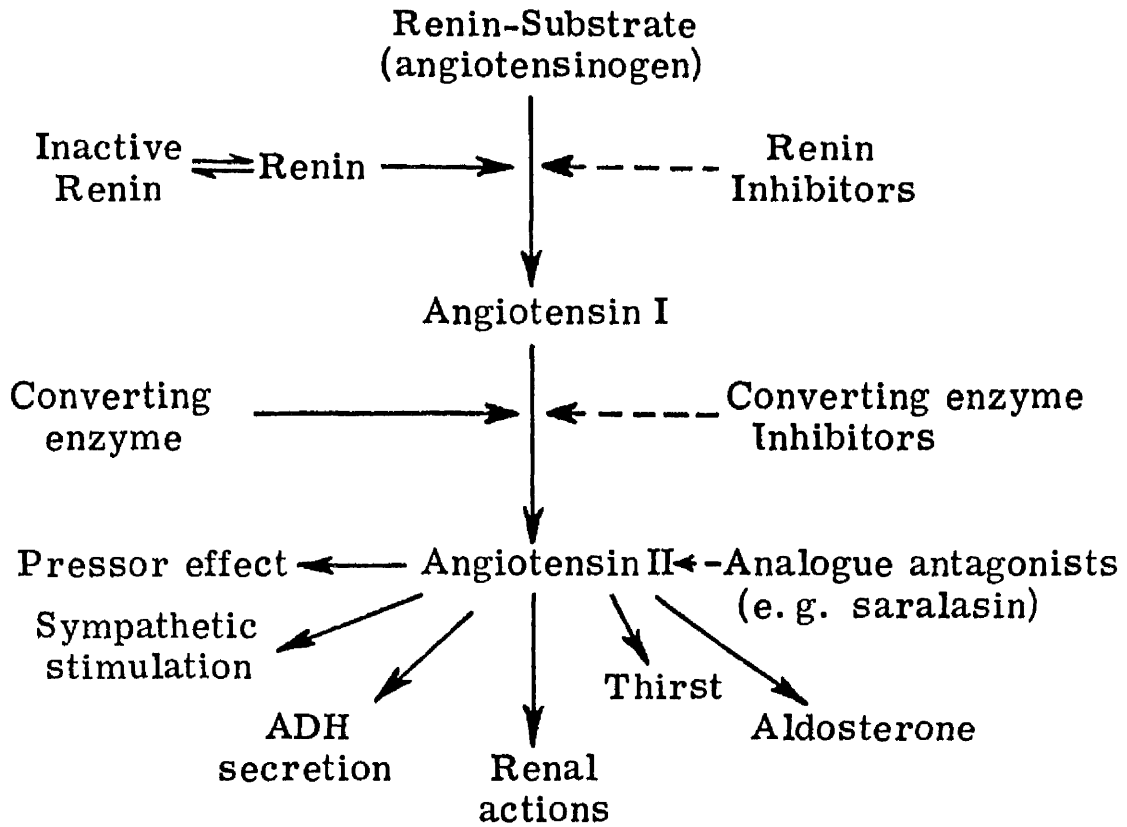


Figure 1.

Components and actions of the renin-angiotensin system.

and fluorometric assays of angiotensin converting enzyme activity in tissue extracts were then developed^(29,30). It has subsequently been shown⁽³¹⁾ that converting enzyme is identical to the enzyme kininase II which inactivates the peptide bradykinin. These aspects are discussed in depth in later chapters describing the development of inhibitors of the renin-angiotensin system.

II. ACTIONS OF THE RENIN-ANGIOTENSIN SYSTEM

The enzyme renin, stored in cytoplasmic granules of the renal afferent arteriolar cells, reacts with its substrate in the alpha-2 fraction of plasma. The decapeptide product of this reaction is angiotensin I, probably largely, if not entirely, devoid of physiological actions.

Angiotensin I is converted in the circulation to the active octapeptide angiotensin II which has been shown to have a wide variety of pharmacological effects (32-35) (Figure 1).

(a) Direct pressor action.

Angiotensin II acts directly on receptors on the smooth muscle vascular cell to produce immediate vasoconstriction⁽³⁶⁾; this direct contractile effect displays the phenomenon of tachyphylaxis which is probably due to receptor occupation by angiotensin⁽³⁷⁻⁴⁰⁾. There

are also indirect neurogenic effects of angiotensin but these, at least in acute studies, seem to play a relatively minor role^(41,42) (see below).

In addition angiotensin exhibits a slow pressor effect; infused in small doses over long periods, it not only raises blood pressure but also resets its own dose-response curve⁽⁴³⁾. This has important implications when considering the long-term hypotensive effects of angiotensin inhibitors.

(b) Renal actions.

The effects of angiotensin II on the kidney have been determined by intravenous infusion studies and the responses while complex are consistent, although there is some species variation.

When low doses of angiotensin II are infused in man there is a decrease in renal blood flow, a lesser decrease in glomerular filtration rate (and hence an increase in filtration fraction), and a decrease in urine flow and sodium excretion even when the pressor response is not marked. These changes are enhanced with increasing infusion rates and the changes are proportional to the log of the dose⁽⁴⁴⁻⁴⁸⁾. The decrease in diuresis and natriuresis may be entirely due to the changes in renal haemodynamics but an effect on the tubules has been suggested⁽⁴⁹⁾. Despite these effects the kidney retains

its ability to autoregulate even when renal blood flow is reduced by up to 50%⁽⁵⁰⁻⁵¹⁾.

Infusions of large doses of angiotensin in experimental animals elicits a complex series of responses (52,53). The initial marked renal vasoconstriction and fall in urine flow is replaced within minutes by a diuresis and natriuresis which does not appear to be related to the pressor response⁽⁵⁴⁻⁵⁹⁾. These observations and the finding that there are obvious increases in the tubular rejection fraction of sodium suggests that high doses of angiotensin are capable of causing a decrease in the tubular reabsorption of sodium which is not accounted for by haemodynamic alterations^(60,61).

(c) Aldosterone secretion.

In 1960, it was discovered, firstly, that a potent aldosterone-stimulating hormone was secreted by the kidney^(62,63) and, secondly, that synthetic angiotensin II increased the rate of aldosterone secretion and excretion in man⁽⁶⁴⁻⁶⁶⁾.

In addition to angiotensin II, aldosterone secretion is altered by corticotrophin (ACTH) and sodium or potassium depletion or loading⁽⁶⁷⁾. Exogenous angiotensin II appears to inhibit ACTH release⁽⁶⁸⁾.

(d) Sympathetic and central nervous system.

Stimulation of suprapontine structures⁽⁶⁹⁾ and the area postrema in the lower medulla⁽⁷⁰⁾ with infused angiotensin II causes a pressor response. In addition, angiotensin stimulates thirst⁽⁷¹⁾ and the release of vasopressin⁽⁷²⁾. However, the pressor effects are only observed in relatively large doses and have only minor effects on blood pressure when infused into the carotid artery. The long-term effects on fluid balance mediated by thirst and vasopressin stimulation could be of great importance in long-term blood pressure regulation through alteration of the renal threshold of fluid excretion in relation to blood pressure⁽⁷³⁾.

At doses which are not directly pressor angiotensin has effects on the peripheral sympathetic nervous system with facilitation of transmission in sympathetic ganglia; enhancement of biosynthesis and release, with inhibition of reuptake, of noradrenaline at sympathetic nerve endings^(41,42). This is fully discussed in a later chapter on the mechanism of action of the angiotensin inhibitors.

III. ANTAGONISTS AND INHIBITORS OF THE SYSTEM

(a) Renin antagonists.

Renin has no known physiological effect but acts only to cleave its substrate, angiotensinogen. The

complete purification of pig^(74,75), canine⁽⁷⁶⁾ and human⁽⁷⁷⁻⁷⁹⁾ kidney renin has now been accomplished. Once renin is purified, its unique structural features may be recognised by specific antibodies and antibodies to dog renin have been raised⁽⁸⁰⁾. However, even though the renin-specific antibody used in these studies has been shown to have a very high degree of selectivity, it seems unlikely that the prolonged or repetitive use of antibodies in man will be possible.

Consequently an agent has been sought with the potential of high selectivity as well as compatibility with human use. Renin is a remarkably fastidious protease in its substrate specificity and it was hoped that the unique sequence of amino-acids around the cleavage site could be used to construct a very specific enzyme inhibitor. Skeggs⁽⁸¹⁾ in 1968 defined the minimum octapeptide sequence from natural protein substrate that interacts strongly with renin; it was also the smallest fragment of the substrate still to be cleaved by renin at a significant rate.

Kokubu⁽⁸²⁾ synthesised a tetrapeptide ester which acted as a weak competitive inhibitor in vitro of rabbit renin although it was inactive in vivo; the first in vivo inhibitor of renin^(83,84) was a tetrapeptide which possessed only modest inhibitory potency (IC_{50} 800 μ M). Then more potent in vitro inhibitors of renin based on an octapeptide were developed but some of these were

inactive in plasma and all were devoid of activity in vivo^(85,86).

Haber and his colleagues⁽⁸⁷⁾ have described a decapeptide derived from equine substrate as an effective inhibitor of renin in vitro and in vivo in primates. Leckie and coworkers⁽⁸⁸⁾ have synthesised a new octapeptide inhibitor of canine renin, H77, which is effective in vivo and in vitro and active in normal and hypertensive human plasma (IC_{50} 0.027 μ M). Infusion studies in dogs have shown reduced circulating concentrations of angiotensin I and angiotensin II and reduced mean arterial pressure in proportion to changes in plasma angiotensin II.

Very recently, Szelke and Leckie⁽⁸⁹⁾ have produced a further series of highly active and species-specific in vitro inhibitors of endogenous human renin cleaving renin substrate in human plasma. The octapeptide H-112 of human angiotensinogen is only a weak inhibitor of human plasma renin (IC_{50} 313 μ M), the analogue H-113 in which the scissile Leu-Val bond has been reduced has IC_{50} 0.19 μ M. Extension of H-113 at the N- and C-termini, giving the decapeptide H-142, has increased the inhibitory potency further (IC_{50} 10 nM). H-113 and H-142 are strongly species specific, and are highly specific for renin among acid proteases: H-142 has no significant inhibitory effect in vitro on human cathepsin-D or renal acid protease.

These extremely interesting inhibitors will hopefully shed light upon many unanswered questions about the biosynthesis, physiology and pathophysiology of renin; therapeutic applications in man are as yet an exciting possibility only.

(b) Aldosterone antagonists.

At least four stimuli govern aldosterone secretion, angiotensin II, corticotrophin (ACTH) and the balances of sodium and potassium^(66,67). Normally each stimulus is kept in bounds by negative feedback.

Spiro lactone.

Kagawa⁽⁹⁰⁾ found that spironolactone blocked the fall in urinary sodium/potassium ratio seen in adrenalectomised rats injected with aldosterone. Liddle⁽⁹¹⁾ showed that spironolactone also blocked the renal effects of aldosterone in man. The findings of these workers suggested that spironolactone acts on the renal tubules as a reversible competitive inhibitor. Salassa⁽⁹²⁾ gave spironolactone to patients with primary hyperaldosteronism and reported an increase in urinary sodium excretion and potassium retention; plasma potassium rose and there was a fall in the exchangeable sodium/exchangeable potassium ratio. Other workers confirmed these acute metabolic effects^(93,94) and spironolactone was shown to produce a fall in exchangeable sodium and in plasma volume^(95,96) in extracellular fluid volume

and in body water⁽⁹⁵⁾ as well as a rise in exchangeable potassium^(95,96) in patients with primary hyperaldosteronism. Mobley⁽⁹⁷⁾ and Flanagan⁽⁹⁸⁾ reported that prolonged usage resulted not only in reversal of the electrolyte abnormalities but also in a fall in blood pressure.

Effective in primary aldosteronism it has a more modest hypotensive effect in essential hypertension. A small but perceptible hypotensive effect is noted in low-renin hypertension where aldosterone levels are not raised in absolute terms but may be raised in relation to exchangeable sodium⁽⁹⁹⁾. In hypertension with secondary hyperaldosteronism and sodium depletion, spironolactone is ineffective in lowering blood pressure though it does correct hypokalaemia.

Trilostane.

Trilostane is not strictly an aldosterone inhibitor but a reversible competitive inhibitor of 3 β -hydroxysteroid dehydrogenase/ Δ 5-3-oxosteroid isomerase which converts pregnenolone to progesterone in the biosynthetic pathway of aldosterone. Accumulation of precursors of adrenal steroids does not complicate therapy because pregnenolone and those of its derivatives which occur before 3 β -HSD blockade have no significant physiological action. Used mainly in the treatment of Cushing's syndrome it has also been used in primary aldosteronism and low-renin hypertension. Variable

falls in blood pressure are associated with a fall of plasma aldosterone and a rise in serum potassium and plasma renin activity ⁽¹⁰¹⁾.

(c) Angiotensin II antagonists.

Saralasin (1-sar-8-ala-angiotensin II) is the most significant of several synthetic analogues of angiotensin II which, administered intravenously, act as specific antagonists but which also have intrinsic agonist activity. For several years following its development in 1970, it was widely used experimentally to assess the effects of the renin-angiotensin system in the maintenance of blood pressure ^(104,105).

However, while its agonist properties are markedly less than angiotensin II, the agonist effects become significant when plasma levels of endogenous angiotensin II are low. Indeed Brown ⁽¹⁰⁶⁾ has shown it to have similar properties to angiotensin II when infused long-term in rats with a slow rise and increased diurnal variation in blood pressure. Thus it has little value in the investigation of the renin-angiotensin system in the large majority of hypertensive patients, who have low levels of angiotensin II and no depressor response to the drug ^(107,108).

Conversely, when levels of endogenous angiotensin II are high, the agonist properties of saralasin become

relatively insignificant. It has thus provided much useful information to the investigation of the mechanisms of hypertension in experimental and human renal artery stenosis, often associated with high levels of circulating renin and angiotensin⁽¹⁰⁹⁻¹¹¹⁾.

It is now of lesser value, as an experimental or therapeutic agent, having been largely superseded by the orally active inhibitors of converting enzyme.

(d) Converting enzyme inhibitors.

Before 1968 the only known inhibitors of angiotensin converting enzyme were non-specific metal chelators such as EDTA and phenanthroline. Earlier, Ferreira⁽¹¹²⁾ showed that a non-toxic ethanol extract of the venom of the Brazilian arrowhead viper, *Bothrops jararaca*, potentiated smooth muscle contraction, hypotension and increased capillary permeability induced by bradykinin. Various properties of this 'bradykinin potentiating factor' (BPF) indicated that it was a peptide or mixture of peptides which appeared to inhibit the enzymatic degradation of bradykinin in plasma⁽¹¹³⁾. Several years later it became clear that angiotensin converting enzyme was the bradykininase inhibited by BPF. Ferreira⁽¹¹⁴⁾ and Ondetti⁽¹¹⁵⁾ isolated several venom peptide inhibitors of angiotensin converting enzyme leading to the development of the biologically stable synthetic nonapeptide, teprotide (SQ 20881).

Teprotide.

The pentapeptide BPP_{5a} was found to be the most potent venom peptide inhibitor of angiotensin converting enzyme of rabbit lung^(116,117) but its use was limited by biological instability and a very short duration of activity. The nonapeptide SQ 20881 (teprotide) was a slightly less potent inhibitor of the rabbit lung converting enzyme but it was shown to be stable and effectively inhibited the vasopressor action of angiotensin I and augmented the vasodepressor action of bradykinin^(118,119). It was also the most effective of the venom peptides for lowering blood pressure in animal models of hypertension, and was eventually shown to be markedly effective for treatment of human hypertension when administered parenterally⁽¹²⁰⁻¹²⁴⁾. Acute reductions of blood pressure were associated with a fall in plasma angiotensin II in animals⁽¹²⁵⁾ and man^(126,127), plasma aldosterone was also shown to fall, though not consistently^(126,128). In hypertensive patients the fall in blood pressure was found to be in proportion to the pre-treatment plasma renin activity^(124,126,128-130).

As discussed above, converting enzyme was shown initially to be a bradykininase and inhibitors were able to potentiate the hypotensive effects of exogenous bradykinin. The significance of this effect in the hypotensive effects of teprotide is still unclear, measurement of blood kinins has produced conflicting and

confusing information in man^(126,127) and animals^(131,132).

Captopril.

Although SQ 20881 (teprotide) was an effective antihypertensive drug its lack of oral activity restricted its use to diagnostic testing and hypertensive emergencies. Thus in 1974, the efficacy of ACE inhibitors as antihypertensive drugs had been demonstrated but the development of an orally effective agent for use in chronic therapy seemed many years away. This situation was dramatically altered by a new approach to the development of ACE inhibitors led by Cushman and his group⁽¹³³⁾.

Increased understanding of the chemical and enzymatic properties of angiotensin-converting enzyme made it possible to design simple non-peptide molecules that would react with great affinity at the active site of this enzyme⁽¹³⁴⁻¹³⁶⁾. The substrate specificity and other properties of ACE suggested that it was a zinc metallopeptidase similar in mechanism to carboxypeptidase A, an enzyme whose active site had been well characterised by x-ray crystallography and other methods⁽¹³⁷⁾. Byers and Wolfenden⁽¹³⁸⁾ demonstrated that D-2-benzylsuccinic acid was a markedly potent competitive inhibitor of carboxypeptidase A. They postulated that this inhibitor was a "biproduct analog" that owed its great affinity for the active site of carboxypeptidase A to multiple binding interactions characteristic of the two products of carboxypeptidase

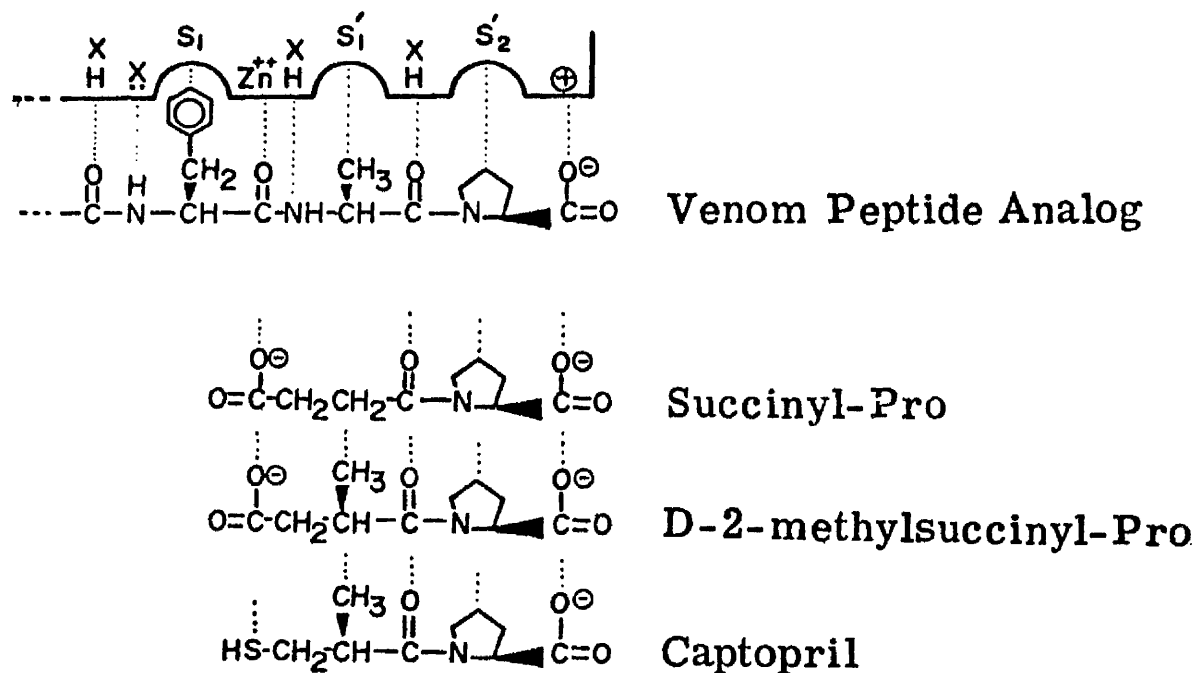


Figure 2.

- Hypothetical binding of competitive inhibitors to the active site of angiotensin-converting enzyme. S_1 , S'_1 and S'_2 are "subsites" that interact with side chains of terminal, penultimate and antepenultimate amino acids, respectively, of a peptide substrate or competitive inhibitor. X-H is a residue donating a hydrogen bond; X is a residue accepting a hydrogen bond (Adapted from Am J Cardiol, 1982, 49: 1393).

action. Applying similar principles, Cushman synthesised succinyl-L-proline (Figure 2) as a potential biproduct analog inhibitor of angiotensin-converting enzyme. It was only a modest inhibitor of the rabbit lung converting enzyme ($IC_{50} = 330 \mu M$), but it specifically inhibited the action of angiotensin I on guinea pig ileum strip and augmented that of bradykinin, actions characteristic of specific inhibitors of the enzyme^(135, 137). The addition of a D-2-methyl group, making the resulting inhibitor a biproduct analog of the tightly binding terminal dipeptide Ala-Pro of bradykinin-potentiating peptide 5a, enhanced inhibitory activity 15- to 20-fold; and replacement of the putative zinc binding 4-carboxyl function by sulphhydryl led to the extremely potent and specific competitive inhibitor captopril which had an IC_{50} value of $0.023 \mu M$.

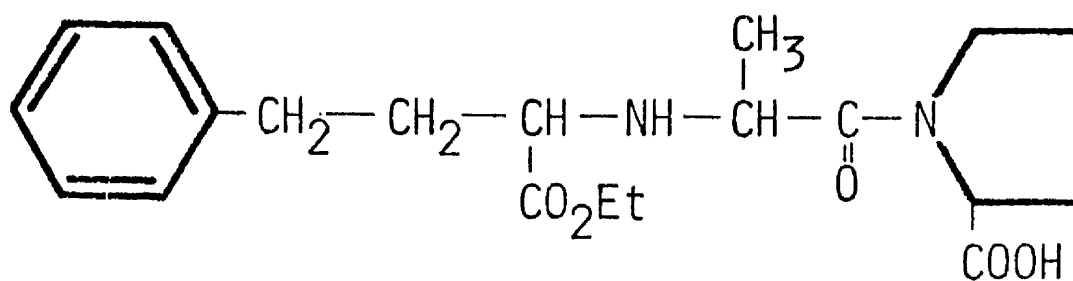
Five important binding interactions are postulated between captopril and the active site of angiotensin-converting enzyme (Figure 2); each has been studied extensively by comparing inhibitory activities of analogs with specific structural modifications⁽¹³⁴⁻¹³⁶⁾. Four of captopril's active-site binding interactions are essentially the same as those postulated for the terminal dipeptide residue Ala-Pro of the most favourable analog of the snake-venom peptides. But captopril, unlike these venom peptides, cannot be cleaved by angiotensin converting enzyme, and the interaction of its sulphhydryl

group with the enzyme-bound zinc ion contributes greatly to its overall enzyme binding affinity. Captopril is an extremely potent competitive inhibitor, with a K_i value (enzyme dissociation constant) of 2×10^{-9} M. It is markedly specific for inhibition of angiotensin converting enzyme both in vitro and in vivo and is an orally effective antihypertensive drug in both animal models of hypertension and in hypertensive patients (136, 137, 139). However, the design and subsequent optimisation of chemical structure that led to captopril was based entirely on enzyme inhibitory activity and not on the antihypertensive action of intermediate compounds. Thus although the consequences of inhibition of angiotensin converting enzyme in vivo may be manifold and complex, it is highly probable that all antihypertensive actions of captopril have as a common denominator inhibition of this biologically important enzyme.

Enalapril maleate (MK421) and lysine analogue (MK521).

In 1976 captopril was the first orally active converting enzyme inhibitor, opening the way for long-term blockade of the renin-angiotensin system in the therapeutics of hypertension. Reference will be made in Chapter 3 to the effectiveness of captopril in the treatment of hypertension of various causes and severity. However, it rapidly became apparent that the undoubted therapeutic benefits were associated with a high incidence of side-effects which limited its use to patients who had failed to respond to conventional therapy.

MK 421



MK 521

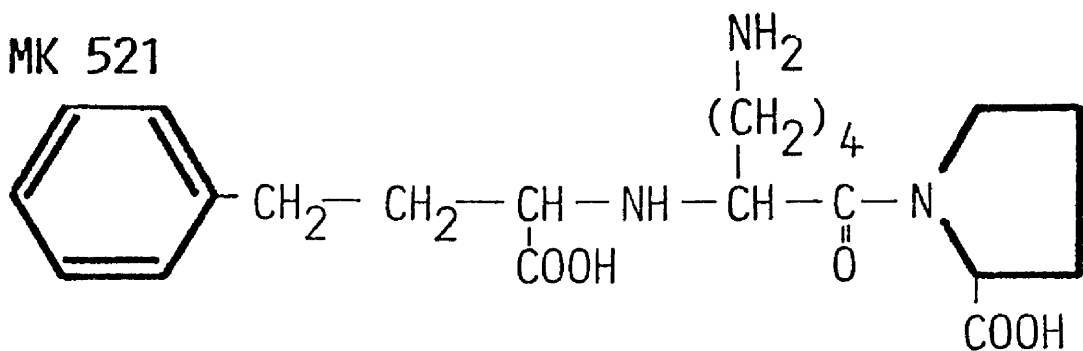


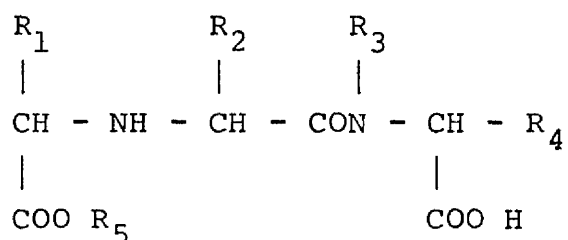
Figure 3.

The chemical structures of N-{(S)-1-(Ethoxycarbonyl)-3-phenylpropyl}-L-Ala-L-Pro (MK421) and lysine analogue (MK521).

It was therefore logical and desirable to seek new inhibitors which were both effective and well tolerated. Many of the side-effects of captopril are shared by penicillamine, which like captopril has an active sulphhydryl group in its molecule. As the active-site binding interactions of captopril were so well understood, it was quickly possible using computer modelling techniques to design numerous potential inhibitors which were devoid of a sulphhydryl group.

Many reached the level of animal testing before being discarded; a handful have survived for human evaluation. The two most promising compounds to emerge for detailed study in man have been developed in the Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey.

Workers in this centre have developed a novel series of substituted N-carboxymethyl-dipeptides^(140,141). These are transition state inhibitors which are active at nanomolar levels and based on the generic formula:



The biological profile of three of these inhibitors was assessed in rats and dogs by the antag-

onism of the pressor response to angiotensin I. High converting-enzyme inhibitory activities in vitro ($IC_{50} < 1.2 \times 10^{-6} M$) were confirmed after intravenous administration. Given orally in dogs they showed good activities with a duration of action of at least 6 hours.

Their long duration of action and apparent specificity thus made them potentially useful anti-hypertensive drugs in man and two were selected for evaluation in man. These were enalapril maleate (MK421) and its lysine analogue (MK521) (Figure 3).

Other orally active inhibitors.

At the time of writing two other converting enzyme inhibitors, RHC 3659^(142,143) and SA 446⁽¹⁴⁴⁾, are in the early stages of evaluation in man. Neither appears to share the potency or duration of action of enalapril and detailed discussion of their properties is outwith the scope of this thesis.

IV. THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN THE MAINTENANCE OF BLOOD PRESSURE

(a) • Normal man.

The renin-angiotensin system developed in early animals during adaptation from aquatic to terrestrial life; thus equipping them with a physiological system

to resist the effects of sodium deprivation, dehydration and haemorrhage⁽¹⁴⁵⁾. Later, it helped early man to assume the erect posture. This was made possible because of its unique dual controlling mechanism; the immediate pressor effects of angiotensin II complemented by long-term control of extracellular volume by regulation of aldosterone, thirst and ADH release. A detailed review of the role of the renin-angiotensin system in normal and hypertensive animals and man is given by Brown et al.⁽¹⁴⁶⁾.

At very low levels of sodium intake, kidney secretion of renin increases and plasma angiotensin II rises⁽¹⁴⁷⁻¹⁴⁹⁾. This prevents hypotension by its direct vasoconstrictor effect and by directly diminishing renal sodium excretion^(150,151). Conversely, when salt intake is excessive, the secretion of renin decreases markedly and the opposite effect occurs. However, if the angiotensin level in the blood is kept constant by continuous infusion of angiotensin II, changes in salt intake from very low to very high cause very large changes in arterial pressure. This demonstrates the very important active feedback mechanism that allows precise control of arterial pressure within narrow limits despite extreme changes in salt intake.

When an animal with a normally functioning renin-angiotensin system, but with a blocked nervous system,

haemorrhages severely, the arterial pressure can return partially to normal within a short time (155,156). However if the renin-angiotensin system is blocked, this recovery is largely prevented, demonstrating the importance of angiotensin in preventing sustained hypotension (157).

Evidence for the role of the system in normal subjects on a normal intake of sodium is more difficult to assess. Studies with graded infusions of angiotensin II with its assay in plasma show that normal circulating levels of angiotensin II do have a small effect on arterial pressure⁽¹⁴⁶⁾. Inhibitors of the system do lower blood pressure, though only slightly, in supine, sodium replete, normal man though the effects are more marked in the upright position⁽¹⁵⁸⁾. However, the relevance of this observation is open to question. It is possible that, in normal man, the effects of angiotensin suppression are within the range of compensation from other homeostatic mechanisms and an effect that can be masked by compensation through other mechanisms cannot be dismissed as unimportant.

(b) Essential hypertension.

• The role of the renin-angiotensin system, and of its inhibitors, has been extensively investigated in patients with essential hypertension with largely inconclusive results⁽¹⁵⁹⁻¹⁶²⁾.

Several authors have sub-classified essential hypertension on the basis of plasma renin and depressor responses to inhibitors. This approach has several flaws. Firstly, as plasma renin in patients with essential hypertension is distributed in an essentially smooth unimodal fashion, it is apparent that dividing lines for classification into 'low' and 'high' renin hypertension are arbitrary. In addition plasma renin levels are partly dependent on sodium intake which must be known to permit classification⁽¹⁵⁹⁾. Having considered these factors, simple assay of plasma renin and angiotensin II does not provide complete information on the pressor effects of the system, which also requires knowledge of the individuals' angiotensin II dose-response curve⁽¹⁴⁶⁾.

Similarly, studies using inhibitors of the system must be assessed with caution. Blood pressure fall is directly related to the pre-treatment circulating plasma renin and angiotensin II but most hypertensive patients have unremarkable values⁽¹⁴⁶⁾. In addition, it is almost certain that converting enzyme inhibitors have antihypertensive mechanisms in addition to reduction of angiotensin II⁽¹⁶³⁻¹⁶⁶⁾. Saralasin, as previously discussed, has agonist effects which become significant when circulating levels of angiotensin II are low.

Finally, differences in natural history or response to treatment between the sub-groups of essential hypertension, even if proven, do not necessarily indicate differences in the mechanism of hypertension.

(c) Renal artery stenosis.

Renal artery stenosis may cause hypertension in man; the two most common causes are atheroma and fibromuscular hyperplasia of the renal artery, and it is usually unilateral.

It is known from animal studies that renal artery constriction leads to an acute rise of blood pressure and an acute rise in plasma renin and angiotensin II. This pressure rise is probably due to acute vasoconstriction and blood pressure returns to normal on removal of the constriction or of the affected kidney^(167, 168).

If the constriction is maintained the blood pressure remains high but plasma renin and angiotensin II concentrations fall⁽¹⁶⁹⁾. Nevertheless, removal of the constriction, or the affected kidney, restores blood pressure to normal. Angiotensin II remains active in maintaining hypertension, partly by vasoconstriction, and partly by its slow pressor mechanism via modification of its own pressure dose-response curve⁽¹⁷⁰⁾.

A third phase develops after several weeks when removal of the abnormal kidney fails to reduce blood pressure. Prolonged infusions of inhibitors reduce blood pressure but this fall is not related to pre-treatment plasma levels of renin and angiotensin; the likely mechanism is suppression of the slow pressor effects of angiotensin II⁽¹⁷¹⁻¹⁷³⁾.

Unilateral renal artery stenosis in man is usually first seen in the second phase when surgical correction of the artery or removal of the affected kidney will restore blood pressure to normal. The use of converting enzyme inhibitors in renal artery stenosis is discussed fully in a later chapter.

(d) Primary hyperaldosteronism.

Conn's syndrome is associated with excessive secretion of aldosterone from a solitary adrenal adenoma, or diffuse hyperplasia. Hypertension is associated with a marked increase in body sodium and extracellular fluid and suppression of renin and angiotensin. Inhibitors of the renin-angiotensin system have little effect and inhibitors of aldosterone are the treatment of choice⁽¹⁷⁴⁻¹⁷⁶⁾.

(e) Chronic renal failure.

Hypertension in renal failure is associated with disproportionate elevations of angiotensin II in relation

to exchangeable sodium and this may be associated with normal or very high concentrations of circulating angiotensin II. Inhibitors of the renin-angiotensin system will therefore be more effective in lowering blood pressure in the latter group^(177,178).

CHAPTER 3

CAPTOPRIL AS A THERAPEUTIC AGENT:

A REVIEW OF THE LITERATURE

I. ANIMAL STUDIES

The rapid and outstanding work which led to the development of captopril has been described in the previous chapter. The potential therapeutic value of such an agent was readily apparent and a rapid programme of animal experimental work got under way.

(a) Normotensive animals.

The inhibitory effects of captopril *in vitro* were demonstrated by incubation studies using brain or plasma angiotensin-converting enzyme⁽¹⁷⁹⁾; it was also shown to inhibit the contractile effects of exogenous angiotensin I on guinea-pig ileum^(180,181).

In vivo studies were then conducted to study the effects of captopril on the renin-angiotensin system in normotensive and hypertensive animal models, usually rats and dogs. It consistently reduced plasma concentrations of angiotensin II⁽¹⁸²⁻¹⁸⁴⁾ with corresponding increases in plasma renin release and activity, due to loss of feedback inhibition⁽¹⁸⁵⁻¹⁸⁷⁾. It blocked the pressor response to infused angiotensin I⁽¹⁸⁸⁻¹⁹⁰⁾ and

reduced plasma aldosterone concentration^(185,191-193) and urinary aldosterone secretion⁽¹⁸⁴⁾. The effects of captopril in magnitude and duration tended to be dose-related, being most pronounced in sodium depleted animals with high plasma renin activity^(190,194,195).

The haemodynamic effects in normal animals were also studied. Captopril had little or no effect on blood pressure in normotensive sodium-replete rats^(191,196,139), but dose-related falls of up to 20% in mean arterial pressure were noted in similar dog models⁽¹⁸³⁾. Blood pressure fell by 20-30% in all sodium depleted animals studied^(191,196,197). Blood pressure reduction was associated with a fall in peripheral vascular resistance^(198,199) but no increase in heart rate. Significantly reduced baroreceptor sensitivity (heart rate response to changes in blood pressure) was shown in rabbits infused with phenylephrine and nitroprusside⁽²⁰⁰⁾. In dogs and rats with normal renal function captopril significantly increased renal blood flow and reduced renal vascular resistance^(185,197,201,202). In dogs with experimentally induced renal failure captopril also increased blood flow with variable effects in glomerular filtration^(185,197).

•

(b) Hypertensive animals.

The hypotensive effects of captopril were demonstrated in studies primarily designed to establish the

relative influence of the renin-angiotensin system in the hypertensive models studied. Such studies usually reported rapid, marked falls of blood pressure in renin dependent models of hypertension, but a slower or reduced effect in models thought to be independent of the renin-angiotensin system.

Thus, captopril caused marked reduction in blood pressure in 2-kidney renal hypertensive rats^(196,203-206) and in rats made hypertensive by aortic stricture⁽²⁰⁷⁾. It also appeared to alter the natural course of 2-kidney 1-clip hypertension, preventing or delaying the development of hypertension which occurred in untreated animals⁽²⁰⁸⁾. Addition of a thiazide diuretic produced a greater fall of blood pressure, with increased survival, than with captopril alone⁽²⁰⁴⁾.

Captopril was shown to be less active in animal models of hypertension thought not to be dependent on the renin-angiotensin system. Single doses did not alter blood pressure in 1-kidney renal hypertensive rats⁽²⁰⁵⁾, though over several days there was a gradual, small reduction in blood pressure, associated with natriuresis and diuresis⁽¹⁹⁶⁾. In the DOCA/salt hypertensive rat model captopril did not significantly alter blood pressure in most studies, or prevent development of hypertension when used prophylactically⁽²⁰⁹⁻²¹¹⁾.

In the spontaneously hypertensive rat, considered to be a model for essential hypertension in man, long-term therapy induced progressive falls in blood pressure (212). Given in high doses to young rats, captopril prevented the onset of hypertension, though this occurred in the usual way when the drug was discontinued^(213,214). In association with its hypotensive effects left ventricular hypertrophy was prevented or reversed with captopril therapy⁽²¹⁵⁾. Also in this model the effects of captopril were attenuated following nephrectomy⁽²¹⁶⁾.

These, and many other animal experiments, confirmed the potential of captopril as a therapeutic agent in man, and attention was directed to its experimental use in normal volunteers.

II. MECHANISMS OF ACTION OF CAPTOPRIL

Captopril has a potent antihypertensive effect, inhibiting the conversion of the inactive decapeptide angiotensin I to the pressor octapeptide angiotensin II⁽²¹⁷⁾. However, it is uncertain whether the suppression of plasma angiotensin II and its acute pressor effect is the sole mechanism whereby captopril exerts its hypotensive action.

(a) Suppression of angiotensin II.

In sodium depleted dogs⁽²¹⁸⁾, a close relationship has been demonstrated between the decrease in blood

pressure and the decrease in angiotensin II, suggesting that suppression of angiotensin II is an important factor in the acute hypotensive response of captopril in the sodium depleted state, and supports the view⁽²¹⁹⁾ that angiotensin II plays an important role in maintaining blood pressure during sodium deprivation. Similar experiments in sodium replete dogs demonstrated a smaller fall in blood pressure which did not correlate with the decrease in plasma angiotensin II concentration. Similar results have been observed after chronic oral administration of captopril to normal man under varying states of sodium balance^(220,221).

Experiments with saralasin and teprotide have shown that inhibition of the renin-angiotensin system in the normal or salt loaded state has a negligible effect on blood pressure^(217,222-226). This has led to the belief that suppressed plasma levels of angiotensin II after salt loading are unimportant in maintaining blood pressure. However, it should be re-emphasised that results from studies using saralasin are difficult to interpret owing to its intrinsic agonist properties.

One interpretation of these findings is that the net effect of angiotensin II in blood pressure is determined by the relative sodium balance existing at the time. However, an experiment by Morton in sodium deplete and replete dogs⁽²¹⁸⁾ has shown that some other mechanism of action is almost certainly involved.

Captopril reduced blood pressure and plasma angiotensin II and subsequent infusion of angiotensin II caused an increase in arterial pressure and plasma angiotensin II. If captopril decreases blood pressure by reducing plasma angiotensin II concentration alone, infusion of angiotensin II in the presence of captopril should restore the relationship between arterial pressure and angiotensin II. In fact this does not happen and the dose-response curve is shifted downwards, with plasma angiotensin II concentrations having to be fivefold greater to return blood pressure to basal values.

(b) The kallikrein-kinin system.

Angiotensin-converting enzyme (kininase II) inhibition by captopril reduces bradykinin metabolism in vitro and in vivo^(227,228). However, circulating bradykinin levels after angiotensin-converting enzyme inhibition have been reported in both experimental animals and man to increase transiently^(229,230), remain the same^(231,232), or even be reduced⁽²³³⁾. Although this failure to observe consistent increases in bradykinin levels may be a consequence of either increased activity in alternative metabolic degradative pathways or simultaneous reduction in bradykinin production after kininase II inhibition, an alternative explanation is that circulating levels do not reflect increased local tissue levels of kinins.

The kallikrein-kinin system has been regarded as a local hormonal system important in regulation of regional blood flow^(234,235). Plasma kallikrein is an enzyme that circulates in an inactive form and is involved in the haemostatic cascades. Tissue kallikrein is a different enzyme, occurring in exocrine glands where it may form kinins locally from kininogen⁽²³⁶⁾. Thus kinins may be both local and circulating hormones and by regulating regional blood flow and resistance they may be important in circulatory homeostasis and blood pressure control.

The kidney contains large amounts of kallikrein and kininase II and urine contains kallikrein, kininogen and kinins thought to arise from renal tissue^(235,236). Bradykinin causes marked renal vasodilation, diuresis and natriuresis^(234,235) and the ability of captopril to potentiate the effects of infused bradykinin has been discussed in Chapter 1.

(c) The prostaglandins.

Animal experiments have shown that bradykinin may induce the release of a prostaglandin-like substance which is significantly increased by angiotensin-converting enzyme inhibition and antagonised by indomethacin^(237,238), a prostaglandin synthetase inhibitor.

To determine whether prostaglandins contribute to the depressor response of angiotensin-converting enzyme inhibitors, plasma prostaglandin levels have been measured by radioimmunoassay in normal and hypertensive subjects⁽²³⁹⁾. The hypotensive response to captopril is accompanied by significant increases in the metabolite of prostaglandin E₂ (PGE₂-M) and these increases directly correlate with the fall in blood pressure. Pre-treatment with indomethacin completely eliminates the captopril-induced prostaglandin increase, without changing bradykinin or angiotensin II responses, but with significant attenuation of the hypotensive response.

(d) Baroreceptor and sympathetic nervous system function.

A haemodynamic feature of captopril is that it reduces arterial blood pressure without a concomitant increase in heart rate⁽²⁴⁰⁻²⁴²⁾ and plasma noradrenaline levels⁽²⁴³⁾ which suggests that under this circumstance baroreceptor inhibition of autonomic nerve activity may be preserved rather than reduced, and that baroreflex potentiation may represent another antihypertensive mechanism of the drug.

• Effects on the peripheral sympathetic nervous system in animals and man have also been observed with captopril. The influence on pressor responses to exogenously administered vasopressor substances has been investigated in normal subjects⁽²⁴³⁾, in whom captopril

has been shown to attenuate the response to norepinephrine, but not to angiotensin II. However, attenuation of the pressor responses to norepinephrine does not occur when a subpressor dose of angiotensin II is infused in addition to captopril.

Similar effects have been observed with in vitro and in vivo experiments in rats using both captopril and teprotide⁽²⁴⁴⁾. These effects of captopril have been confirmed in other experiments using pithed rat and rat mesenteric artery preparations^(245,246) but interestingly, they were not observed when other converting enzyme inhibitors were used (teprotide, MK421 and SA446).

Vasoconstriction in vivo is governed by post-synaptic α_1 and α_2 -adrenoreceptors and noradrenaline has no preference for either class. Using a beta-blocked, pithed rat model⁽²⁴⁷⁾, it has been shown that captopril significantly reduces the pressor response to infused noradrenaline, and that this attenuated response is unchanged by pretreatment with the selective α_1 -antagonist prazosin. On the other hand, after selective blockade of that part of noradrenaline's vasoconstriction mediated by α_2 -adrenoreceptors with the antagonist rauwolscine, captopril is devoid of antagonist-ic activity against the residual vasoconstriction to noradrenaline. Stimulation of postsynaptic α_2 -adrenoreceptors with B-HT 920⁽²⁴⁸⁾ shifts the dose-response

curve to the right following previous treatment with captopril. However, infusion of angiotensin II restores the vasoconstriction due to B-HT 920, implying a modulatory role of angiotensin II in postsynaptic α_2 -adrenoreceptor activation in vivo.

(e) Renal responses.

In healthy, recumbent, sodium replete animals and man neither saralasin nor teprotide influence renal perfusion or glomerular filtration rate⁽²⁴⁹⁻²⁵²⁾. However, captopril does increase renal blood flow in the dog⁽²⁵³⁾, rabbit⁽²⁵⁴⁾, rat⁽²⁵⁵⁾ and in man⁽²⁵⁶⁾, when the renin-angiotensin system has been suppressed by a liberal salt intake. The mechanism of this action will be largely that of suppression of angiotensin II with the probability of contribution of other systems, especially the kallikrein-kinin or prostaglandin system.

That teprotide does not share the same specificity of action as captopril is of great interest. It may be important that there appear to be converting enzyme inhibitors which differ not only in their pharmacokinetics, but also in their specificities of action. It cannot be assumed that the physiological and therapeutic properties of captopril will be shared by other orally active inhibitors, one of which, enalapril, is to be examined in this thesis.

III. CAPTOPRIL IN HUMAN HYPERTENSION

(a) Renal hypertension.

It was likely from the results of animal studies that captopril should prove most effective in hypertensive patients whose levels of renin and angiotensin II are highest. Thus, the antihypertensive effects of captopril might be expected to be most apparent in patients with renal artery stenosis, the pathophysiology of which has been discussed in Chapter 2.

Indeed, the use of captopril in renal artery stenosis has confirmed these earlier animal experimental findings. There is an early fall in blood pressure which is directly proportional to the pre-treatment levels of renin and angiotensin II, and which parallels the initial fall in circulating angiotensin II⁽²⁵⁷⁻²⁵⁹⁾. However, continued treatment leads to a progressive lowering of blood pressure over several weeks and this fall is not related to the pre-treatment levels of renin and angiotensin II⁽²⁵⁸⁻²⁶⁰⁾. In addition, captopril has returned blood pressure to normal in patients with renal artery stenosis whose peripheral levels of renin and angiotensin II are within the normal range^(258,259,261); subsequent reconstructive surgery has also been curative in some of these patients^(258,259,261).

Prolonged treatment with captopril is associated with a sustained reduction in plasma angiotensin II and aldosterone, with converse increases in circulating renin and angiotensin I⁽²⁵⁷⁻²⁶²⁾. Plasma potassium may be raised⁽²⁵⁸⁻²⁶¹⁾ and, in the presence of renal impairment, this may reach dangerous levels⁽²⁶³⁾. Total exchangeable and total body sodium and potassium are not significantly changed with long-term treatment^(258,259,264).

Some patients with chronic renal failure undergoing regular haemodialysis remain hypertensive despite sodium depletion⁽²⁶⁵⁾. Although circulating levels of renin and angiotensin II are not always above the normal range, it appears that renin secretion from the diseased kidneys is inappropriately high in relation to exchangeable sodium^(266,267). Captopril has proved effective in these patients, and also in those with less severe renal impairment, supporting the hypothesis that these latter patients may also have a disproportionate elevation of angiotensin II in relation to exchangeable sodium⁽²⁶⁸⁾.

Severe hyponatraemia and hypertension with renal artery stenosis is a rare syndrome associated with hypersecretion of renin, progressive sodium depletion, a disproportionate increase in angiotensin II and secondary hyperaldosteronism. Captopril has been used in this

condition with rapid correction of both the hypertension and the electrolyte abnormalities^(257,269).

(b) Essential hypertension.

The role of the renin-angiotensin system in essential hypertension has been discussed in Chapter 2. Plasma angiotensin II tends to be within or near a range having a distinct, though modest, effect on blood pressure. As discussed earlier the principal hypotensive mechanism of captopril is probably inhibition of angiotensin II formation, so a modest hypotensive effect in essential hypertension might be anticipated.

In fact captopril has been shown to have approximately the same hypotensive effect as standard doses of beta-blocker or diuretic^(270,271), though up to half of patients with mild to moderate hypertension require the addition of a diuretic to achieve blood pressure control^(270,271).

In combination with a diuretic captopril has revolutionised the treatment of severe resistant hypertension, being as effective as standard triple therapy in most instances⁽²⁷²⁻²⁷⁶⁾. Sodium is removed and the compensatory rise in plasma angiotensin II is prevented. Many failures have been reported with the combination of captopril and thiazide but the success rate is improved if a loop diuretic is used , with a beta-blocker

necessary in some cases⁽²⁷⁶⁾.

As with renovascular hypertension there are consistent rises in plasma renin and angiotensin I, with suppression of aldosterone and angiotensin II^(258,259,264). Although aldosterone secretion and excretion are known to be depressed there is conflicting evidence on the presence of an early natriuresis^(260,262,278). Serum potassium is usually, but not always, increased during long-term treatment^(258,259,260,264). Plasma catecholamines are unchanged though cardiac output tends to increase slightly⁽²⁷⁸⁻²⁸²⁾. Renal vascular resistance is decreased, with increased renal blood flow, but glomerular filtration remains unchanged⁽²⁸³⁻²⁸⁵⁾.

(c) Malignant phase hypertension.

When captopril is given there is an immediate fall in blood pressure, which may be minimal or profound, and which is directly related to the level of circulating angiotensin II. Malignant phase hypertension, related to essential or to secondary hypertension, is usually associated with very high levels of circulating renin and angiotensin II. Thus captopril is especially effective in reducing blood pressure^(259,286) but extreme caution should be used.

The absolute values for cerebral blood flow are the same for both hypertensive and normotensive man⁽²⁸⁷⁾.

However, in studies which directly measured cerebral blood flow during hypotension, it has been clearly shown that the lower limit of autoregulation in patients with severe, untreated hypertension was shifted to higher blood pressure levels than in normotensive subjects, and that the onset of cerebral dysfunction occurred at higher blood pressure level in hypertension⁽²⁸⁸⁾. Thus, when challenged with hypotension, hypertensive patients are more susceptible to cerebral hypoxia due to relative inability to compensate fully for the fall in blood pressure⁽²⁸⁹⁾.

As will be described later, captopril may cause precipitous falls in arterial pressure when the first dose is given, with potentially serious effects on cerebral circulation. For these reasons it is probably better to avoid captopril in malignant phase hypertension and to rely on conventional oral antihypertensive therapy.

Benefit has been obtained in patients with scleroderma and hypertension, especially when suffering renal crises. It is also reported to improve cutaneous circulation^(290,291).

•

IV. CAPTOPRIL IN CONGESTIVE CARDIAC FAILURE

It has recently become apparent that peripheral factors play an important role in the pathophysiology of congestive cardiac failure⁽²⁹²⁾. Generally agreed factors are circulatory congestion, related in part to renal sodium retention, and increased aortic input impedance (afterload), associated with systemic vasoconstriction^(293,319). Systemic venoconstriction may also contribute to the cardiac volume load by shifting blood centrally due to reduced venous capacitance.

The role of afterload as a primary determinant of cardiac performance has been well demonstrated⁽³¹⁹⁾, and subsequently decreasing arterial impedance to ventricular ejection has been shown to substantially improve cardiac output in patients with acute and chronic congestive heart failure^(308,320,321). While this is usually achieved by administration of either direct smooth muscle relaxants, or by alpha-adrenergic blockers, the use of captopril appears logical since it directly inhibits the production of the potent arteriolar vasoconstrictor angiotensin II.

The mechanisms controlling sodium retention, venoconstriction and arterial constriction in congestive cardiac failure have not been entirely clarified. The renin-angiotensin system clearly plays an important role:

plasma renin activity may be elevated in certain forms of experimental heart failure⁽²⁹⁴⁻²⁹⁵⁾ and is frequently elevated in patients with chronic congestive heart failure⁽²⁹⁷⁻³⁰²⁾. The mechanism by which the renin-angiotensin system is stimulated in heart failure is not fully understood but probably represents a combined effect of both neurohumoral and haemodynamic factors on the kidney. Increased circulating angiotensin II may then produce systemic vasoconstriction and decreased sodium excretion either by a direct renal vasoconstrictor effect, or by stimulation of aldosterone production.

Teprotide and captopril consistently produce a fall in arterial pressure associated with increased cardiac output and cardiac index, a fall in total peripheral resistance and right atrial pressure, and a largely unchanged pulse rate⁽³⁰³⁻³⁰⁶⁾. This haemodynamic response is similar to that noted with other vasodilating drugs which do not however significantly reduce right atrial pressure⁽³⁰⁷⁻³⁰⁸⁾.

Patients, all with moderate to severe congestive cardiac failure, have a wide range of values for plasma renin activity⁽³⁰²⁾. It is of interest that there is no consistent relationship between plasma renin and haemodynamic or renal function⁽³⁰³⁻³⁰⁶⁾ although plasma renin and plasma urea have been shown to be correlated⁽²⁹⁹⁾. A negative correlation between plasma

renin and serum sodium is consistently demonstrated but this relationship is not peculiar to congestive failure, existing similarly in patients with essential hypertension⁽³⁰⁹⁾. Angiotensin II stimulates water drinking and secretion of antidiuretic hormone^(310,311); these factors may contribute to a low serum sodium in patients with high renin levels. The intrarenal mechanisms that control renin activity are complex, but a reduction of serum sodium may reduce sodium availability to the macula densa and stimulate renin secretion⁽³¹²⁾. Regardless of the mechanism of the relationship it appears that plasma renin activity and serum sodium do change inversely in sequential studies in the same patient. Spontaneous changes in serum sodium are observed in patients with heart failure and are accompanied by striking inverse changes in plasma renin⁽³⁰³⁾.

The absence of a correlation between plasma renin activity and systemic vascular resistance cannot necessarily be assumed to indicate that angiotensin II plays no role in the systemic vasoconstriction of congestive heart failure. Other mechanisms may contribute to the elevated systemic resistance in heart failure, including catecholamines⁽³¹³⁾, sodium and water accumulation in the vascular walls⁽³¹⁴⁾, and perhaps ADH⁽³¹⁵⁾.

Reference has been made to changes in right atrial pressure in response to captopril. As angiotensin II is a potent arterial vasoconstrictor with relatively little effect on the venous capacitance bed^(313,316), this response is paradoxical. If captopril were exerting its effect exclusively by reduction of circulating angiotensin II, then one would not expect a change in venous capacitance. There are several possible explanations; the haemodynamic effects of captopril may not be related to suppression of angiotensin II, or alternatively, angiotensin II, in the presence of congestive failure, is exerting a venoconstrictor effect. Another explanation could be that captopril therapy results in reduced sympathetic outflow. This is unlikely as no relationship has been observed between changes in right atrial pressure and the decrease in plasma norepinephrine^(317,318).

It is undoubtedly true that captopril produces marked symptomatic and haemodynamic improvement in most patients with cardiac failure⁽³⁰³⁻³⁰⁶⁾. This is, however, largely palliative, and is not necessarily associated with increased survival in a disease complicated by arrhythmias, electrolyte imbalance, digoxin toxicity and infection⁽³²²⁾. It may even be detrimental at the end-stage of the disease when arteriolar vasoconstriction is essential to maintain adequate blood pressure⁽³²³⁾.

V. CAPTOPRIL: OTHER INDICATIONS

It has been suggested that angiotensin converting enzyme inhibition may clarify the pathogenesis of, or provide specific treatment for, essentially every clinical state with abnormalities of the renin-angiotensin-aldosterone system. However, with the exception of scleroderma crisis, which is much akin to malignant hypertension, there are only sporadic case reports of the use of captopril available. Thus, the therapeutic use of captopril for conditions other than hypertension and congestive heart failure is as yet unproven⁽³²⁴⁾.

Plasma renin levels are usually elevated in hepatic cirrhosis and the hepatorenal syndrome, acute renal failure and scleroderma renal crisis⁽³²⁵⁻³²⁷⁾. An inappropriate response of the renin-angiotensin system to upright posture may play a part in the pathogenesis of idiopathic oedema⁽³²⁸⁾. Patients with Bartter's syndrome are normotensive or even hypotensive, despite activation of the renin-angiotensin system, perhaps due to hyposensitivity to angiotensin II⁽³²⁹⁾. Serum levels of angiotensin converting enzyme are elevated in about half of the patients with sarcoidosis⁽³³⁰⁾, and are raised in patients with viral hepatitis and some nonsarcoid pulmonary diseases⁽³³¹⁾. Because of its vasodilating properties, captopril may well prove useful in the management of ischaemic heart

disease and peripheral vascular disease. There are suggestions of improved haemodynamics and reduced mortality, in animals and man, in haemorrhagic shock⁽³³²⁾.

Detailed discussion of the use of captopril in these conditions is outwith the scope of this thesis.

VI. CAPTOPRIL AND THE BRAIN

(a) Converting enzyme and angiotensin in the brain.

Converting enzyme is present in the brain at concentrations lower than in lung tissue but higher than in most other organs^(333,334). High concentrations are found in the striatum, the pituitary gland, the cerebellum, the caudate nucleus, the choroid plexus and the microvessels of the brain. Converting enzyme is also present in the cerebrospinal fluid of rats and dogs⁽³³⁵⁾. Captopril can block the biological effects elicited by intracranial injections of renin or angiotensin I, but not the central blood pressure responses to angiotensin II, demonstrating that the converting enzyme is active in vivo in brain tissue⁽³³⁶⁻³³⁹⁾.

The other components of the system (renin substrate, renin, angiotensin and angiotensinases) have also been found in the brain⁽³⁴⁰⁾, and in the CSF^(335,341). The central effects of angiotensin II include increase of

blood pressure, stimulation of drinking behaviour with selective salt uptake, release of antidiuretic hormone and ACTH from the pituitary and stimulation of catecholamine synthesis and turnover^(340,342). These effects are mediated by specific angiotensin II receptors localised mainly in the vicinity of the brain ventricles and which can be blocked by saralasin^(343,344). Thus, stimulation of brain angiotensin II biosynthesis by intraventricular renin, or application of angiotensin II into the brain, is followed by several neurohumoral responses which may each contribute to the elevation of blood pressure⁽³⁴⁵⁾.

(b) Interactions with other enzyme-peptide systems in the brain.

As previously discussed, angiotensin converting enzyme is identical with kininase II, which degrades bradykinin. Bradykinin has been demonstrated in the brain^(346,347) but in contrast to its peripheral depressor effects, it increases blood pressure when injected into the CSF of rats^(346,348). In the periphery, potentiation of the depressor action of kinins by inhibiting kininase II is a mechanism which could explain in part the antihypertensive properties of captopril, which can also potentiate the pressor effects of centrally administered bradykinin. This obviously does not contribute to the blood pressure lowering effect of captopril but it may influence other complex interactions

induced by captopril in the brain⁽³⁴⁹⁾.

Possibly more important, in terms of central anti-hypertensive actions, is the fact that converting enzyme has properties in common with an enkephalin degrading dipeptidyl-carboxypeptidase⁽³⁵⁰⁾, although a more specific enkephalinase is apparently not identical with converting enzyme⁽³⁵¹⁾. Enkephalins are widely distributed in the brain⁽³⁵²⁾, and, in addition to other biological functions, the opioid peptides act on the cardiovascular system in a complex way, depending on the length of the peptide chain, and on the site of application or action. For example, leucine-enkephalin is pressor when injected into the ventricles or intravenously⁽³⁵³⁾, but the analogue D-ala-met-enkephalin is depressor in a similar way to beta-endorphin⁽³⁵³⁾. In spontaneously hypertensive rats many tissues contain less met-enkephalin activity than normotensive controls⁽³⁵⁴⁾, and the opiate receptor antagonist naloxone increases blood pressure in haemorrhagic shock⁽³⁵⁵⁾.

It has been shown that, in vitro, teprotide and captopril inhibit both converting enzyme activity and enkephalinase activity from mouse and rat brain preparations⁽³⁵¹⁾ although both inhibitors have 1000-fold higher inhibitory potencies against converting enzyme. In addition, captopril has been shown to inhibit the degradation of 3H-Met-enkephalin in the rat striatum,

and causes prolonged analgesia which can be prevented by intraperitoneal injection of naltrexone⁽³⁵⁶⁾.

In view of these findings, it can be assumed that converting enzyme inhibitors interfere in vivo with the degradation of opioid peptides. Thus, potentiation of their cardiovascular effects due to inhibition of opioid peptide degradation in the periphery, or in the brain, could be a mechanism involved in the antihypertensive actions of the inhibitors.

Experiments in the cat and rats have been performed to investigate whether captopril can penetrate the blood brain barrier. Intravenous captopril does not inhibit the central pressor response to intraventricular angiotensin I, though it markedly diminishes the response to intravenously administered angiotensin I. Conversely, when captopril is given into the ventricular system the pressor effects of angiotensin I infused into the ventricle are significantly inhibited. Only in very high doses does captopril enter the blood from CSF⁽³⁵⁷⁾.

These experiments have been performed in single dose studies in animals. It is still not clear whether long-term captopril therapy in man is associated with significant levels of drug in the central nervous system. Possible effects of captopril on endogenous opioid metabolism are important for two reasons. Firstly, such

effects may be relevant in the antihypertensive effects of captopril. Secondly, and perhaps more importantly, enhancement of endogenous opioid activity may theoretically be associated with increased well-being or mood enhancement. This latter, possible effect is the subject of an experiment described in a later chapter.

CHAPTER 4

ENALAPRIL AS A THERAPEUTIC AGENT:

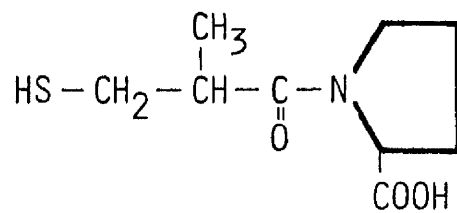
A REVIEW OF THE LITERATURE

In Chapter 2 the work leading to the design and synthesis of enalapril and its analogues was described. Since the work on this thesis was begun, the first experiments describing the pharmacology and therapeutic efficacy of these drugs have been published and a summary of the most significant is outlined here.

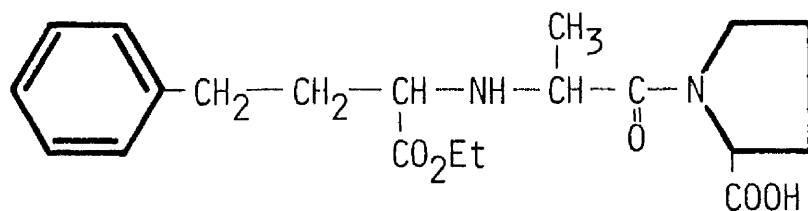
Captopril has several side-effects which may be related to its sulphhydryl group. Although the serious side-effects of leucopenia and glomerulonephritis are unlikely to occur if low doses are used, it is not yet clear whether the incidence of skin rash and taste loss is lessened by dosage reduction. Thus the development of new inhibitors, devoid of a sulphhydryl group, is possibly of great practical importance and certainly of interest.

The evaluation of captopril has been hampered by difficulties in the measurement of plasma converting enzyme and drug concentrations. Enalapril maleate and its lysine analogue are the first inhibitors which can easily be measured in plasma. In addition, the drug/converting enzyme complex is stable in storage, readily permitting measurement of converting enzyme activity⁽³⁵⁸⁾. Thus,

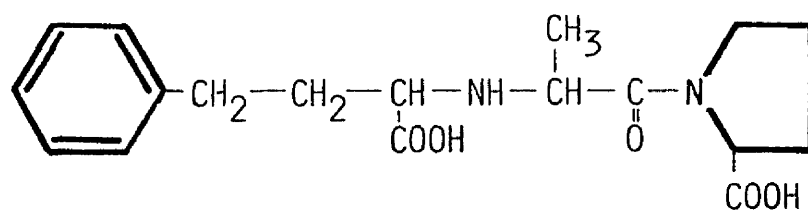
CAPTOPRIL



MK 421



MK 422



MK 521

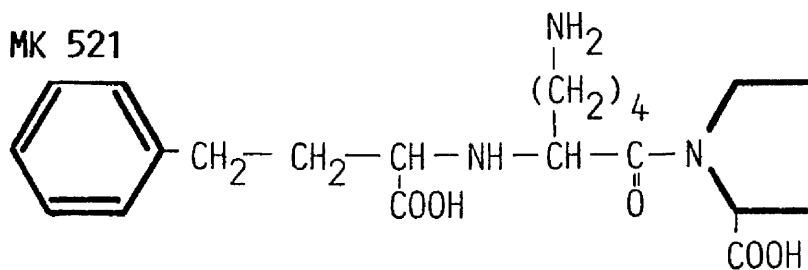


Figure 4.

The chemical structures of captopril, enalapril (MK421), active diacid enalaprilic acid (MK422) and lysine analogue (MK521).

the pharmaco-kinetic properties of these drugs may be ascertained with detailed analysis of their effects on the renin-angiotensin system.

Pre-clinical experiments have shown that both compounds block the pressor effects of infused angiotensin I in animals^(140,359) and man^(143,360), and lower blood pressure in normal and hypertensive rats⁽³⁶¹⁻³⁶⁶⁾. The effects on converting enzyme activity are still apparent after 72 hours although the rate of disappearance of drug is more rapid, and the blockade of angiotensin I pressor responses has disappeared after 24 hours. Despite this there is a close relationship between the levels of active metabolite and plasma converting enzyme activity. In addition to sharing a longer duration of action, both drugs are more potent than captopril on a weight and molar basis^(140,359).

Pharmaco-kinetic studies have been performed in normal subjects. The largely inactive enalapril maleate (MK421) is absorbed and de-esterified, yielding the highly active, but poorly absorbed diacid (MK422) (Figure 4). MK521 (the lysine analogue of MK422) is orally absorbed and requires no bioactivation. MK421 has an absorption peak at 1 hour with a minimum absorption of about 60%. MK521 is absorbed more slowly with a peak at 6-8 hours and a minimum absorption of about 30%. There is no significant metabolism of either

compound apart from bioactivation of MK421⁽³⁶⁷⁾.

Their effects on blood pressure, the renin-angiotensin system, baroreceptor and sympathetic nervous system function, renal function and sodium excretion have been compared in placebo-controlled single dose studies in normal subjects⁽³⁶⁸⁻³⁷⁶⁾. Both drugs have been shown to fully suppress converting enzyme activity, with peak effects occurring at 2-6 hours after drug administration but with distinct effects still apparent after 24 hours. The detailed findings of these studies will be outlined when discussing the experimental results described in later chapters.

Studies in small numbers of patients with essential hypertension have shown them to be effective, with a prolonged duration of action, especially when combined with a thiazide diuretic⁽³⁷⁷⁻³⁸⁸⁾. It has also been shown that their antihypertensive effects are similar when given in a once or twice daily dosage regime⁽³⁸⁹⁾. Studies of their use in congestive cardiac failure have shown similar haemodynamic responses to captopril⁽³⁹⁰⁻³⁹⁴⁾.

The question of side effects requires much longer study in many more subjects before conclusions can be drawn. However, in the literature to date the reports of side effects and adverse reactions have been remarkably low and require confirmation⁽³⁹⁵⁻³⁹⁸⁾.

CHAPTER 5

MATERIALS AND METHODS

I. MEASUREMENT OF BODY COMPOSITION

(a) Total exchangeable sodium and potassium.

These measurements depend upon the principle of isotope dilution, known amounts of ^{43}K and ^{24}Na are given to the individual patient by the oral route, and equilibration with stable endogenous sodium and potassium allowed to occur. Sodium equilibration usually takes 12 hours but may take up to 24 hours in oedematous subjects; potassium equilibration takes 40-44 hours. After equilibration the ratio of radioactive element to endogenous element is calculated and the total exchangeable element derived. The technique is described in detail by Davies and Robertson⁽³⁹⁹⁾. The results quoted in this work are expressed as a percentage of exchangeable element in mmol/Kg expected for a normal individual of the same leanness index where this index is the ratio of height³ to weight.

(b) Total body sodium estimation.

This was determined by neutron activation analysis using sealed-tube neutron generators and a shadow-shield whole-body counter as described by Boddy et al. (400,401). Two sealed-tube 14MeV neutron generators are

installed in a large concrete shield, one above and one below a tunnel through the shield. The patient lies on a couch which is driven through the tunnel and is irradiated during a single pass through the neutron generators. An average dose equivalent of 1 rem is delivered to the body during the irradiation of each subject. After irradiation, the subject is transferred to a dual detector, shadow-shield, whole-body counter. Spectra are then collected from the two detectors separately, and analysed. Energy ranges have been specified to include the total absorption peak of the major radiation from the activated sodium. The counts in the appropriate energy range are then corrected for natural background and for stray neutron radioactivity. Using this information the amount of total body sodium is calculated.

(c) Measurement of total body potassium.

The natural radioisotope ^{40}K occurs in potassium in the order of 0.012 per cent and emits gamma rays which may be detected using whole-body radioactivity counters. The observed counting rate can be related to the total body content of potassium. The method is described in detail by Boddy et al. (402).

II. MEASUREMENT OF COMPONENTS OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

(a) Plasma active renin.

The international reference preparation of renin (National Institute for Biological Standards and Control) was used⁽⁴⁰³⁾. The method used for radioimmunoassay of plasma renin concentration was based on the antibody trapping method of Poulsen and Jorgensen^(404,405). The normal laboratory range is 10-50 μ U/ml for normal volunteers on a free sodium intake.

(b) Angiotensins I and II.

Angiotensin I in blood is measured by radioimmunoassay using the method described by Waite⁽⁴⁰⁶⁾, and modified by Morton⁽⁴⁰⁸⁾.

In the technique for measurement of plasma angiotensin II concentration, when approximately 80% of labelled angiotensin II is bound to antibody, the assay system becomes extremely sensitive to the addition of small amounts of unlabelled angiotensin. Dusterdieck and McElwee made use of this fact in describing their method⁽⁴⁰⁷⁾, subsequently slightly modified by Morton et al.⁽⁴⁰⁸⁾. In both these methods it is important that the blood sample is taken directly into inhibitor (EDTA/orthophenanthroline) so that generation of further angiotensin II from angiotensin I is immediately stopped.

Under normal circumstances there is a clinically unimportant degree of cross-reactivity between angiotensin II antibody and angiotensin I - less than 1%. However, when converting enzyme inhibitors have been given to the patient, angiotensin I builds up in the circulation and correction for angiotensin I may be necessary⁽⁴⁰⁹⁾.

The normal range of angiotensin II in peripheral plasma is 5-35 pg/ml.

(c) Plasma aldosterone.

This is measured by radioimmunoassay using the method of Fraser et al.⁽⁴¹⁰⁾. The normal range is less than 18 ng/100 ml.

(d) Angiotensin-converting enzyme activity.

This is measured by an enzyme kinetic technique using hippuryl-histidyl leucine synthetic substrate as described by Cushman and Cheung⁽⁴¹¹⁾.

III. MEASUREMENTS OF RENAL FUNCTION

(a) Intravenous urography and arteriography.

These were performed using standard techniques.

(b) Renal vein sampling.

Patients who have renal vein sampling receive a standard ward diet of normal sodium content, and remain off all antihypertensive therapy for at least 1 week. After recumbency for at least 1 hour, both femoral veins are catheterised under local anaesthesia by the Seldinger technique. The catheters are then sited in both renal veins under fluoroscopic control. Samples are drawn from the right and left renal veins, simultaneously and quickly, but without application of excessive suction. Three sets of samples for plasma renin concentration are obtained over about 5 minutes. Additional samples are drawn from the inferior vena cava (below the renal veins) before and after sampling from the renal veins.

(c) Bilateral ureteric catheterisation studies.

The study conditions are as those for renal vein sampling. With the patient in the lithotomy position, and under epidural anaesthesia, ureteral catheters are passed via a cystoscope and left in situ. The cystoscope is withdrawn and a Foley catheter inserted to detect any urine leak past an ill-fitting ureteric catheter.

Before the ureteral catheters are passed a loading intravenous dose of 20% para-amino-hippurate (PAH) is given, followed by a steady intravenous infusion.

Following a run-in period, four timed urine collections are obtained from each kidney. Each collection period is 5 minutes with blood samples drawn in the middle of each collection period for measurement of plasma creatinine and PAH concentration.

The volume of each urine collection is measured with measurements of sodium, creatinine and PAH. Creatinine and PAH clearances are then calculated and expressed in ml/min. Creatinine clearance is taken as an estimate of glomerular filtration rate, and PAH clearance as an estimate of effective renal plasma flow.

(d) ^{123}I -Hippuran renography.

Patients are given an intravenous bolus of ^{123}I -hippuran and renal images recorded and analysed, using a gamma camera, computer and colour television display. The total effective renal plasma flow (E.R.P.F.) is calculated using the method described by Ram et al. (412).

IV . STATISTICAL METHODS

These are described in the appropriate experimental method sections of each study.

CHAPTER 6

THE FIRST DOSE HYPOTENSIVE EFFECT OF CAPTOPRIL

SUMMARY

The blood pressure response to the first dose of captopril (6.25 mg, 12.5 mg, or 25 mg) was measured in 65 treated, severely hypertensive patients. Mean supine blood pressure was 187/108 mmHg immediately before captopril was given. Twenty one patients experienced a fall in supine systolic pressure greater than 50 mmHg, including five whose pressure fell more than 100 mmHg and two whose pressure fell more than 150 mmHg. Six patients developed symptoms of acute hypotension, including dizziness, stupor, dysphasia, and hemiparesis. Percentage reductions in blood pressure were greatest in those with secondary hypertension ($p < 0.05$), high pretreatment blood pressure ($p < 0.05$), and high concentrations of plasma renin and angiotensin II ($p < 0.01$). No significant correlation was found between fall in blood pressure and serum sodium concentration, age, renal function, and the dose of captopril given.

. A severe first dose effect cannot be consistently predicted in individual patients who have received other antihypertensive drugs for severe hypertension. Such patients should have close medical supervision for at least three hours after the first dose of captopril.

PATIENTS

When captopril is first given, there may be a precipitous fall in blood pressure which is largely unpredictable. To study this problem I designed a protocol so that all patients given captopril could be observed under standard conditions following the first dose of drug.

Sixty-five patients with severe resistant hypertension, including 39 women, were studied in this way. All had been extensively investigated previously and no underlying cause for the hypertension had been found in 36 patients. The remaining 29 had secondary hypertension: 20 had renal artery stenosis or occlusion and the remainder had other forms of renal disease. They were considered for captopril having failed to respond to their previous therapy. Fifty-two were receiving a diuretic, and all had received one in the past. Fifty-six patients were receiving a beta-blocker, while 33 were also receiving a third, and 13 a fourth. antihypertensive drug. Eight were receiving bethanidine alone to facilitate certain investigative procedures.

PROTOCOL

MONDAY

Morning: (a) Patients were admitted to ward and all antihypertensive treatment was stopped.

Afternoon: (b) Whole body neutron activation analysis was performed for assessment of total body sodium (21 patients).

TUESDAY

Morning: (a) Patients lay supine for 45 minutes after insertion of a forearm intravenous cannula.

(b) A solution of angiotensin II (Hypertensin; Ciba) was made up in 5% dextrose to a concentration of 1 µg/l. A variable speed infusion pump was prepared in the event of a dangerous fall in blood pressure.

(c) Blood pressure was measured with a standard sphygmomanometer and blood drawn for measurement of serum sodium and creatinine, plasma active renin concentration, and plasma angiotensin II concentration.

(d) Captopril was given by mouth in a dose of 6.25 mg (20 patients), 12.5 mg (21 patients), or 25 mg (24 patients). The dose of captopril was not randomly allocated - those patients who were suspected of having the greatest risk of hypotension were given the lowest doses.

(e) All subjects remained supine for a further 3 hours with blood pressure checked every 10 minutes.

Statistical analyses of blood pressure were all based on the percentage change in mean arterial pressure - that is, diastolic pressure plus one third of the pulse pressure. All correlations quoted are Spearman's rank correlations. The falls in blood pressure in the group with essential hypertension and the group with secondary hypertension were compared using Welch's modification of Student's t-test, which does not require the assumption of equal variance in the two groups. As well as considering variables individually stepwise regression analysis was used to assess the relations of several combinations of variables to the percentage fall in blood pressure.

For the purpose of this analysis patients with demonstrable renal or renovascular disease were considered to have secondary hypertension, although this assessment was not confirmed in every case by operative intervention.

RESULTS

First dose effect.

Blood pressures immediately before the first dose of Captopril ranged from 130/96 to 254/145 mmHg (mean 187/103 mmHg). Mean plasma active renin concentration was significantly higher in patients with secondary hyper-

tension, but there were no significant differences in the mean values before captopril of blood pressure or serum sodium or creatinine concentration (Table 1).

The reduction in blood pressure in the whole group ranged from 12/0 to 174/82 mmHg, the nadir occurring at a mean of 110 minutes of the first dose of captopril (range 27-330 minutes). The average fall in blood pressure was significantly greater in those with secondary hypertension (52/29 mmHg) than in those with essential hypertension (33/17 mmHg) ($p < 0.05$).

Twenty-one patients experienced a fall in supine systolic pressure greater than 50 mmHg, including five whose pressure fell by more than 100 mmHg and two whose pressure fell by more than 150 mmHg. While supine six patients developed symptoms related to the acute hypotension, including dizziness, dysphasia, hemiparesis, drowsiness, and stupor. One patient developed electrocardiographic changes of acute myocardial ischaemia. Hypotension was corrected promptly by infusion of angiotensin II in five patients and more gradually by giving 0.9% saline in one (Table 2).

Case Reports.

Case 1. Figure 5 shows serial measurements of blood pressure in this 67 year old woman with renal artery occlusion. A combination of atenolol and frusemide

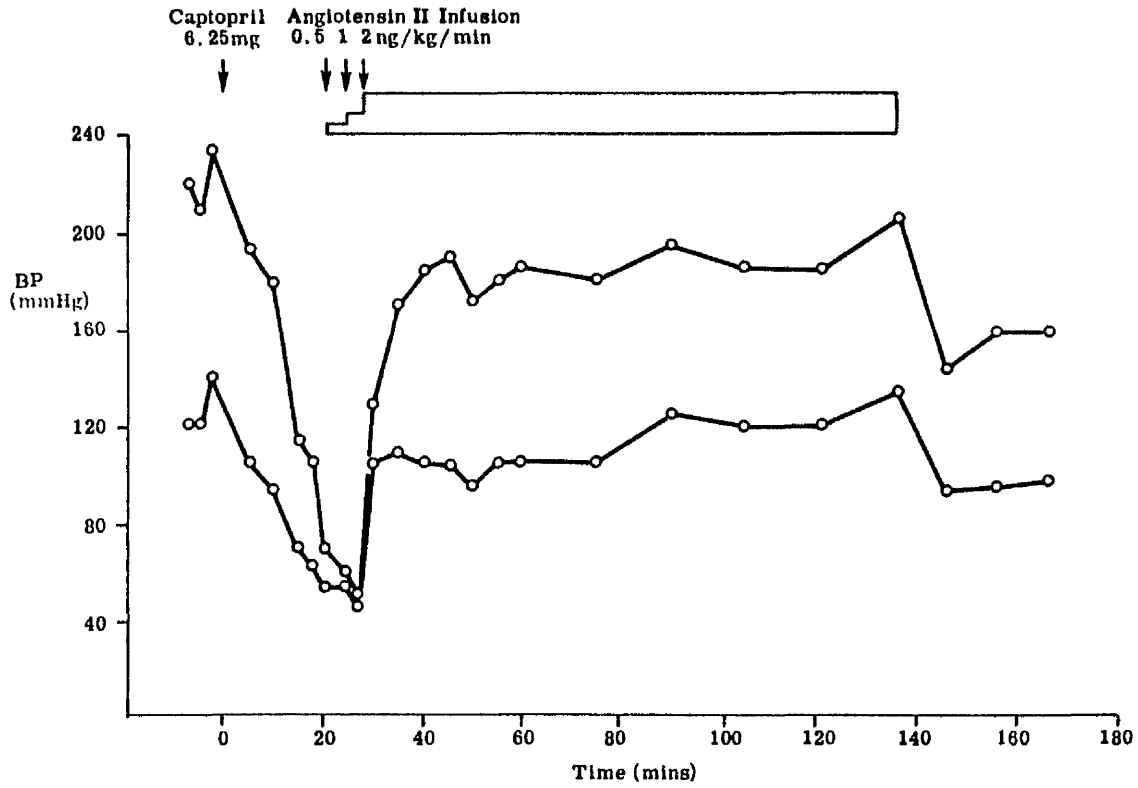


Figure 5.

Serial measurements of systolic and diastolic blood pressure in a 67 year old woman with renal artery stenosis. Blood pressure fell precipitously following an oral dose of captopril 6.25 mg, but was immediately restored with a graded infusion of angiotensin II.

| | Essential | Secondary |
|--------------------------------------|---------------|---------------|
| Supine B.P. (mm Hg) | 180/105 ± 5/2 | 195/112 ± 6/4 |
| Serum Sodium (mmol/l) | 141 ± 0.6 | 139 ± 0.9 |
| Serum Creatinine (μmol/l) | 183 ± 30 | 206 ± 30 |
| Plasma Active Renin conc. (μU/ml) | 59 ± 20 | 349 ± 134* |

Mean ± S.E.M. * p < 0.05

Table 1.

Comparison of blood pressure and biochemistry before captopril in patients with essential and secondary hypertension.

| Diagnosis | Basal B. P. (mm Hg) | Nadir B. P. (mm Hg) | % Δ MAP | Plasma Active Renin concentration (μ U/ml) | Serum Sodium (mmol/l) | Serum Creatinine (μ mol/l) | Dose Captopril (mg) |
|--------------------------|------------------------|------------------------|-------------------|--|-----------------------------|---------------------------------------|---------------------------|
| 1 Renal artery occlusion | 222/128 | 48/44 | 72 | 575 | 137 | 109 | 6.25 |
| 2 Renal artery stenosis | 214/136 | 76/58 | 60 | 884 | 136 | 119 | 6.25 |
| 3 Essential hypertension | 144/102 | 60/40 | 60 | 150 | 139 | 141 | 6.25 |
| 4 Renal artery stenosis | 250/112 | 90/58 | 57 | 2756 | 122 | 87 | 25 |
| 5 Renal artery stenosis | 186/120 | 110/50 | 51 | 2318 | 132 | 209 | 6.25 |

Table 2. Blood pressure and biochemical data in 5 patients who required urgent correction of hypotension by graded infusions of angiotensin II.

had failed to control blood pressure adequately. Immediately before captopril was given plasma active renin concentration was 575 mU/l, serum sodium concentration 137 mmol/l, and serum creatinine concentration 136 μ mol/l. Her blood pressure started to fall shortly after an oral dose of 6.25 mg captopril and reached a nadir of 48/44 mmHg after 27 minutes. She became profoundly drowsy until blood pressure was restored by an infusion of angiotensin II (2 ng/kg/min), which was continued for a further two hours. An infusion of 1.5 litres 0.9% saline was given over the next 24 hours, when a further 6.25 mg captopril was given. Blood pressure fell on this occasion from 230/126 to 156/80 mmHg at one hour, but she developed no symptoms of hypotension. Blood pressure six months later was only moderately well controlled at 200/90 mmHg with a combination of captopril 100 mg, frusemide 160 mg and metoprolol 50 mg daily.

Case 6. This 55 year old man with essential hypertension had previously been receiving bendrofluazide, timolol, and hydralazine, but blood pressure has remained inadequately controlled. He had intermittent claudication and bilateral carotid artery bruits, though he had had no previous symptoms of cerebral ischaemia. All treatment was stopped on the day before captopril was given. Immediately before an oral dose of 25 mg captopril supine blood pressure was 206/132 mmHg, plasma

active renin concentration was 71 mU/l, serum sodium concentration was 140 mmol/l, and serum creatinine concentration was 100 μ mol/l. On administration of captopril blood pressure fell progressively and reached a nadir of 130/88 mmHg after 120 minutes. He then became dysphasic with loss of power in his left arm. An infusion of 1 litre 0.9 saline was given over 120 minutes when blood pressure had returned to 186/96 mmHg. After 24 hours his neurological signs had resolved. Cerebral dysfunction was possibly due to reduced perfusion pressure across stenosed carotid arteries and treatment with captopril was suspended pending further investigation.

Factors correlated with first dose effect.

Positive correlations (Table 3) were observed between the percentage fall in blood pressure after the first dose of captopril and pre-treatment values of both plasma active renin and angiotensin II concentrations ($p < 0.01$). Patients with a higher basal pressure were likely to have a greater percentage fall in pressure ($p < 0.05$), as were those with secondary hypertension ($p < 0.05$). There was a positive correlation between the percentage fall in blood pressure and the dose of diuretic in those with secondary hypertension ($p < 0.01$) but not in those with essential hypertension (Figure 6). There were negative correlations between the percentage fall in blood pressure and both serum sodium concen-

| | Essential | Secondary | Total | N |
|-----------------------|-----------|-----------|---------|----|
| Plasma Renin | 0.315 | 0.592** | 0.466** | 55 |
| Plasma AII | 0.563* | 0.454 | 0.519** | 34 |
| Basal B. P. | 0.170 | 0.322 | 0.253* | 65 |
| Serum Na ⁺ | 0.097 | -0.242 | -0.060 | 65 |
| TBNa | -0.301 | -0.055 | -0.212 | 22 |
| Age | -0.015 | 0.396* | 0.197 | 65 |
| Serum Creat. | 0.218 | -0.107 | 0.083 | 65 |
| Diuretic dose | -0.269 | 0.534** | 0.121 | 65 |

* p < 0.05
** p < 0.01

Table 3.

Spearman's rank correlations comparing several pre-treatment variables with percentage change in mean arterial pressure following the first dose of captopril.

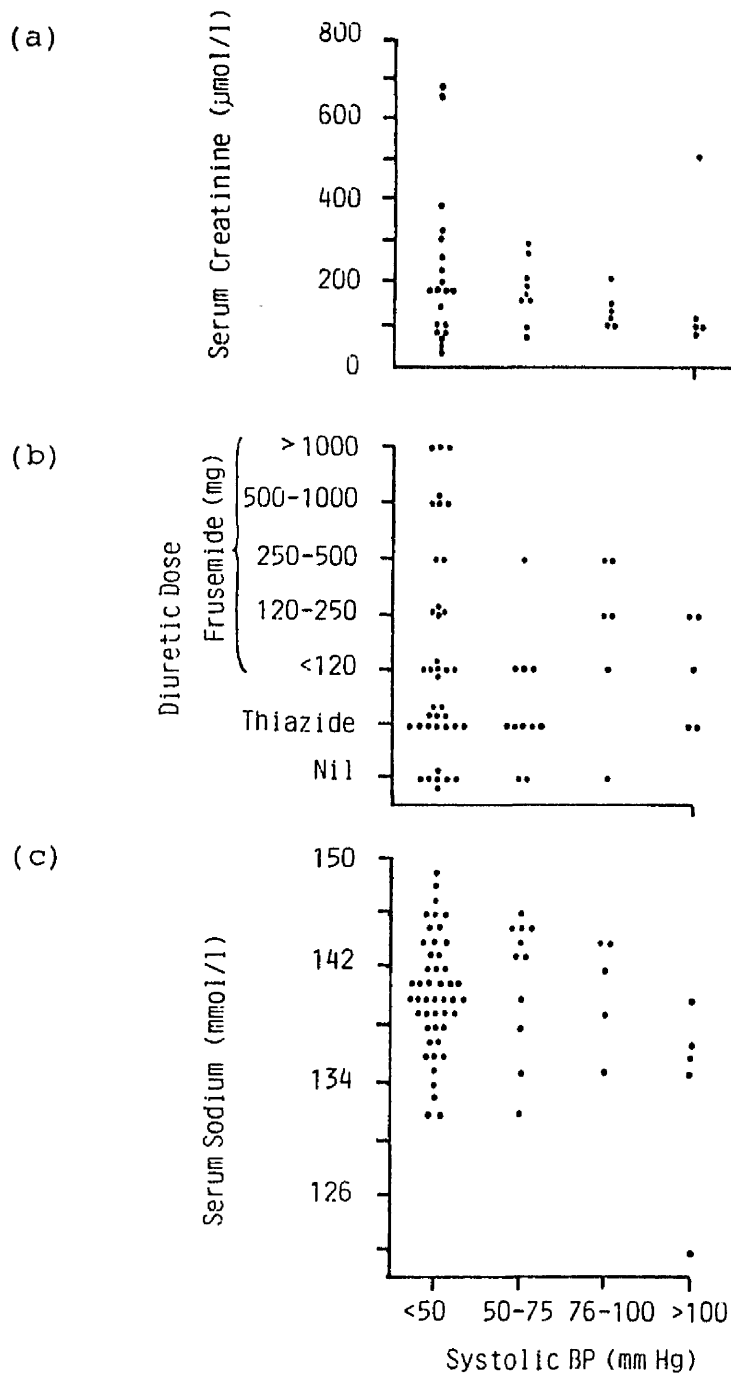


Figure 6.

The relationships in 65 severely hypertensive patients between the acute fall in systolic blood pressure following captopril and pre-treatment (a) renal function, (b) previous diuretic dose and (c) serum sodium.

tration and total body sodium, but these were not significant. Age and renal function did not appear to influence the fall in pressure, which was also independent of the dose of captopril given.

Correlations between some physiologically related variables were also examined. There was a significant negative correlation of serum sodium concentration with plasma renin concentration ($r = -0.447$, $p < 0.001$) but not with plasma angiotensin II ($r = -0.247$), total body sodium ($r = 0.184$), serum creatinine concentration ($r = 0.081$), or the dose of diuretic given as previous treatment. Plasma renin concentration correlated significantly with plasma angiotensin II concentration ($r = 0.79$, $p < 0.01$) but not with total body sodium ($r = 0.052$) or serum creatinine concentration ($r = 0.248$).

Testing different combinations of measurements against the percentage fall in blood pressure did not show any increase in the ability of individual measurements to predict the fall.

CHAPTER 7

CAPTOPRIL AND DIURETIC IN THE LONG-TERM TREAT- MENT OF SEVERE HYPERTENSION

SUMMARY

The efficacy and tolerability of the long-term use of captopril and diuretic was assessed in 70 patients with severe hypertension. Forty-four patients were treated for 6 months when supine blood pressure had fallen from $186/109 \pm 4/2$ mmHg on previous therapy to $149/93 \pm 3/2$ mmHg on captopril. Two (4%) patients achieved good blood pressure control on captopril alone while seventeen (38.6%) were controlled on a combination of captopril and diuretic. Seven (18%) required the addition of a third antihypertensive drug and 16 (36.3%) required a beta-blocker for drug-induced tachycardia.

Captopril was discontinued in 14 (20%) of the 70 patients because of drug related side-effects which included taste loss (13 patients), skin rash (4 patients) and nephrotic syndrome (3 cases). There was no significant change in mean serum sodium, serum potassium or serum creatinine.

Despite the high incidence of side-effects and adverse reactions captopril was effective and well-tolerated.

PATIENTS

These 70 patients, aged 15-73, including 44 women, were studied after various combinations of conventional antihypertensive agents had failed to control their blood pressure (arbitrarily defined as less than 160/100 mmHg supine), or had caused intolerable side-effects.

Forty-four patients were treated for 6 months, of whom 28 had essential hypertension and 16 had a secondary form of hypertension. Of these, 8 had renal artery stenosis or occlusion and 8 had other forms of renal disease, including scleroderma, analgesic nephropathy, diabetic nephropathy and shrunken kidneys of unknown aetiology. Twelve patients had previously had malignant phase hypertension.

Before captopril was given, the 44 patients were receiving an average of 2.9 antihypertensive drugs daily, not including potassium supplements given to 8 patients. Forty-one patients were receiving a diuretic and 40 patients were receiving a beta-blocker, 24 patients were receiving a third drug and 9 required a fourth drug.

This was an open study of a consecutive heterogeneous series of severe hypertensives. Other than the

presence of known glomerulo-nephritis or proteinuria in excess of 3G daily there were no treatment exclusion criteria.

PROTOCOL

INPATIENT

- (a) The patients were admitted to the ward and all antihypertensive drugs were stopped.
- (b) Routine evaluation including chest x-ray, cardiograph, full blood count, serum electrolytes, serum creatinine and urine protein was made.
- (c) On the following day the first dose of captopril was given under the standard conditions and with the precautions outlined in Chapter 6.
- (d) In the absence of a precipitous fall in blood pressure the patients were mobilised and prescribed the maximum dose of captopril with reductions in the presence of renal impairment.

The initial recommended maximum dose of 150 mg t.i.d. was given to the first 19 patients. This maximum was successively reduced to 100 mg t.i.d.,

- 100 mg b.d. and then to 50 mg b.d. in the face of several reports suggesting equal efficacy, with increased tolerability, in low-dose regimens.

- (e) After stabilisation in the ward the patients were discharged on captopril alone to be observed in the out-patient clinic.

OUT-PATIENT

- (a) Patients were seen at 1, 3, 6 and 12 weeks after discharge and then at 3 monthly intervals subsequently (with intermediate visits if required).
- (b) Therapy was adjusted to achieve a target blood pressure of less than 160/100 mmHg sitting. No change in the dose of captopril was made - blood pressure control was achieved by the addition of, initially, a thiazide diuretic or, if subsequently necessary, a loop diuretic (frusemide).
- (c) The maximum daily dose of frusemide given was 500 mg for patients with good overall renal function (serum creatinine $<125 \mu\text{mol/l}$), 1,000 mg for patients with mild to moderate renal impairment (serum creatinine 125-250 $\mu\text{mol/l}$) and 2,000 mg daily, in the presence of moderate to severe renal failure (serum creatinine $>250 \mu\text{mol/l}$). Thiazide diuretics were given once daily while frusemide was given twice daily (morning and noon).
- Increases of diuretic therapy were precluded in the presence of unacceptable adverse effects, including biochemical or symptomatic evidence of

volume depletion, and intolerable urinary frequency or nocturia.

- (d) If, despite treatment with the maximum/tolerable doses of captopril and diuretic, blood pressure remained poorly controlled, a third antihypertensive agent (prazosin, maximum dose 10 mg b.d.) was added.
- (e) In the presence of drug induced tachycardia, a beta-blocker (atenolol 100 mg i.d.) was added to therapy whether blood pressure was controlled or not. Two patients had angina and received atenolol 100 mg and nifedine 10 mg t.i.d., respectively, throughout the study.
- (f) Blood pressure and heart rate were measured in the out-patient clinic after 5 minutes sitting, and after 2 minutes standing, using a standard mercury sphygmomanometer.
- (g) After 6 weeks, and at 3 monthly intervals subsequently, safety analyses including white cell count, serum electrolytes and creatinine, and urine testing for protein were made. If significant proteinuria (Albustix greater than "trace")
 - was demonstrated on fresh sample voided in the clinic, a 24 hour urine collection was then performed for accurate quantitation.

RESULTS

FIRST-DOSE HYPOTENSIVE RESPONSE

The response to the first dose of captopril is described in Chapter 6. Of the patients who suffered a profound fall of blood pressure, all except one (Case 6) were subsequently stabilised on captopril therapy without continued hypotension.

LONG-TERM TREATMENT

Seventy patients entered the study. Within 6 months of starting captopril, 9 patients with renal artery stenosis or occlusion underwent nephrectomy; 14 were withdrawn because of drug-related side-effects, 1 died of purulent meningitis and 2 were lost to follow-up.

(a) Blood pressure.

After 6 months of therapy, blood pressure for the remaining 44 patients had fallen from $186/109 \pm 4/2$ mmHg to $149/93 \pm 3/2$ mmHg. Corresponding figures in the standing position were $174/108 \pm 6/3$ falling to $134/95 \pm 8/2$ mmHg ($p < 0.001$ for both comparisons) (Figure 7). In the group with essential hypertension blood pressure fell from $179/106 \pm 6/3$ to $146/92 \pm 3/2$ mmHg. Those with secondary hypertension had a fall in blood pressure

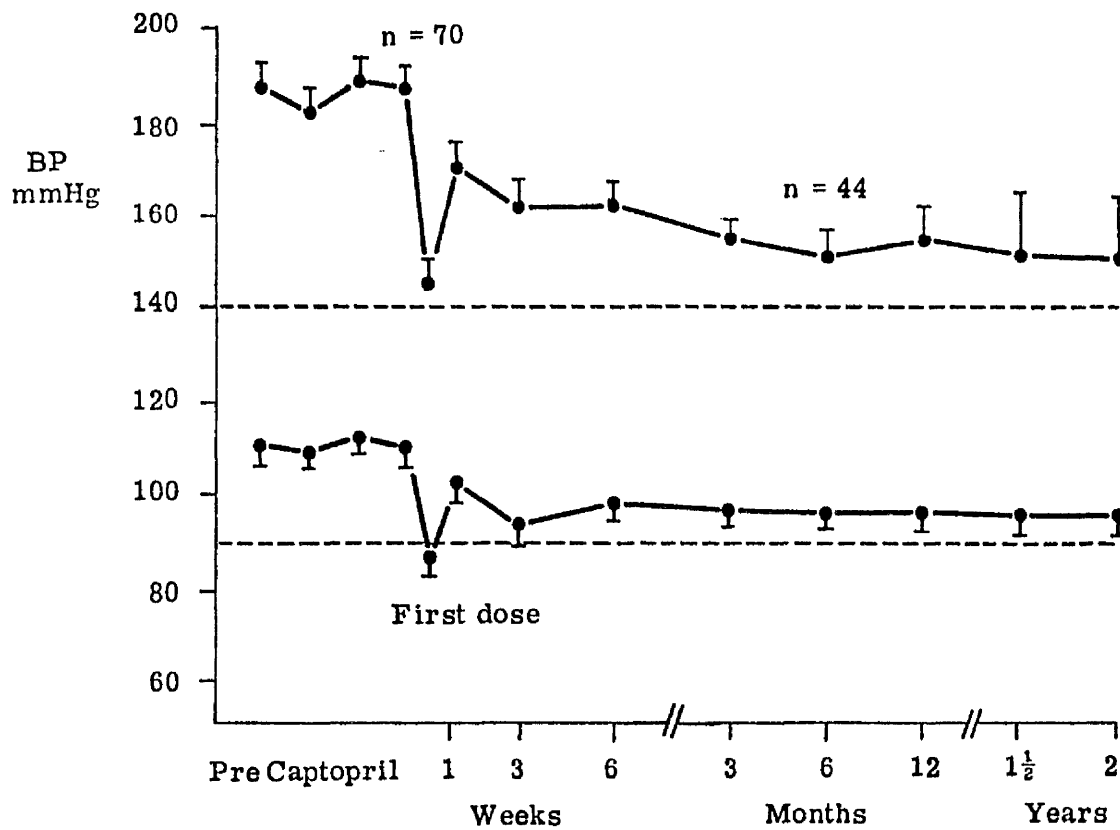


Figure 7.

The long-term blood pressure response of 70 patients treated with captopril and diuretic. The first 3 points are the last outpatient recordings on previous therapy. The next 3 points are recordings made, respectively, immediately before and after the first dose of captopril, and immediately before discharge from the ward.

from 193/112 \pm 7/5 mmHg to 155/95 \pm 6/4 mmHg with comparable reductions in the standing position. No postural effects were observed.

The quality of blood pressure control was not related to renal function and was independent of the dose of captopril given (range 25-450 mg daily). After 6 months treatment the group were receiving an average of 2.6 drugs daily, excluding potassium supplements or sparing agents given to 4 patients.

Two (4%) patients, both with renal artery stenosis, had achieved good blood pressure control after 6 months on captopril alone, while 17 (38.6%) were controlled on a combination of captopril and diuretic. Seven (18%) required the addition of prazosin (range 1-12 mg daily, in divided doses) because of poor blood pressure control.

Two patients required a beta-blocker because of ischaemic heart disease but 16 (36.3%) required a beta-blocker for tachycardia. Tachycardia was defined as a persistent heart rate in excess of 100 beats/minute, with or without palpitations or symptoms of volume depletion. In all cases it occurred in patients receiving loop diuretic in addition to captopril.

Five (11%) patients required a thiazide diuretic to control blood pressure but 36 (82%) required a loop diuretic, usually in high dose (mean dose frusemide 370 mg daily).

(b) Renal function and serum electrolytes.

The renal function of the group overall did not change significantly over the 6 month period (mean 218 ± 30 $\mu\text{mol/l}$ before captopril, 215 ± 26 $\mu\text{mol/l}$ after 6 months captopril). In 2 patients there was a significant deterioration in renal function related in one case to the development of drug-induced glomerulonephritis (described in detail later).

There were no significant changes in serum sodium (140 ± 0.5 mmol/l before captopril, 140 ± 0.6 mmol/l after 6 months), or in serum potassium (3.9 ± 0.1 mmol/l before captopril, 4.0 ± 0.06 mmol/l after 6 months).

(c) Side-effects.

Captopril was discontinued in 14 (20%) patients because of drug-related side-effects. Taste loss occurred in 13 patients after an average of 6 weeks on captopril (range 2-16 weeks). In 9 of these there was no improvement when captopril was continued at a lower dose, and taste returned slowly to normal for up to 3 months after stopping the drug. No cases of the

"scalded mouth" syndrome were seen. A maculopapular rash developed in 4 patients between 4 weeks and 4 months of therapy, there being complete resolution within 1 week of stopping captopril in 3 cases. In the fourth patient, captopril was continued and the skin rash resolved during treatment with a topical steroid.

There were no cases of leucopenia but 3 cases became nephrotic and 2 patients also taking cimetidine developed polyneuropathy. These and the relationships between side-effects, drug dosage and renal function are discussed in Chapter 8.

CHAPTER 8

CAPTOPRIL: RELATIONSHIP BETWEEN SIDE-EFFECTS, DAILY DOSE AND RENAL FUNCTION

SUMMARY

The side effects experienced by 100 consecutive patients taking captopril for severe hypertension or cardiac failure were analysed in relation to daily dose and to renal function. Three cases of proteinuria and two of neuropathy developed in patients who were taking high doses of captopril and had impaired renal function. Taste loss (14 cases) and skin rash (7) occurred independently of daily dose and of renal function in this study. Our current practice is to use a maximum of 100 mg captopril daily in patients with normal renal function, and to reduce the dose further in the presence of renal failure.

PATIENTS AND METHODS

Seventy-two patients, including 45 females, with severe hypertension received captopril. Sixty-three were also given a diuretic and 29 required a third anti-hypertensive drug, either atenolol or prazosin. Forty-three patients had essential hypertension, 20 had renovascular disease, hypertension in the remainder being due to other causes, including diabetic nephro-

pathy, chronic pyelonephritis and analgesic nephropathy. Twenty-eight patients, including 12 females, with grade 3 or 4 congestive cardiac failure (New York Heart Association criteria)⁽⁴¹³⁾ resistant to digoxin and frusemide together, also received captopril. The cause of heart failure was considered to be ischaemic heart disease (15 patients), valvular heart disease (6 patients), alcoholic cardiomyopathy (2 patients) and idiopathic cardiomyopathy (5 patients). The mean age for the whole series was 53 years (range 15-79 years) and the mean serum creatinine at the start of treatment was 170 $\mu\text{mol/l}$ (range 54-895 $\mu\text{mol/l}$). The first 19 hypertensive patients were given captopril 450 mg daily; latterly the maximum dose was reduced to 100 mg daily, with lower doses for patients with renal impairment. Patients with cardiac failure, most of whom were normotensive, received lower doses of captopril (mean \pm SD = 86 \pm 42 mg daily) than those with hypertension (246 \pm 145 mg daily). At the time of writing, 65 patients had taken captopril for at least 6 months. Statistical analysis was performed using GLIM 3 (generalised linear interactive modelling release 3)⁽⁴¹⁴⁾ on an ICL 2976 computer.

RESULTS

Mean blood pressure for the hypertensive patients fell from 183/109 mmHg to 150/93 mmHg six months after

starting captopril, and most tolerated their therapy well. Similarly, clinical improvement occurred in 25 out of 28 patients with congestive cardiac failure following the addition of captopril. Twelve patients, all of whom had severe vascular disease, died during the study. There were, in addition, 28 withdrawals from treatment. Improved blood pressure control after nephrectomy or the start of regular dialysis led to withdrawal of captopril in 12 hypertensive patients, while one patient with congestive cardiac failure secondary to mitral valve disease was able to discontinue the drug postoperatively; in 14 others captopril was stopped because of drug-related side-effects.

Minor side-effects.

Taste loss occurred in 14 patients (14%) after a mean of 6 weeks on captopril (range 1 week to 4 months). In 5 patients captopril was immediately discontinued. In the remaining 9 patients there was no improvement when captopril was continued at a lower dose, and taste returned slowly to normal for up to 3 months after stopping the drug.

• Skin rash developed in 7 patients (7%). In 6 of these it was an early side-effect occurring between 10 days and 4 months of therapy, while in 1 patient its appearance was delayed until 11 months. The skin

rash disappeared completely within 1 week of stopping captopril in 6 cases. In the 7th patient, captopril was continued and the rash resolved during treatment with a topical steroid. Moreover, skin rash did not recur in 3 patients when captopril was reintroduced at a lower dose, and in one other who was treated subsequently using enalapril. No cases of the recently reported "scalded mouth" syndrome were seen⁽⁴¹⁵⁾.

Serious side-effects.

Agranulocytosis. No case was seen in this study.

Neuropathy developed in 2 patients taking captopril and cimetidine⁽⁴²⁶⁾. Since there have been no other reports of neurological dysfunction, a causal relationship with either drug or with the combination is uncertain.

Proteinuria in excess of 3 grammes daily developed in 3 patients during captopril treatment, with proteinuria of 7.3, 17.7 and 12.2 g daily, first noted after 2, 3 and 11 months respectively (Table 4). Two of these patients had pre-existing but milder proteinuria (<1.5 g daily) and both underwent renal biopsy. The features on light microscopy were those of marked ischaemia, presumed secondary to long-standing hypertension. Immunofluorescence showed granular staining for IgG and C3 around capillary loops in one of the biopsies, but no significant deposition of immunoglobulins or

CAPTOPRIL INDUCED PROTEINURIA

| <u>Patient</u> | <u>Diagnosis</u> | <u>Daily dose (mg)</u> | <u>Proteinuria (g/24 hr)</u> | <u>Onset</u> | <u>Serum creatinine (μmol/l)</u> | <u>Biopsy</u> |
|----------------|-----------------------|------------------------|------------------------------|--------------|---|---|
| M.S. | Essential | 450 | 1.5 + 17 | 3 months | 200 + 188 | LM: Non-specific changes IF: Granular IgG, C ₃ . EM: Not done. |
| M.C. | Renal artery stenosis | 450 | 0.21 + 7.3 | 2 months | 200 + 340 | LM: Ischaemic changes IF: Negative EM: Negative |
| M.F. | Renal artery stenosis | 300 | 0 + 10G | 11 months | 110 + 340 | None obtained. |

Table 4. Changes in renal function and protein excretion with biopsy appearances in 3 patients with nephrotic syndrome induced by captopril.

LM : light microscopy
EM : electron microscopy.

IF : immunofluorescence

complement in the other (a nephrectomy specimen from a patient with renovascular disease). No glomeruli were present in either of the tissue cores submitted for electron microscopy.

Case report.

This 43 year old Dominican woman was first discovered to be hypertensive with blood pressure 180/115 mmHg in 1970, at which time investigations including urinalysis, U & E, IVP and renal arteriography were normal. Failure to achieve adequate control of blood pressure on a variety of medications including beta-blockers, vasodilators, methyldopa and diuretics led to a trial of captopril. On captopril 450 mg daily and frusemide 500 mg daily, blood pressure fell to 134/96 mmHg. However, three months later she complained of pain in her right loin and was found to have developed nephrotic syndrome. The results of further investigations included negative antinuclear factor, serum C3 1,678µg/ml (normal range 720 to 1,800), serum C4 1,364 µg/ml (normal range 199 to 574) and normal immunoglobulins. Renal venography showed no evidence of renal vein thrombosis. Renal biopsy was undertaken at which time captopril was withdrawn. The features on light microscopy were those of marked ischaemia presumed secondary to long-standing hypertension. Immunofluorescence showed granular staining for IgG and C3 around capillary loops, consistent with early membranous nephropathy.

No glomeruli were present in the tissue core submitted for electron microscopy. This patient's serum albumin has since returned to within the normal range although proteinuria remains variable from 1.9 to 12.2 g daily (Fig. 8). When last reviewed at the clinic she felt generally well although her blood pressure was once again poorly controlled at 188/128 despite minoxidil 10 mg daily, metoprolol 150 mg daily and frusemide 240 mg daily.

Relationship between side-effects, dose of captopril and renal function.

Patients experiencing serious side-effects (proteinuria and neuropathy), minor side-effects (taste loss and skin rash) and no side-effects are shown separately in Figure 9, where the continuous line gives the maximum daily dose of captopril recommended by the manufacturers for a particular level of renal function.⁽²⁷¹⁾ Clearly, serious side-effects occurred at doses of captopril greater than those now recommended for patients with renal impairment. The relationship between minor side-effects, daily dose of captopril and renal function (as measured by the reciprocal of serum creatinine) was investigated by the method of log linear modelling (GLIM 3). Neither dose ($x^2 = 0.06$; 1df) nor reciprocal creatinine ($x^2 = 0.18$; 1df) was related to minor side-effects when each was considered separately. Moreover, there was no improve-

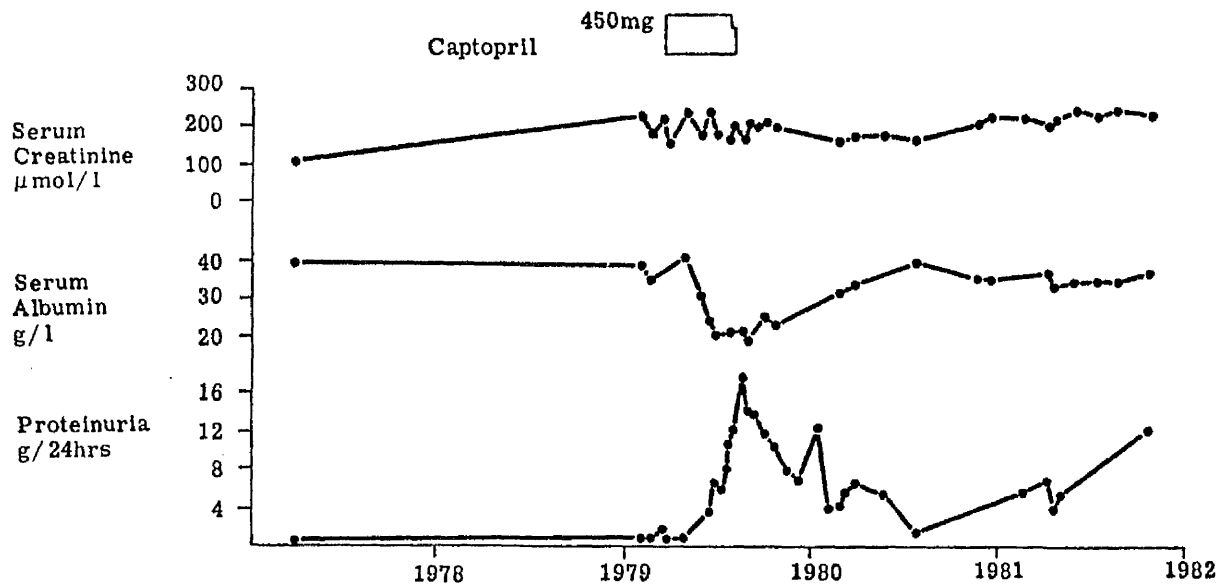


Figure 8.

Serial measurements of serum creatinine, serum albumin and urine protein in a 43 year old woman with nephrotic syndrome induced by captopril.

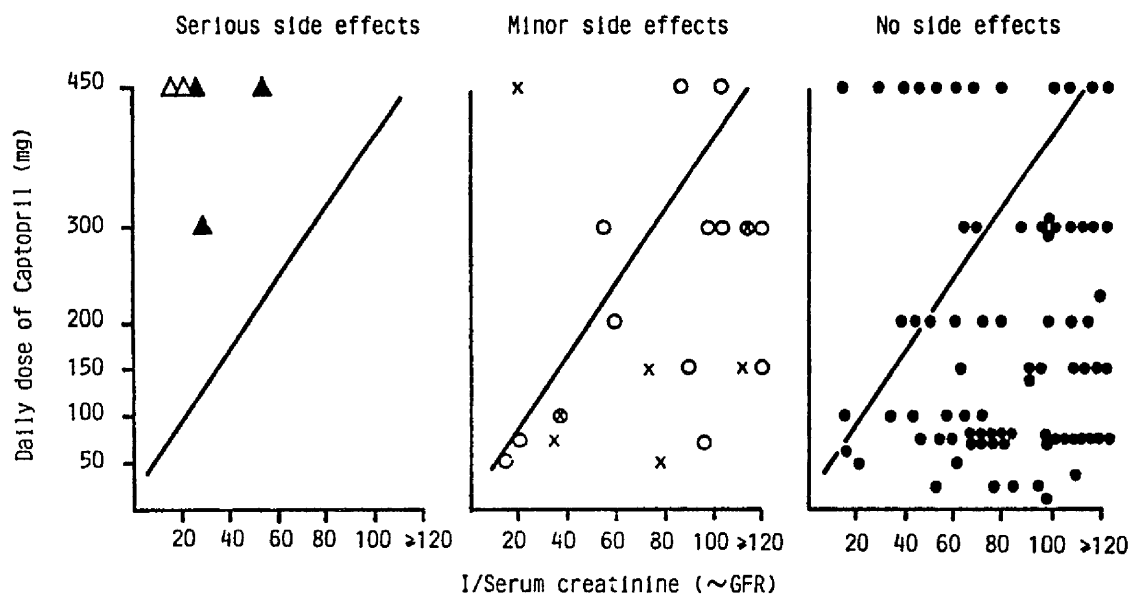


Figure 9.

The relationship between renal function, side-effects and daily dose of captopril in 100 patients. The continuous line gives the maximum daily dose of captopril recommended by the manufacturers for a particular level of renal function.

Key. ● = no side-effects, O = taste loss,
 X = skin rash, ⊗ = rash and taste loss
 in same patient, Δ = neuropathy,
 ▲ = nephrotic syndrome.

ment in fit when both variables were combined either as separate main effects ($\chi^2 = 0.25= 2df$) or as two main effects and an interaction term ($\chi^2 = 0.48; 3df$). In other words, these statistical techniques suggest that the minor side-effects, taste loss and skin rash, occurred independently of dose and renal function in this study.

CHAPTER 9

MOOD CHANGES DURING CAPTOPRIL THERAPY FOR HYPERTENSION

SUMMARY

A small double-blind pilot study was mounted to address the question whether captopril treatment in hypertension has a euphoriant effect. Eight patients were maintained on constant therapy of atenolol and bendrofluazide for at least 4 weeks before and throughout the study. Captopril 25 mg three times daily or matching placebo was administered double-blind for 6 weeks, with cross-over to placebo or captopril from weeks 7 to 12. Psychiatric assessment was made at weeks 3, 6, 9 and 12. During the captopril phase, blood pressure was reduced, plasma angiotensin II lowered, and plasma renin raised. Mood was slightly, but significantly, lower during captopril administration. There was thus provided no evidence of a euphoriant effect of captopril. This pilot trial also indicates the feasibility of the approach, and such studies of hypertensives under therapy could and should be usefully extended and refined.

PATIENTS

Eight patients (4 female and 4 male, mean age 51), with moderately severe hypertension, entered the study

after informed consent was obtained. Patients were excluded if there was a history of cerebro-vascular accident; evidence of organic brain damage or impairment; a history of schizophrenia or affective psychosis; if they had received any psychotropic medication other than benzodiazepines within 3 months of entry; if they had heart failure; or severe renal impairment.

Apart from captopril, the only antihypertensive drugs used during the study were atenolol and bendrofluazide. At least 4 weeks before the start of the trial, antihypertensive therapy was standardised and remained fixed for each subject throughout. One patient also received diazepam 5 mg/day for 4 weeks before the start and throughout the study.

PROTOCOL

- (a) Patients were seen at the ward and placed supine. Captopril/placebo 25 mg p.o. was given and blood pressure measured at 15 minute intervals for a minimum of 2 hours.
- (b) During the introduction of captopril/placebo facilities for reversal of acute hypotension were available (but were not required in the event).

- (c) Patients and observers were unaware of the treatment code though this was available for immediate use if necessary.
- (d) After 3 weeks patients were seen at the ward for safety analysis and measurement of blood pressure.
- (e) At 6 weeks patients were seen at the ward and therapy crossed over under identical conditions to week zero.
- (f) At 9 weeks patients were again seen for safety analysis and blood pressure measurement.
- (g) At 12 weeks the trial ended and antihypertensive therapy was changed, if necessary, at the discretion of the investigator.

Withdrawal criteria.

- (i) Blood pressure >120 mmHg diastolic.
- (ii) Intolerable side-effects - skin rash and taste loss were not absolute indications to stop therapy.
- (iii) Proteinuria or neutropenia.
- (iv) Development of heart failure, cerebral ischaemia or diminishing renal function.
- (v) Antihypertensive therapy changes (other than modifications of diuretic dose).

Safety analysis (weeks 0, 3, 6, 9, 12).

- (i) Erect and supine blood pressure with pulse rate.
- (ii) FBC, U & E, urine protein.

Blood measurements.

At weeks 3 and 9 after 30 minutes supine and 2 hours post-drug, blood was drawn for measurement of plasma renin and angiotensin II.

Tablet count.

At weeks, 3, 6, 9 and 12 a tablet count was made to confirm compliance.

Psychological assessment.

Psychological investigation was performed by Dr. John Callender, Department of Psychiatry, Duke Street Hospital, Glasgow. He assessed patients at weeks 3, 6, 9 and 12 before blood pressure measurements and blood samples were obtained. He and I were in separate rooms and there was no exchange of clinical information between us throughout the trial.

1. Sixty item Goldberg General Health Questionnaire (Table 5).

This scale was developed for the detection of psychiatric morbidity in general practice and hospital.

HAVE YOU RECENTLY:

| | | | | |
|--|-------------------|--------------------|------------------------|-----------------------|
| 1 - been feeling perfectly well and in good health? | Better than usual | Same as usual | Worse than usual | Much worse than usual |
| 2 - been feeling in need of a good tonic? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 3 - been feeling run down and out of sorts? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 4 - felt that you are ill? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 5 - been getting any pains in your head? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 6 - been getting a feeling of tightness or pressure in your head | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 7 - been able to concentrate on whatever you are doing? | Better than usual | Same as usual | Less than usual | Much less than usual |
| 8 - been afraid that you were going to collapse in a public place? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 9 - been having hot or cold spells? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 10 - been perspiring (sweating) a lot? | Not at all | NO more than usual | Rather more than usual | Much more than usual |
| 11 - found yourself waking early and unable to get back to sleep? | Not at all | NO more than usual | Rather more than usual | Much more than usual |

Table 5.

The first 11 questions of the sixty item Goldberg General Health Questionnaire. From left to right the columns are allocated respective scores of 0, 1, 2 and 3.

Subjects respond to 60 questions by selecting the most appropriate of 4 possible responses. The test provides a measure of psychological well-being in relation to depression, anxiety, somatic symptoms and ability to carry out normal functions⁽⁴¹⁶⁻⁴¹⁸⁾. It has been used to assess psychiatric morbidity before entry to the large Medical Research Council treatment trial of mild hypertension, and also the effect of participation in the trial on these aspects^(419,420).

2. Paced Auditory Serial Addition Task.

This is a test of recent memory and of ability to integrate information quickly. It assessed alertness and concentration with only slight dependence on arithmetical ability and general intelligence⁽⁴²¹⁾.

3. Rating Scale for Mania.

This detects and quantifies hypomanic features⁽⁴²²⁾.

RESULTS

Three of the eight patients received captopril before placebo; the other 5 placebo first.

No subject required intervention because of poor blood pressure control. There was no instance of proteinuria, leucopenia, electrolyte disturbance or skin rash. Taste impairment occurred in one patient

at week 11 while taking captopril.

Tablet counts were in all instances correct. In all patients plasma renin concentrations were higher and angiotensin II concentrations lower, during the period of captopril therapy. Comparing captopril with placebo overall, mean blood pressures were significantly lower ($164/98$ mmHg \pm $9/3$ SEM versus $176/101$ \pm $8/2$; $p < 0.05$), plasma active renin concentration was higher (50 ± 18 μ U/ml versus 23 ± 7 ; $p < 0.05$) and angiotensin II reduced (9.3 ± 1 pg/ml versus 15.1 ± 3 ; $p < 0.05$).

Psychological testing.

1. Goldberg Questionnaire.

With this method, a high score indicates psychiatric morbidity and a tendency towards mood diminution and depression.

(a) Overall score.

All except one subject had higher mean overall scores during the captopril than the placebo phase, and this difference was statistically significant ($p < 0.05$). (Figure 10 and Table 6).

(b) Subdivision of symptom scales.

Analysis was further pursued by considering separately those items addressing

| | <u>PATIENT</u> | <u>WEEK OF TREATMENT</u> | | | |
|-----|----------------|--------------------------|----|-----------|----|
| | | 3 | 6 | 9 | 12 |
| (a) | J.G. | 37 (34.5) | 32 | 18 (18.0) | 18 |
| | R.C. | 49 (44.5) | 40 | 33 (30.0) | 27 |
| | P.McC. | 20 (23.5) | 27 | 23 (22.5) | 22 |
| (b) | W.McG. | 59 (61.5) | 64 | 79 (85.0) | 91 |
| | J.McN. | 36 (50.0) | 64 | 55 (60.5) | 66 |
| | A.G. | 62 (68.0) | 74 | 72 (71.0) | 70 |
| | P.McB. | 40 (43.0) | 46 | 34 (32.5) | 31 |
| | M.J. | 23 (22.0) | 21 | 52 (68.5) | 85 |

Table 6.

Goldberg General Health Questionnaire: scores after 3, 6, 9 and 12 weeks of study. Patients (a) received captopril followed by placebo and patients (b) received the converse. Figures in parentheses are the mean scores at weeks 3 and 6, and 9 and 12 respectively.

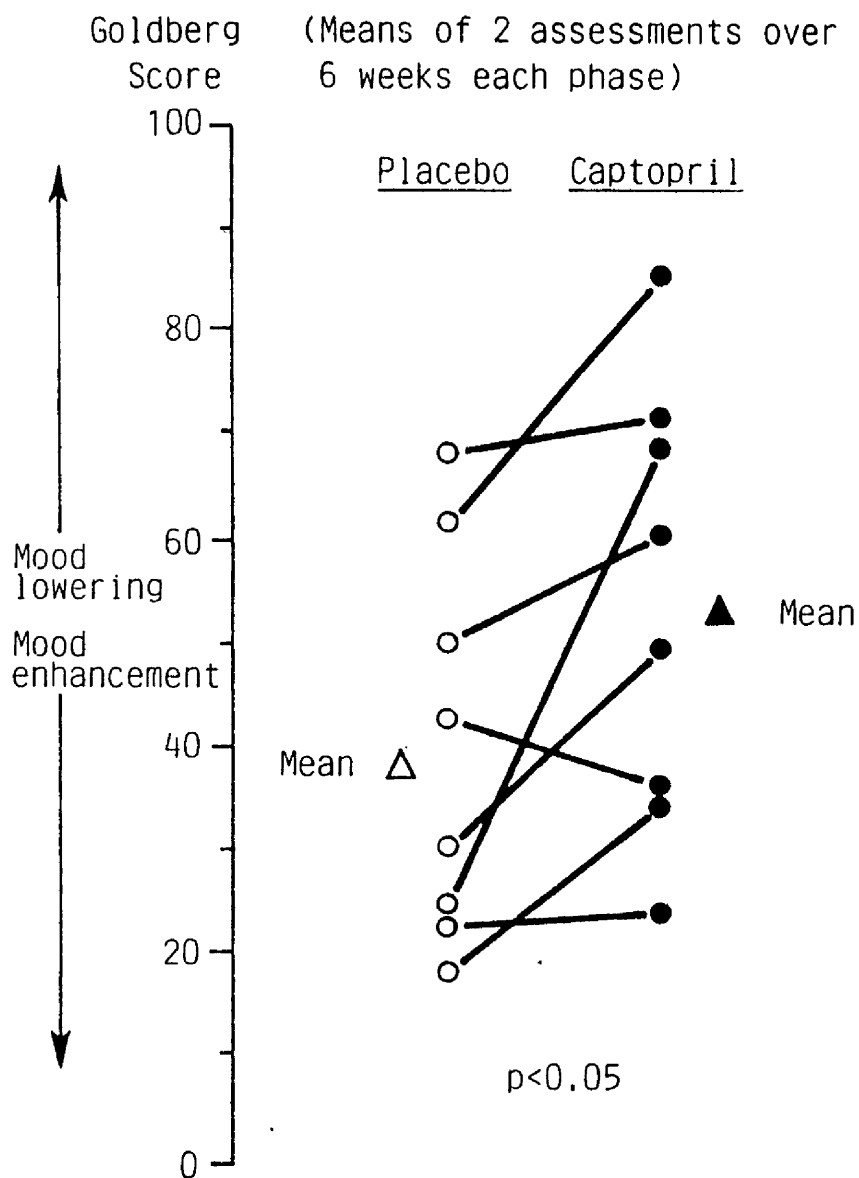


Figure 10.

The mood changes in 8 patients assessed by the Goldberg General Health Questionnaire.

- i) Somatic symptoms
- ii) Anxiety and insomnia
- iii) Social dysfunction
- iv) Severe depression.

In all these subgroups of questions, except those dealing with depression, average scores were higher during the captopril than during the placebo phase, although the differences did not achieve conventional levels of statistical significance. Only the subscale of questions dealing with depression appeared to be making no substantial contribution to the total.

2. Paced Auditory Serial Addition Task.

This test provided no evidence of any differences between captopril and placebo.

3. Mania Rating Scale.

No subject at any time had a score on this scale suggestive of hypomanic illness.

CHAPTER 10

ENALAPRIL (MK421) AND ITS LYSINE ANALOGUE (MK521): A COMPARISON OF ACUTE AND CHRONIC EFFECTS ON BLOOD PRESSURE, RENIN-ANGIOTENSIN SYSTEM AND SODIUM EX- CRETION IN NORMAL MAN

SUMMARY

The immediate and long-term effects of enalapril (MK421) and its lysine analogue (MK521), in once daily dosage, were compared in a study of 12 normal subjects.

Both compounds lowered blood pressure equally throughout 24 hours without causing tachycardia. The biochemical changes with MK521 were more sustained than with MK421, but this did not affect the magnitude of blood pressure reduction. There was an early natriuresis with each compound but this effect was no longer apparent after 8 days of continuous therapy.

Both MK421 and MK521 were well tolerated with no serious side-effects.

SUBJECTS

Twelve healthy, normotensive male volunteers, aged 23-39 years, weighing between 61 and 90 kg (mean 73 ± 2

kg) were included in the study, which was approved by the hospital Ethical Supervisory Committee. The nature of the experiment was explained to each volunteer and written informed consent was obtained. Each had a medical history taken and a physical examination performed. None had a family history of hypertension. For safety purposes, routine laboratory investigations were performed before and during administration of the experimental drugs.

PROTOCOL

- (a) For three days before, and for the eight days of the experiment, the subjects were maintained on a fixed metabolic diet containing sodium 150 mEq and potassium 70 mEq daily.
- (b) Meals were given at 0900, 1200 and 1800, and the constituents were the same throughout each treatment period. Alcohol and strenuous exertion were prohibited.
- (c) On the morning of days 1 and 8 the subjects attended the ward at 0800 after an overnight fast and lay supine. At 0850 a blood sample was drawn. Blood pressure and pulse rate were then measured, supine, and after 2 minutes standing, using a Hawksley random zero sphygmomanometer. The bladder was then emptied and the urine discarded.

- (d) The experimental drug was given at 0900, following which the subjects were mobilised and given a light breakfast.
- (e) At 1400 the subjects again lay supine. At 1500 a further blood sample was drawn, followed by measurement of pulse rate and blood pressure.
- (f) The subjects were again mobilised to attend the ward at 0800 on the following morning for repeat measurements as above. Drug was given at 0900 on all study days for 8 days.
- (g) On days 1 and 8 at times zero and again 6 hours and 24 hours after drug administration, measurements were made of plasma active renin concentration, blood angiotensin I, plasma angiotensin II, plasma aldosterone, plasma cortisol and serum converting enzyme activity (ACE). As the antibody used for the angiotensin II assay reacts mol for mol with angiotensin III, the angiotensin II assay gives also some assessment of angiotensin III.
- (h) Urine was collected for 24 hours, from 0900 to 0900 on days 1 and 8, and each collection divided into two time periods, 0-8 hours and 8-24 hours.
- Measurement was made of urine volume, sodium, potassium, creatinine and protein. Five subjects completed continuous 24 hour urine collections

for each of the 8 days of the experiment.

DRUG ADMINISTRATION

Capsules containing 10 mg of enalapril maleate (MK421), lysine analogue (MK521) or placebo were supplied by Merck, Sharp & Dohme and Company, Rahway, New Jersey. Each subject received an 8 day course of all 3 drugs allocated to a Latin Square design in strictly double-blind fashion. A single oral dose was given at 0900 for 8 days with a minimum period of 3 weeks between each experimental period.

STATISTICAL METHODS

The results were initially analysed using repeated measures analysis of variance, and the results presented are the follow-up multiple comparisons which are based on paired t-tests after logarithmic transformation of the data where appropriate. Each test compares the changes on one drug with the changes on another drug, with one test, for example, comparing the change in supine systolic blood pressure from day 1, 0 hours, to day 1, 6 hours, on MK421 with the corresponding change on placebo. Significant results are stated either as being significant as an individual test or as being significant after making a Bonferroni correction to allow for the fact that 15 comparisons are made on each variable. The analysis of the

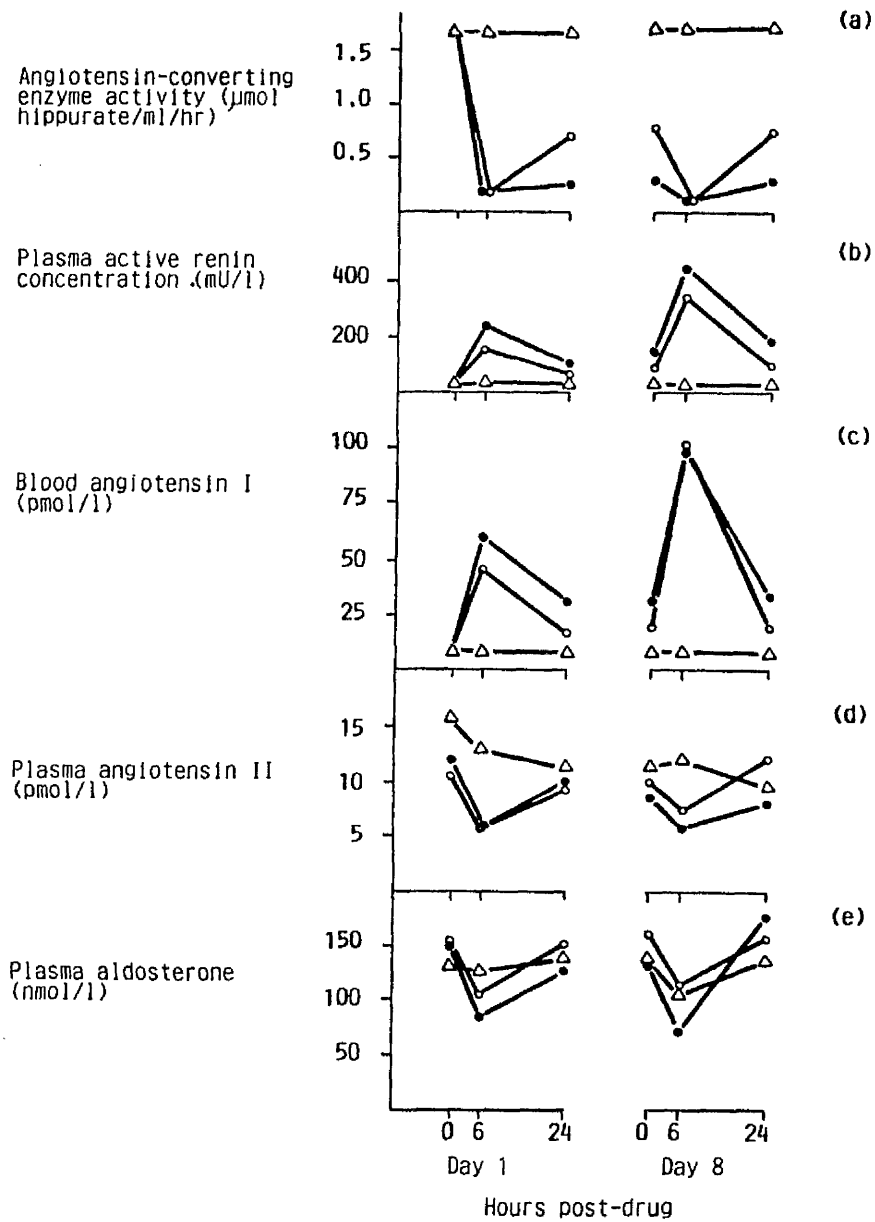


Figure 11.

Changes in components of the renin-angiotensin system during the administration of placebo, MK421 and MK521 in 12 subjects.

Key. placebo $\Delta - \Delta$
 MK421 $\circ - \circ$
 MK521 $\bullet - \bullet$

| | DAY 1 | | | DAY 8 | | |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | Basal | 6 hours | 24 hours | Basal | 6 hours | 24 hours |
| ACE μmol hippurate /ml/hr | 1.66 (0.10) | 1.62 (0.09) | 1.63 (0.09) | 1.63 (0.11) | 1.62 (0.11) | 1.64 (0.10) |
| RENIN mU/l | 27 (5) | 31 (8) | 24 (4) | 20 (3) | 19 (3) | 23 (3) |
| ANGIOTENSIN I pmol/l | 7.2 (0.6) | 8.8 (1.2) | 7.9 (0.7) | 7.5 (1.0) | 7.8 (0.8) | 6.8 (0.8) |
| ANGIOTENSIN II pmol/l | 15.5 (4.2) | 12.9 (1.3) | 11.3 (1.2) | 11.3 (1.2) | 12.2 (1.5) | 9.6 (0.8) |
| ALDOSTERONE pmol/l | 130 (18) | 125 (15) | 138 (20) | 138 (18) | 103 (13) | 138 (23) |
| CORTISOL nmol/l | 423 (41) | 228 (17) | 379 (39) | 404 (52) | 240 (19) | 400 (26) |
| SUPINE SYSTOLIC B.P. mmHg | 108.5 (2.9) | 112.3 (1.7) | 107.7 (2.6) | 106.2 (2.7) | 107.8 (2.6) | 108.8 (2.4) |
| SUPINE DIASTOLIC B.P. mmHg | 66.0 (1.9) | 69.0 (2.5) | 64.8 (1.6) | 61.5 (2.6) | 66.3 (2.3) | 64.0 (2.0) |
| SUPINE PULSE min ⁻¹ | 63.0 (2.9) | 65.2 (3.5) | 61.0 (1.9) | 62.2 (2.4) | 58.8 (1.9) | 60.7 (2.7) |
| ERECT SYSTOLIC B.P. mmHg | 107.0 (3.2) | 112.8 (3.0) | 111.8 (3.2) | 110.0 (3.9) | 105.8 (2.6) | 109.3 (2.5) |
| ERECT DIASTOLIC B.P. mmHg | 71.0 (2.3) | 76.7 (2.3) | 75.5 (2.1) | 72.7 (3.0) | 67.0 (4.5) | 72.2 (2.1) |
| ERECT PULSE min ⁻¹ | 77.7 (4.2) | 80.0 (4.0) | 83.5 (3.8) | 79.3 (4.5) | 81.6 (3.5) | 86.3 (5.5) |

Table 7. Mean (±SEM) changes in components of renin-angiotensin system, blood pressure and pulse rate during placebo.

| | DAY 1 | | | DAY 8 | | |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | Basal | 6 hours | 24 hours | Basal | 6 hours | 24 hours |
| | | | | | | |
| ACE μmol hippurate /ml/hr | 1.66 (0.10) | 0.15 (0.02) | 0.69 (0.08) | 0.80 (0.12) | 0.15 (0.02) | 0.78 (0.07) |
| RENIN mU/l | 24 (3) | 147 (24) | 59 (5) | 93 (15) | 336 (67) | 92 (10) |
| ANGIOTENSIN I pmol/l | 7.2 (0.6) | 46.6 (8.5) | 17.3 (2.5) | 21 (3.7) | 102 (32) | 19.4 (3.1) |
| ANGIOTENSIN II pmol/l | 10.5 (1.3) | 5.5 (1.0) | 9.3 (0.9) | 10.0 (1.0) | 7.3 (0.9) | 12.2 (0.9) |
| ALDOSTERONE pmol/l | 155 (25) | 103 (23) | 153 (25) | 160 (35) | 113 (33) | 158 (30) |
| CORTISOL nmol/l | 442 (37) | 218 (16) | 392 (42) | 455 (44) | 224 (19) | 425 (30) |
| SUPINE SYSTOLIC B.P. mmHg | 113.5 (3.8) | 102.3 (2.2) | 102.8 (2.4) | 101.5 (1.8) | 102.6 (2.9) | 102.8 (1.6) |
| SUPINE DIASTOLIC B.P. mmHg | 66.7 (3.5) | 61.5 (1.6) | 58.7 (1.9) | 59.3 (2.0) | 60.5 (2.7) | 62.0 (2.3) |
| SUPINE PULSE min ⁻¹ | 64.0 (2.2) | 65.5 (2.9) | 62.3 (1.9) | 63.3 (2.6) | 66.7 (2.2) | 62.2 (2.9) |
| ERECT SYSTOLIC B.P. mmHg | 103.8 (3.3) | 97.0 (3.8) | 102.0 (3.6) | 102.8 (2.7) | 98.0 (3.1) | 104.7 (2.6) |
| ERECT DIASTOLIC B.P. mmHg | 73.8 (2.5) | 69.2 (3.1) | 68.8 (3.0) | 69.3 (2.7) | 69.8 (2.9) | 69.5 (2.1) |
| ERECT PULSE min ⁻¹ | 82.3 (3.7) | 84.5 (3.3) | 86.5 (4.1) | 86.7 (3.4) | 90.0 (4.5) | 85.7 (3.8) |

Table 8. Mean (\pm SEM) changes in components of renin-angiotensin system, blood pressure and pulse rate during MK421.

| • | DAY 1 | | | DAY 8 | | |
|-----------------------------------|---------------------------------|-------------|-------------|-------------|-------------|-------------|
| | Basal | 6 hours | 24 hours | Basal | 6 hours | 24 hours |
| | ACE μmol hippurate /mL/hr | 1.67 (0.12) | 0.15 (0.02) | 0.27 (0.04) | 0.30 (0.04) | 0.13 (0.02) |
| RENIN mU/l | 26 (3) | 226 (52) | 96 (12) | 158 (38) | 450 (135) | 191 (35) |
| ANGIOTENSIN I pmol/l | 8.0 (0.6) | 69 (21.8) | 32.2 (3.9) | 32.7 (6.2) | 90.2 (24.3) | 36.1 (5.3) |
| ANGIOTENSIN II pmol/l | 11.9 (1.4) | 5.7 (1.1) | 10.0 (2.6) | 6.5 (0.8) | 5.7 (1.0) | 8.1 (0.8) |
| ALDOSTERONE pmol/l | 150 (23) | 83 (20) | 128 (18) | 135 (18) | 68 (18) | 178 (43) |
| CORTISOL nmol/l | 390 (20) | 209 (18) | 383 (40) | 441 (29) | 250 (28) | 412 (31) |
| SUPINE SYSTOLIC B.P. mmHg | 110.2 (2.6) | 102.5 (16) | 99.0 (2.1) | 100.8 (3.4) | 102.8 (2.6) | 103.2 (2.5) |
| SUPINE DIASTOLIC B.P. mmHg | 68.6 (2.5) | 61.8 (2.2) | 58.8 (2.4) | 56.5 (4.1) | 60.3 (1.4) | 62.2 (1.4) |
| SUPINE PULSE min ⁻¹ | 62.6 (2.1) | 63.8 (2.4) | 62.0 (2.7) | 61.7 (2.3) | 61.5 (2.1) | 61.0 (3.0) |
| ERECT SYSTOLIC B.P. mmHg | 110.0 (3.1) | 96.0 (3.3) | 101.5 (2.6) | 96.6 (3.8) | 97.3 (2.0) | 104.0 (2.3) |
| ERECT DIASTOLIC B.P. mmHg | 78.2 (2.5) | 63.7 (3.1) | 69.0 (3.2) | 65.8 (4.3) | 67.0 (2.9) | 69.7 (2.0) |
| ERECT PULSE min ⁻¹ | 83.3 (4.2) | 86.2 (4.5) | 85.5 (4.4) | 84.5 (4.7) | 87.3 (4.2) | 84.5 (3.8) |

Table 9. Mean (±SEM) changes in components of renin-angiotensin system, blood pressure and pulse rate during MK521.

| | Day 1 | | Day 8 | | Day 1, 0 hours → Day 8, 0 hours |
|--------------------------|-----------|------------|-----------|------------|------------------------------------|
| | 0-6 hours | 0-24 hours | 0-6 hours | 0-24 hours | |
| ACE | ** | ** | ** | - | ** |
| Renin | ** | ** | ** | - | ** |
| Angiotensin I | ** | ** | ** | - | ** |
| Angiotensin II | * | - | * | * | - |
| Aldosterone | - | - | - | - | - |
| Cortisol | - | - | - | - | - |
| Supine systolic B.P. | ** | ** | - | - | * |
| Supine diastolic B.P. | * | - | - | - | - |
| Supine pulse | - | - | - | - | - |
| Erect systolic B.P. | * | - | - | - | - |
| Erect diastolic B.P. | * | - | - | - | - |
| Erect pulse | - | - | - | * | - |

Table 10. Comparison of changes on MK421 with changes on placebo.
* Significant at 0.05 level as an individual test.
** Significant at 0.05 level allowing for multiple comparisons.

| | Day 1 | | Day 8 | | Day 1, 0 hours → Day 8, 0 hours |
|--------------------------|-----------|------------|-----------|------------|------------------------------------|
| | 0-6 hours | 0-24 hours | 0-6 hours | 0-24 hours | |
| ACE | ** | ** | ** | - | ** |
| Renin | ** | ** | ** | - | ** |
| Angiotensin I | ** | ** | ** | * | ** |
| Angiotensin II | * | - | * | - | - |
| Aldosterone | * | - | - | - | - |
| Cortisol | - | - | - | - | - |
| Supine systolic B.P. | * | * | - | - | - |
| Supine diastolic B.P. | * | * | - | - | - |
| Supine pulse | - | - | - | - | - |
| Erect systolic B.P. | * | * | - | - | * |
| Erect diastolic B.P. | ** | * | - | - | * |
| Erect pulse | - | - | - | - | - |

Table 11.

Comparison of changes on MK521 with changes on placebo.

* Significant at 0.05 level as an individual test.

** Significant at 0.05 level allowing for multiple comparisons.

| • | Day 1 | | Day 8 | | Day 1, 0 hours → Day 8, 0 hours |
|--------------------------|-----------|------------|-----------|------------|------------------------------------|
| | 0-6 hours | 0-24 hours | 0-6 hours | 0-24 hours | |
| | ACE | - | ** | ** | |
| Renin | - | * | - | - | - |
| Angiotensin I | - | ** | * | - | * |
| Angiotensin II | - | - | - | - | - |
| Aldosterone | - | - | - | - | - |
| Cortisol | - | - | - | - | - |
| Supine systolic B.P. | - | - | - | - | - |
| Supine diastolic B.P. | - | - | - | - | - |
| Supine pulse | - | - | - | - | - |
| Erect systolic B.P. | - | - | - | - | - |
| Erect diastolic B.P. | - | - | - | - | - |
| Erect pulse | - | - | - | - | - |

Table 12.

Comparison of changes on MK421 with changes on MK521.

* Significant at 0.05 level as an individual test.

** Significant at 0.05 level allowing for multiple comparisons.

electrolyte results was based entirely upon paired t-tests, comparing each set of results on an active drug back to the corresponding placebo results. No correction was made to allow for the multiple comparisons.

RESULTS

The magnitude of the difference between the drug effects is shown in Tables 7 - 9, with the significance levels shown in Tables 10 - 12.

Blood pressure and heart rate.

Measurements of pulse and blood pressure obtained with both inhibitors are summarised in Tables 7 to 12. With both compounds there was a fall of approximately 10% in systolic and diastolic blood pressure 6 hours and 24 hours after the first dose. After 8 days of continuous administration, blood pressure remained slightly lower than on placebo at all time points. There was no further fall in blood pressure on standing with either compound.

Supine heart rate did not change with treatment and there were similar rises of pulse rate with both compounds and with placebo on standing.

Plasma converting enzyme activity.

Figure 11(a) illustrates the reduction in plasma converting enzyme activity following the administration

of both compounds. Activity decreased markedly to under 10% of basal values 6 hours after the first dose. At 24 hours 40% recovery of enzyme activity was apparent with MK421 but recovery was minimal with MK521. The differences in enzyme inhibition between the two active drugs at 24 hours were highly significant ($p < 0.01$). After 8 days of continued therapy, the pattern of dose-related change remained apparent.

Plasma renin and angiotensin I.

Figures 11(b) and 11(c) illustrate the rise in plasma active renin and blood angiotensin I with the first dose and after 8 days respectively of each compound. In each case the levels of renin and angiotensin I had failed to return to basal values after 24 hours. The rise associated with MK521 was greater than that with MK421 and for both drugs the rise at 6 hours was much greater at 8 days than on day 1.

At 24 hours after the last dose of each drug, plasma renin concentration was significantly higher on day 8 than on day 1 ($p < 0.025$), although there was no significant change in the concentration of angiotensin I.

Plasma angiotensin II.

Figure 11(d) illustrates the fall in plasma angiotensin II with each compound. Angiotensin II concentrations fell by approximately 50% 6 hours after the

first dose of each drug, with return to basal values after 24 hours. The same pattern was apparent after 8 days of treatment, although at all time points suppression appeared more complete with MK521.

Plasma angiotensin III.

The low levels of plasma angiotensin II during treatment with each drug similarly indicate low concentrations of angiotensin III (see Discussion).

Plasma aldosterone.

Figure 11(e) illustrates the fall in plasma aldosterone with each compound. Significant suppression, similar with each drug, occurred at 6 hours, with return to basal values, after the first dose and after 8 days treatment.

Plasma cortisol.

Plasma cortisol concentrations were significantly and similarly lower after 6 hours (1500 hours) with placebo and active drug in keeping with the known diurnal variation in cortisol secretion.

Electrolyte excretion.

Table 13 illustrated the changes in electrolyte excretion occurring with each compound. With each drug

| | | | Na ⁺ (mmol) | K ⁺ (mmol) | Creatinine (mmol) |
|-------|------------|---------|---------------------------|--------------------------|----------------------|
| DAY 1 | 0-8 hours | Placebo | 47 (5) | 40 (3) | 6.3 (0.6) |
| | | MK421 | 71 (8)** | 39 (3) | 6.4 (0.5) |
| | | MK521 | 63 (8)* | 37 (2) | 6.6 (0.7) |
| | 8-24 hours | Placebo | 63 (9) | 22 (2) | 9.9 (1.1) |
| | | MK421 | 64 (6) | 22 (2) | 10.3 (0.8) |
| | | MK521 | 77 (9) | 25 (2) | 12.4 (1.5) |
| DAY 8 | 0-8 hours | Placebo | 53 (6) | 36 (2) | 6.1 (0.3) |
| | | MK421 | 64 (2) | 41 (3) | 7.5 (0.5) |
| | | MK521 | 67 (10)* | 38 (3) | 5.9 (0.4) |
| | 8-24 hours | Placebo | 73 (5) | 21 (2) | 10.9 (0.5) |
| | | MK421 | 67 (6) | 24 (2) | 11.1 (0.6) |
| | | MK521 | 69 (9) | 25 (3) | 10.1 (0.7) |

Table 13.

Comparison of urine electrolyte and creatinine excretion during placebo, MK421 and MK521 (mean and SEM).

* Significantly different from placebo $p < 0.05$.

** Significantly different from placebo $p < 0.01$.

there was a significant increase in urinary sodium excretion within 24 hours of the first dose, although after 8 days this effect within a few hours of administration had largely disappeared. There was no change in the diurnal pattern of sodium excretion and there were no significant differences in urine creatinine or potassium excretion over any time period.

In five subjects cumulative measurements of urinary sodium excretion over the 8 day period showed a net sodium loss of 48 mmol (not significant) in excess of placebo with each drug (mean daily sodium excretion 133 ± 5 SEM mmol on placebo compared with 139 ± 4 mmol on MK421 and 139 ± 5 mmol on MK521).

Serum electrolytes

After 8 days there was a significant fall in serum sodium concentration during MK521 and a significant rise in serum potassium concentration during MK421 (Table 14).

Side-effects.

The drugs were well tolerated, although one subject noted slight gastric flatulence throughout the period of MK421 administration. Two subjects complained of light-headedness related to posture during administration of both active drugs, although blood pressure did not fall

| | | Na ⁺ (mmol/l) | | K ⁺ (mmol/l) | |
|-------|---------|-----------------------------|-------|----------------------------|--------|
| DAY 1 | Placebo | 142,7 | (0.8) | 3.87 | (0.07) |
| | MK421 | 141.4 | (0.5) | 3.89 | (0.08) |
| | MK521 | 141.4 | (0.5) | 3.95 | (0.10) |
| DAY 8 | Placebo | 141.6 | (0.6) | 3.80 | (0.05) |
| | MK421 | 141.1 | (0.7) | 3.99 ^{**} | (0.06) |
| | MK521 | 139.8 [*] | (0.5) | 3.85 | (0.09) |

Table 14.

Comparison of changes in serum electrolytes during placebo, MK421 and MK521 (mean and SEM).

* Significantly different from placebo $p < 0.05$.

** Significantly different from placebo $p < 0.01$.

measurably on standing. In each subject blood pressure was distinctly lowered and the symptoms were not considered to be specific drug side-effects.

There were no instances of taste loss, skin rash, glycosuria, leucopenia or proteinuria, nor were any biochemical abnormalities detected.

CHAPTER 11

ENALAPRIL (MK421) IN THE TREATMENT OF HYPERTENSION WITH RENAL ARTERY STENOSIS

SUMMARY

The converting enzyme inhibitor enalapril, in single daily doses of 10-40 mg, has been given to 20 hypertensive patients with renal artery stenosis. Enalapril effectively controlled hypertension long-term, and only 2 of the 20 required concomitant diuretic treatment. The blood pressure fall 6 hours from the first dose of enalapril was significantly related to the pre-treatment plasma concentrations of active renin and angiotensin II, and to the concurrent fall in angiotensin II. Blood pressure fell further with continued treatment; the long-term fall was not significantly related to pre-treatment plasma renin or angiotensin II. At 3 months, 24 hours from the last dose of enalapril, blood pressure, plasma angiotensin II and converting enzyme activity remained low, and active renin and angiotensin I high; 6 hours after dosing, angiotensin II had however fallen further. During prolonged therapy, the increase of active renin was proportionately greater than that of angiotensin I. Enalapril alone caused long-term reduction in exchangeable sodium, with slight but distinct increases in serum potassium, creatinine and urea. Enalapril alone did not

impair overall renal function in 5 patients with bilateral renal lesions despite effective blood pressure reduction. Enalapril was well tolerated with no serious side-effects.

Enalapril given once daily is effective in controlling hypertension associated with renal artery stenosis.

PATIENTS AND METHODS

All patients gave informed consent to the study which was approved by the hospital's Ethical Supervisory Committee. Two distinct study protocols were followed. Group 1 consisted of 10 patients with unilateral renal artery stenosis and good overall renal function. Previous antihypertensive therapy was withdrawn to permit detailed measurement of blood pressure, serum and body electrolytes and components of the renin-angiotensin system, during the administration of placebo, and then during long-term enalapril therapy. Group 2 comprised 10 patients with unilateral or bilateral renal artery disease, in whom severe or resistant hypertension necessitated the continuation of previous antihypertensive therapy up to the time of introduction of enalapril.

Group 1.

These 10 patients (aged 37-56; 2 women) all had unilateral renal artery stenosis or occlusion. On renal

arteriography, 9 had radiological evidence of atheromatous stenosis and one had fibromuscular hyperplasia (Figure 12). The diagnosis was reinforced by isotope renography and bilateral renal vein renin measurements in all patients. Seven also had divided ureteric catheter studies performed; this was not done in 2 patients who had renal artery occlusion on arteriography, and in 1 other case the procedure was technically unsatisfactory. All patients had normal serum electrolyte values and satisfactory overall renal function (mean serum creatinine $103 \pm 8 \mu\text{mol/l}$). Three of these patients had proteinuria (respectively 0.3, 0.5 and 1.3 gm/24 hours) at the time of introduction of enalapril. Seven of the 10 patients had previously poor blood pressure control on the combination of beta-blocker and diuretic, together with either hydralazine (3 cases), prazosin (1 case), minoxidil (1 case) or methyldopa (1 case). One patient had received diuretic alone with poor response and 2 patients had received no previous antihypertensive therapy. Mean outpatient blood pressure on previous treatment was $183/109 \pm 10/3 \text{ SEM mmHg}$.

All treatment was stopped at least 14 days before enalapril was started except in one patient who received prazosin alone⁽⁴²³⁾ until 2 days before enalapril was given. The patients were admitted and ate a normal ward diet; we have shown elsewhere that measurements of renin, angiotensin II and aldosterone made on this



Figure 12.

The arteriogram of a 50 year old woman with unilateral renal artery fibromuscular hyperplasia.

regimen are similar to those made on a strictly controlled sodium and potassium intake⁽⁴²⁴⁾. Matching placebo was given (single-blind; unknown to the patient) at 1000 hours each day for 5 days before active drug was administered; thereafter enalapril was given at 1000 hours each morning. Two patients received placebo for only 2 days before enalapril was given because of severely elevated blood pressure.

The first 3 patients were the subject of a dose-finding study and began with 1.25 mg of enalapril, which was then increased to 2.5 mg, 5 mg, 10 mg, 20 mg and 40 mg on successive days; these patients were then discharged taking 40 mg daily (Figure 13). After analysis of blood pressure and biochemical data in these 3, the subsequent patients were started on 10 mg enalapril daily, which was continued for each of the 6 days of inpatient stay. After discharge in all 10 patients the enalapril dose was adjusted until supine and erect blood pressures were below 140 mmHg systolic and 90 mmHg diastolic (phase V) 4 hours after the morning dose, or until a maximum dose of 40 mg daily was reached. No other drugs than enalapril were given in this study. After 3 months of treatment with enalapril the patients were readmitted and again studied under identical circumstances to those at the start of therapy.

ENALAPRIL DOSE-FINDING STUDY n=3

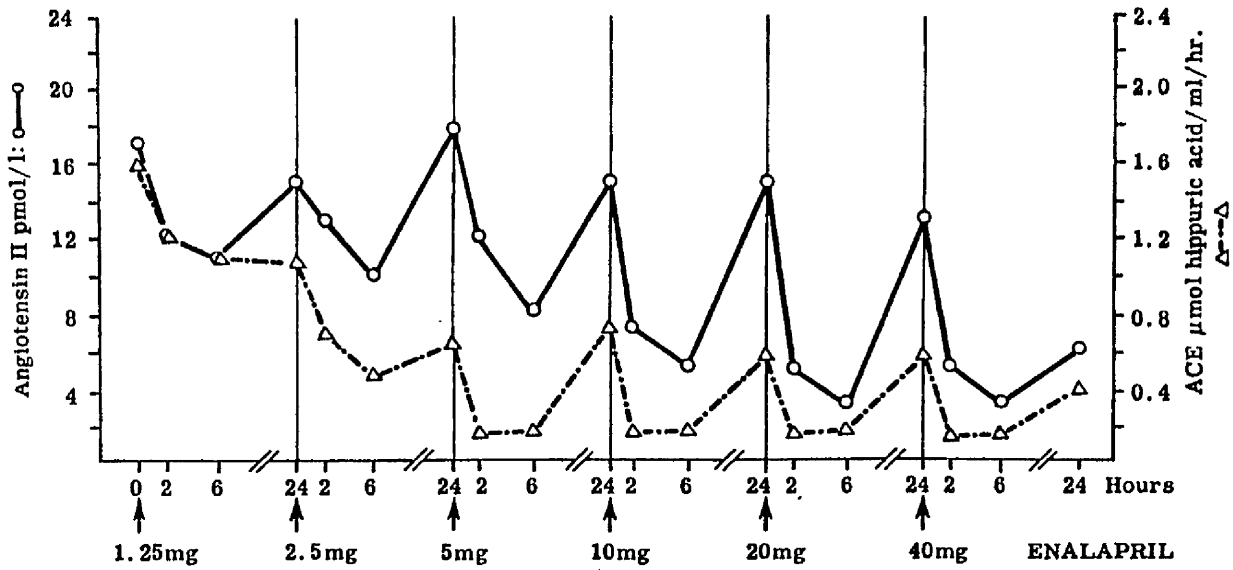


Figure 13.

Dose-finding study in 3 untreated patients. For clarity, mean values only are plotted. Enalapril given in increasing doses as shown as 10.00 a.m. daily with blood samples taken for angiotensin II and converting enzyme activity measurement 2, 6 and 24 hours from the preceding dose.

Group 2.

These 10 patients (aged 15-61; 5 female) were more seriously ill and therefore unsuitable for study according to the above protocol. On arteriography, 5 had unilateral renal artery stenosis or occlusion and 5 had bilateral renal artery disease (Figure 14). Eight had very high blood pressure (4 were in the malignant phase, the optic fundi showing papilloedema, exudates and haemorrhages). One had cardiac failure and recent hypertensive encephalopathy. Three had overall renal impairment (mean \pm SEM serum creatinine for the whole group was $132 \pm 17 \mu\text{mol/l}$). Four had proteinuria of 0.13 to 4.2 g/24 hours. Preceding therapy in the patients of Group 2 included various combinations of thiazide diuretics, loop diuretics, beta-blocking agents, prazosin, hydralazine and nifedipine. Supine blood pressure immediately before enalapril was introduced was $190/104 \pm 15/5$ (SEM) mmHg. Enalapril was given in a starting dose of 10 mg and increased to a maximum of 40 mg daily with lower dosage in those with renal impairment. Over 12 weeks therapy was adjusted and other drugs withdrawn whenever possible.

Peripheral venous blood was taken for measurement of plasma active renin concentration, blood angiotensin I, plasma aldosterone, plasma converting enzyme activity and plasma angiotensin II after 30 minutes of recumbency before the first dose of enalapril and 6 and 24 hours



Figure 14.

- The arteriogram of a 49 year old man with severe hypertension and occlusion of aorta
- and left renal artery.

later. Possible cross-reaction of the antibody used for angiotensin II assay with angiotensin I was corrected for as described earlier⁽⁴⁰⁹⁾. The angiotensin II antibody cross-reacts molecule for molecule with angiotensin III; no correction was applied for this. Supine and erect pulse rate and blood pressure (after 2 minutes standing) were recorded after blood sampling. The Hawksley random-zero sphygmomanometer was employed. These measurements were repeated under identical conditions after 6 days (Group 1 patients only) and after 12 weeks of treatment. Measurements were made of exchangeable sodium and total body potassium in the patients of Group 1 before and during long-term enalapril treatment.

The statistical comparisons are based on paired t-tests, with all biochemical measurements transformed to a logarithmic scale. The correlation coefficients quoted are product-moment correlations, again with biochemical measurements analysed on a logarithmic scale.

The patients who entered the dose-finding study have been excluded from analyses in Group 1 concerning changes at 6 hours and 6 days from the first dose of enalapril.

RESULTS

Dose-finding study (Figure 13).

Suppression of plasma converting enzyme activity and falls of plasma angiotensin II appeared progressively more marked and sustained over the dose-range enalapril 1.25 - 40 mg daily. Plasma angiotensin II was consistently lower at 6 hours than at 2 hours after enalapril administration.

Group 1.

No patient in this group had, before treatment, marked elevation of plasma active renin, angiotensin II or aldosterone, and none had hyponatraemia or hypokalaemia. At the 3rd month, 4 patients were receiving 10 mg, 3 patients 20 mg and 3 patients 40 mg enalapril.

Figure 15 compares blood pressure and various biochemical measurements 6 hours after the daily dose of enalapril on the first and 6th days of therapy, and at 3 months, with those on the final day of placebo at the comparable time after dosing.

- Six hours after the initial dose of enalapril, marked falls in plasma converting enzyme activity and in angiotensin II had occurred, with converse increases in blood levels of angiotensin I and in plasma active

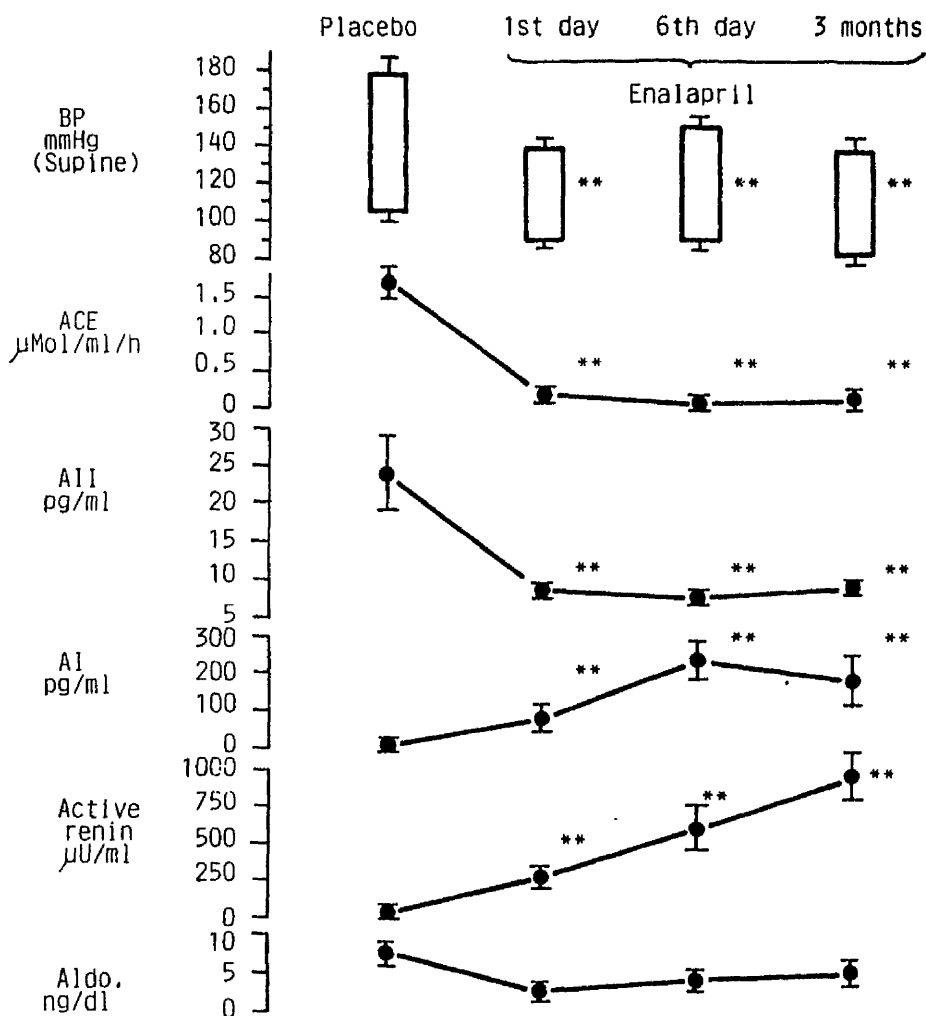


Figure 15.

Patients of Group 1. Measurements made 6 hours after dosing on the final day of placebo, on the 1st day of enalapril therapy, on the 6th day of enalapril therapy and at the 3rd month of enalapril therapy.

- ACE = plasma converting enzyme activity;
 AI = blood angiotensin I concentration;
 AII = plasma angiotensin II concentration;
 Aldo. = plasma aldosterone concentration.

Statistical comparisons with placebo values
 ** = p < 0.01.

renin concentration. The small fall in mean plasma aldosterone was not significant. A highly significant reduction in blood pressure accompanied the fall in plasma angiotensin II and these changes were significantly correlated ($r = 0.67$; $p < 0.05$); the blood pressure fall at 6 hours also correlated with the pre-enalapril plasma concentrations of active renin ($r = 0.76$; $p < 0.05$) and angiotensin II ($r = 0.70$; $p < 0.05$).

With continued enalapril treatment, similar suppression of plasma converting enzyme activity and angiotensin II to that on the first day was seen at 6 days and 3 months, 6 hours from dosing. Active renin concentration increased progressively from the first to the 6th day and from the 6th day to the 3rd month of treatment. While mean blood concentration of angiotensin I also rose further from the first to the 6th day of enalapril treatment, there was thereafter no further rise. The slight fall in mean plasma aldosterone remained insignificant. Blood pressure remained controlled at 6 days and 3 months. However during long-term treatment the blood pressure fall was less closely related than on the first day to either the pre-treatment plasma renin or angiotensin II concentrations or to the long-term fall in plasma angiotensin II. At 3 months, the respective correlation coefficients were $r = 0.44$, 0.42 and 0.56 , none of which were significant.

Figure 16 compares measurements made on the last day of placebo with those at the 3rd month of enalapril treatment, 24 hours from the preceding dose and again 6 hours from the morning dose of drug.

Continued enalapril effect was evident 24 hours from the last dose of the drug, with marked elevation of active renin and angiotensin I, and reduction of converting enzyme activity and angiotensin II. The sustained inhibition of converting enzyme activity was particularly evident from the ratio of angiotensin I to II, which remained some 5:1 at 24 hours from the last dose of enalapril at 3 months, in contrast to a ratio of 1:1 on placebo. Blood pressure also remained significantly reduced 24 hours after dosing in comparison with pre-treatment values.

Nevertheless, following the morning dose of enalapril at the third month, significant further falls in plasma converting enzyme activity and angiotensin II occurred, while mean values for active renin and angiotensin II increased. The small additional lowering of arterial pressure 6 hours from dosing was not statistically significant.

Figure 17 shows systolic and diastolic blood pressures, and heart rates, lying and standing, on placebo, and at the 6th day and 3rd month of enalapril treatment.

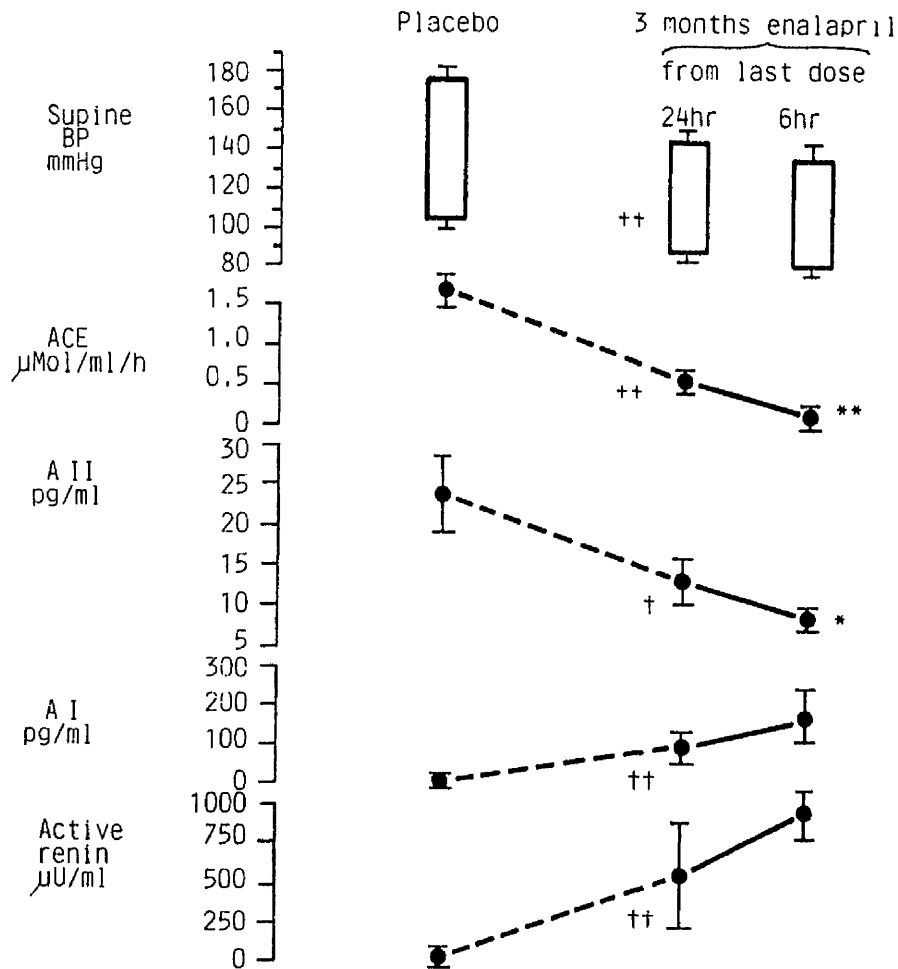


Figure 16.

Patients of Group 1. Comparison of values after 3 months of continuous enalapril therapy alone with final day of placebo. Measurements on enalapril shown 24 hours and 6 hours from the preceding dose. Daggers show comparisons with placebo values; † = p 0.05; †† = p < 0.01. Asterisks show comparisons with 24 hour values; * = p < 0.05; ** = p < 0.01. Other legends as for Figure 2.

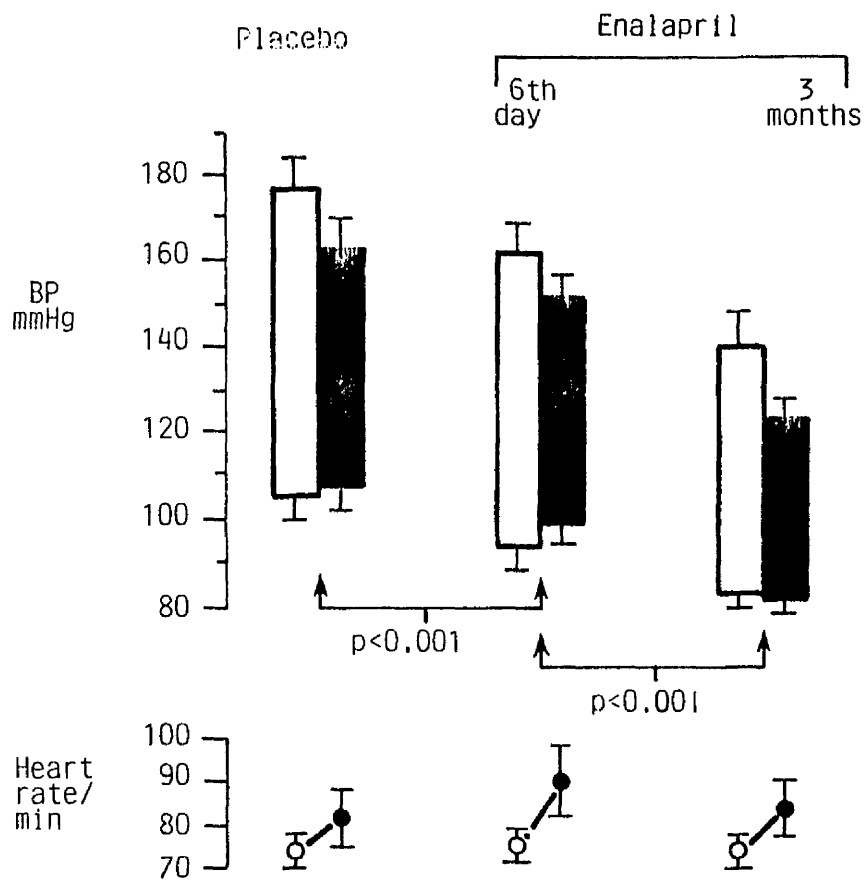


Figure 17.

Patients of Group 1. Comparison of supine (open column) and erect (solid column) blood pressures and pulse rate before and during enalapril therapy. Means (\pm SEM) of measurements made 2, 6 and 24 hours after dosing.

Values are means of measurements made 2, 6 and 24 hours after dosing. A significant fall in all these measurements had occurred by the 6th day of enalapril, and a further highly significant fall by 3 months. Mean heart rate was not altered, although one man experienced symptomatic tachycardia (142/minute) on standing at 6 days; this subsided spontaneously with continued therapy. Figure 17 also shows that the hypotensive effect of enalapril did not have an appreciable postural component.

There was a small but highly significant rise in serum creatinine during long-term enalapril therapy; serum urea also increased (Table 15). Serum potassium rose significantly but in no individual was this marked. There was no change in serum sodium.

There were no measurable long-term changes in total body potassium (Figure 18). Total exchangeable sodium however was reduced during therapy in 9 patients, and unchanged in the tenth (Figure 19). The mean reduction was 140 mmol, and was highly significant ($p < 0.001$). Expressed in terms of predicted normal values, exchangeable sodium fell from a mean of 101% to 96%. Of the 3 patients with proteinuria initially, this had cleared in one and moderated in the others after 3 months.

| | <u>Pre-treatment</u> | <u>1 week</u> | <u>3 months</u> |
|------------|----------------------|---------------|-----------------|
| Sodium | 140.8 ± 0.7 | 140.6 ± 0.5 | 140.4 ± 0.7 |
| Potassium | 3.8 ± 0.1 | 4.0 ± 0.1 | 4.3 ± 0.1 * |
| Creatinine | 103 ± 8 | 117 ± 9 | 127 ± 10 ** |

* p<0.01

** p<0.001

Table 15.

Mean (± SEM) changes in serum electrolytes and creatinine during long-term enalapril therapy.

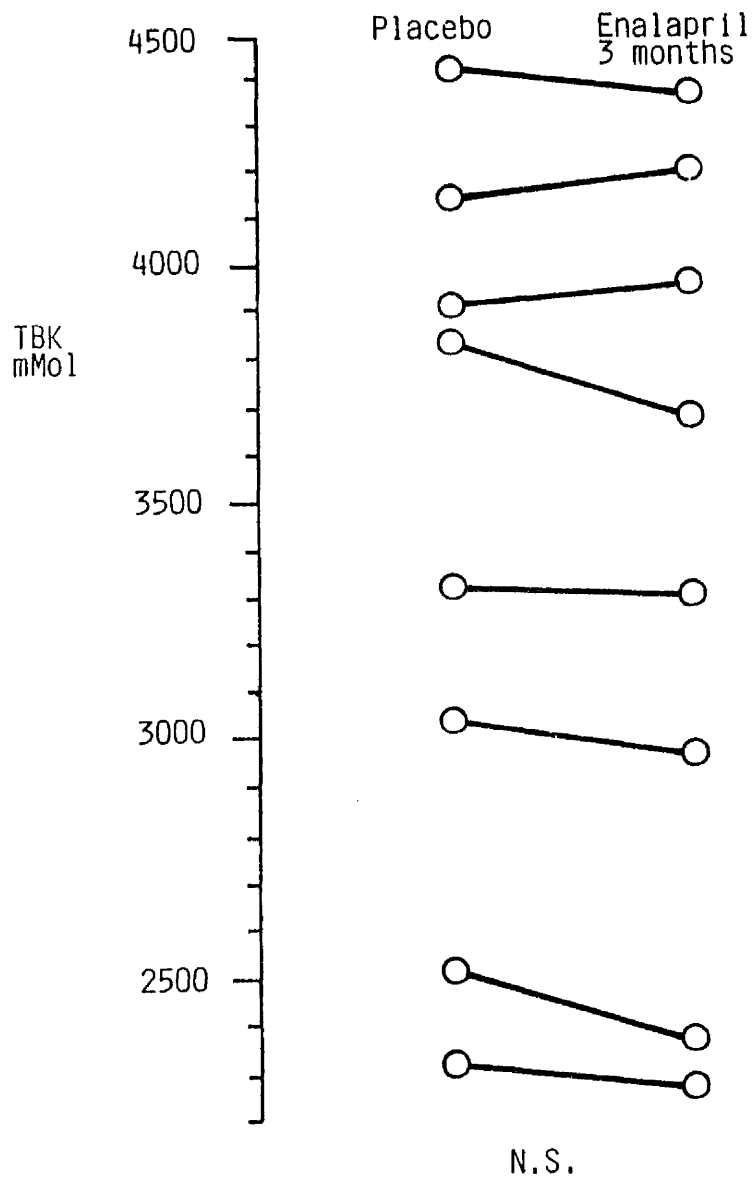


Figure 18.

Patients of Group 1. Total body potassium (TBK) in absolute terms during placebo and after 3 months of continuous enalapril therapy.

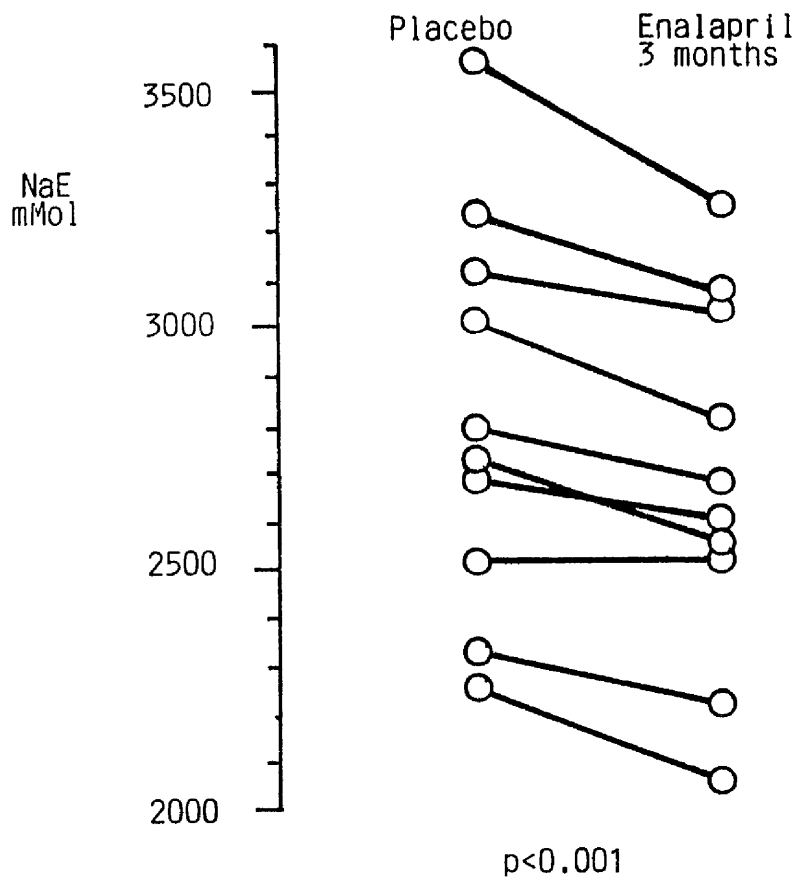


Figure 19.

- Patients of Group 1. Total exchangeable sodium (NaE) in absolute terms on placebo and after 3 months enalapril therapy.

Only one patient in this group has since had operative relief of renal artery stenosis. Average supine blood pressures over 24 hours were 196/104 mmHg on placebo, 150/92 mmHg during long-term enalapril before operation and 148/90 mmHg on no treatment 3 months after transluminal balloon dilatation.

Group 2.

Supine and erect blood pressures, on previous antihypertensive treatment and immediately before enalapril was given, were 190/104 \pm 15/5 mmHg and 179/108 \pm 11/4 mmHg respectively (Figure 20). After 3 months, 4 patients were receiving 10 mg, 3 patients 20 mg and 3 patients 40 mg enalapril daily. The blood pressures of 7 patients were well controlled on enalapril alone. Three required additional therapy. One patient with overall renal impairment continued also on frusemide 500 mg daily, together with atenolol 50 mg daily for control of symptomatic tachycardia. One patient remained on chlorthalidone 25 mg daily, and also atenolol 100 mg daily because of angina. One patient needed atenolol alone, 50 mg daily, for control of symptomatic tachycardia.

In this group of 10 patients, after 3 months of enalapril, supine and erect blood pressures had fallen to 142/84 \pm 5/8 mmHg and 129/85 \pm 7/5 mmHg ($p < 0.01$) respectively (Figure 20). There were no changes in mean

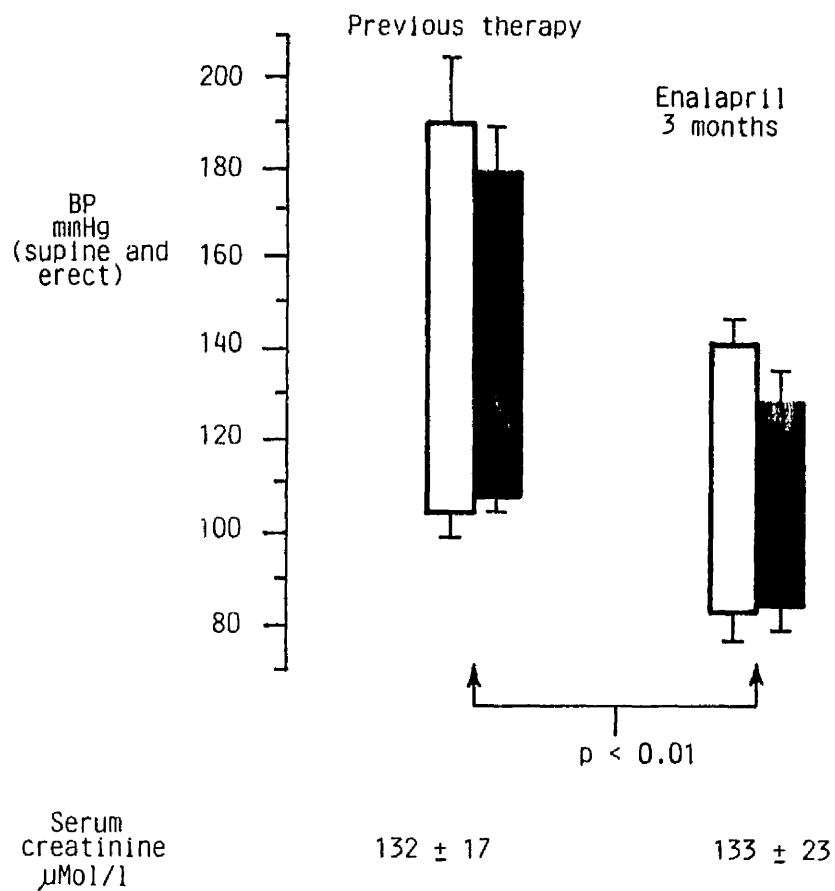


Figure 20.

- Patients of Group 2. Measurements of blood pressure supine (open column) and erect (solid column) during previous therapy immediately before the introduction of enalapril and after 3 months of enalapril therapy.

Also shown are means \pm SEM for serum creatinine at the same times.

serum sodium or potassium and no deterioration in overall renal function (serum creatinine $132 \pm 17 \mu\text{mol/l}$ on previous therapy, $133 \pm 23 \mu\text{mol/l}$ after 4 months of enalapril).

Proteinuria cleared in 1 of the 4 patients in whom it was present before starting enalapril, and was greatly reduced in the other 3.

In the 5 patients with bilateral renal artery lesions, none of whom remained on any therapy other than enalapril, mean recumbent blood pressures were $154/88 \pm 2/3$ SEM mmHg after 3 months of enalapril, compared with $204/110 \pm 21/7$ mmHg on previous therapy. Neither as a group nor individually was there appreciable deterioration of renal function on enalapril (values for serum creatinine and urea respectively before and during treatment 142 ± 34 SEM $\mu\text{mol/l}$ versus 145 ± 35 ; and 7.3 ± 2.4 mmol/l versus 8.4 ± 2.5).

Side-effects.

The drug was well tolerated by all 20 patients and problems were minor. One man recovered previously impaired sexual function. Four patients (one also on a large dose of loop diuretic) developed symptomatic tachycardia in the supine and standing positions; in two cases the tachycardia slowly and spontaneously resolved without additional therapy but two patients,

both in Group 2, required the addition of a beta-blocker.

One patient on enalapril alone developed de novo Raynaud's phenomenon. In another woman, also on enalapril alone, pre-existing Raynaud's phenomenon worsened distinctly.

One patient of Group 1, with a small, poorly functioning kidney, developed complete occlusion of a previously tightly stenosed renal artery during enalapril treatment although he remained asymptomatic with good blood pressure control throughout. His overall renal function did not deteriorate seriously, the rise in serum creatinine over 3 months, from 118 to 127 $\mu\text{mol/l}$, being less than the average for the whole of Group 1.

There were no instances of drug-induced proteinuria; indeed, significant proteinuria in 6 subjects before starting enalapril was reduced markedly in 4 and resolved completely in two.

There were no instances of taste disturbance, skin rash, glycosuria or haematological disorder.

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CHAPTER 12

CAPTOPRIL AND ENALAPRIL: EFFECTS ON ALPHA- ADRENORECEPTOR FUNCTION

SUMMARY

The effects of two converting enzyme inhibitors, captopril and enalapril, on alpha-adrenoreceptors and baroreceptor function were studied in 6 healthy male volunteers.

In this placebo controlled study the pressor responses to graded infusions of phenylephrine (an α_1 -agonist) were identical following pre-treatment with captopril, enalapril and placebo. This suggests that the fall in peripheral resistance with each drug is not a function of reduced vasoconstrictor tone mediated by α_1 -receptor inhibition.

Plotting pulse rate against mean arterial pressure demonstrated the expected linear relationship during placebo. During captopril and enalapril therapy the slope of the regression line was unchanged although shifted with both drugs, indicating baroreflex resetting without loss of sensitivity. This suggests that the lack of tachycardia when blood pressure is lowered with these drugs is due to changes in baroreflex function.

SUBJECTS

The study was performed on 6 healthy male subjects (aged 23-39) after approval by the hospital Ethical Supervisory Committee. The procedure and risks were explained to each subject and their informed consent was obtained.

PROTOCOL

- (a) Each subject received, with a minimum intervening 3 day washout period, 4 graded infusions of phenylephrine, a pure α_1 -receptor agonist without cardiac or central effects.
- (b) To ensure sodium repletion, each subject received slow sodium, 50 mEq daily, in addition to normal sodium intake for each of the 3 days before each infusion.
- (c) Before each infusion, each subject received a random order, one each of placebo, captopril 25 mg, enalapril 10 mg and prazosin 1 mg (a specific α_1 -receptor antagonist). Neither subject nor investigator was aware of the treatment order.
- (d) The subjects arrived at the ward at 0900 having had only water to drink since the previous evening. They lay supine and a heparinised intravenous cannula was inserted into a forearm vein.

- (e) The drug was administered by mouth to the following pattern because of differing absorption and activity characteristics.

| DAY | 1 | 2 | 3 | 4 |
|-------|---------|----------|-----------|-----------|
| 07.30 | Placebo | Placebo | Placebo | Enalapril |
| 09.00 | Placebo | Prazosin | Captopril | Placebo |

- (f) At 10.00, phenylephrine was infused at rates of 0.6, 0.9, 1.35, 2, 3, 4.5 and 6.75 $\mu\text{g}/\text{kg}/\text{min}$, incrementally increased at 5 minute intervals. The phenylephrine was diluted to 30 ml in 5% dextrose and the syringe was attached to an automatic infusion pump which was calibrated on the day before each experiment. The dead space of the forearm cannula was calculated and a time allowance made at the start of each infusion.
- (g) Blood pressure recordings were made using a standard mercury sphygmomanometer and pulse rates were recorded manually over 30 seconds. Recordings were made at 15, 11, 10, 6, 5, 4 and 1 minutes before each infusion to familiarise the subjects, and at 4 and 5 minutes after each infusion rate.
- (h) Close and continuous supervision was maintained and the infusions were stopped when either:
- (a) Pulse rate fell to 40 beats/min or less

(b) Blood pressure increased to greater than 40 mmHg systolic over basal values.

Blood pressure recordings were analysed using a mean of the 4th and 5th minute recordings for each infusion rate. Basal blood pressure was taken as the mean of recordings taken at -4 and -1 minutes. A log-dose pressor response curve was then constructed for each drug studied, blood pressure being plotted as mean arterial pressure.

To assess baroreceptor function mean arterial blood pressure was plotted against pulse rate. No plot could be constructed for the infusion during prazosin treatment because of the specific agonist-antagonist interaction.

The statistical comparisons are based on paired t-tests, data sets being analysed at each phenylephrine dose interval.

RESULTS

The procedures were well tolerated except in one subject who developed slight chest tightness at the end of the highest phenylephrine infusion rate while taking placebo. Recovery was immediate and an ECG performed later was normal. All subjects reported cold

extremities, and scalp and facial paraesthesiae when infused at the highest rates.

Following prazosin administration, 2 subjects developed marked, though asymptomatic, hypotension during phenylephrine 1.35 $\mu\text{g}/\text{kg}/\text{min}$ in each case. This "first dose" effect is a well known characteristic of prazosin therapy and occurred despite the infusion of phenylephrine, a direct antagonist. In each case transient hypotension was followed by a transient rise in blood pressure during the next infusion rate of 2 $\mu\text{g}/\text{kg}/\text{min}$. Table 16 shows the mean changes as recorded; for clarity the values marked with an asterisk are averaged in the accompanying figure.

The log-dose pressure response curves demonstrated the expected linear relationship during the placebo period with a marked shift to the right during prazosin therapy (Figure 21). The curves for captopril and enalapril were exactly similar, with no shift to the right, indicating no demonstrable α_1 -adrenoreceptor antagonism.

The plot of mean arterial blood pressure against pulse rate demonstrated a linear relationship for the infusion during placebo. The relationships during captopril and enalapril were also linear, parallel to placebo and significantly shifted to the right equally with both drugs ($p < 0.05$), indicating baroreflex resetting (Figure 22). Baroreflex sensitivity (calculated as the slope of the linear regression line⁽⁴²⁵⁾), was unimpaired by either converting enzyme inhibitor.

PHENYLEPHRINE ($\mu\text{g}/\text{kg}/\text{min}$).

| MAP (mmHg) | 0 | 0.6 | 0.9 | 1.35 | 2 | 3 | 4.5 | 6.75 |
|---------------------------|------------|------------|------------|-------------|-----------------|-----------------|----------------|----------------|
| Placebo | 80 \pm 1 | 89 \pm 2 | 93 \pm 2 | 102 \pm 2 | 106 \pm 2 | 112 \pm 2 | - | - |
| Captopril | 73 \pm 1 | 81 \pm 2 | 87 \pm 2 | 94 \pm 2 | 101 \pm 3 (5) | 104 \pm 3 (2) | - | - |
| Enalapril | 75 \pm 1 | 81 \pm 2 | 84 \pm 2 | 91 \pm 2 | 98 \pm 2 | 106 \pm 2 (4) | - | - |
| Prazosin | 74 \pm 1 | 77 \pm 2 | 77 \pm 2 | 66 \pm 9* | 87 \pm 4* | 83 \pm 4 (5) | 87 \pm 2 (5) | 94 \pm 2 (5) |
| Pulse rate (beats/min) | | | | | | | | |
| Placebo | 65 \pm 5 | 59 \pm 5 | 62 \pm 4 | 48 \pm 2 | 44 \pm 2 | 41 \pm 1 (3) | - | - |
| Captopril | 57 \pm 4 | 56 \pm 4 | 51 \pm 3 | 45 \pm 2 | 41 \pm 1 (5) | 40 \pm 1 (2) | - | - |
| Enalapril | 58 \pm 4 | 55 \pm 4 | 52 \pm 3 | 48 \pm 3 | 43 \pm 2 | 40 \pm 2 (4) | - | - |
| Prazosin | 62 \pm 5 | 61 \pm 5 | 59 \pm 6 | 57 \pm 6 | 55 \pm 6 | 56 \pm 5 (5) | 53 \pm 6 (5) | 49 \pm 4 (5) |

Table 16. Mean (\pm SEM) arterial blood pressure and pulse rates in 6 normal subjects during graded infusion of phenylephrine. Figures in parentheses indicate number of subjects completing each infusion rate (6 unless otherwise stated).

* see text.

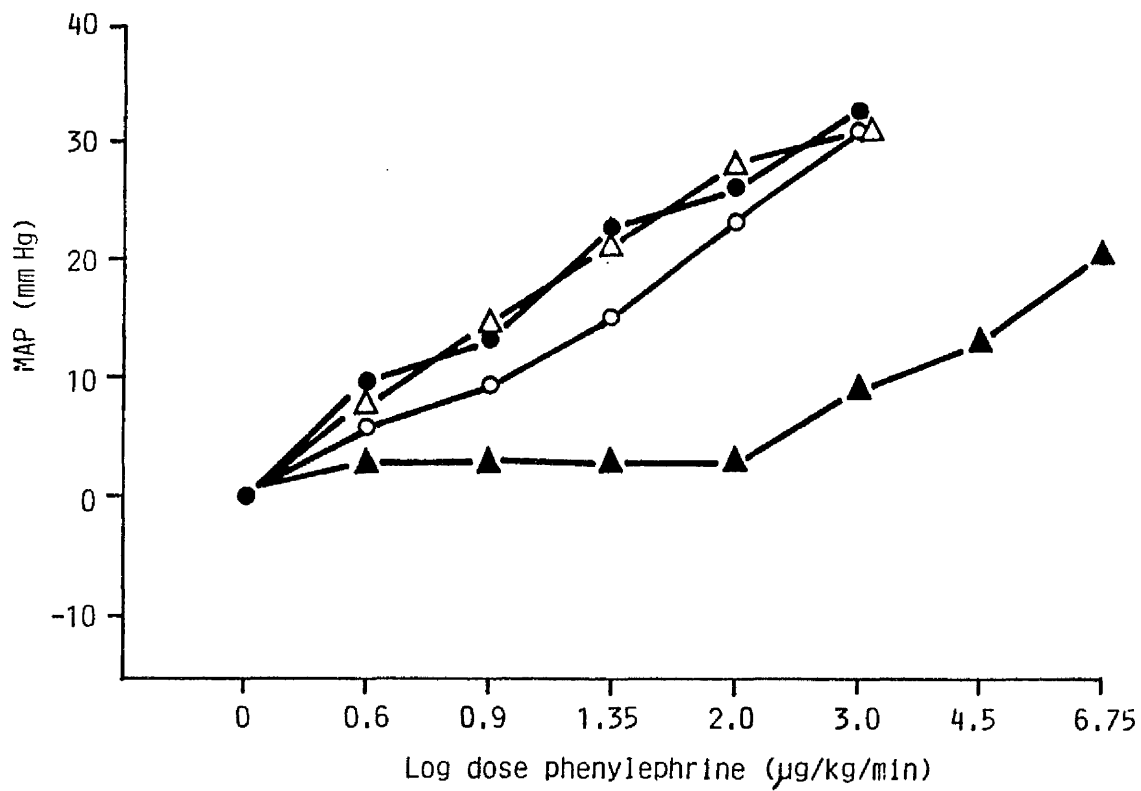


Figure 21.

Absolute change in mean arterial pressure in 6 subjects during graded infusions with phenylephrine.
 • Mean values only are plotted for clarity.

Key. placebo O — O
 captopril Δ — Δ
 enalapril ● — ●
 prazosin ▲ — ▲

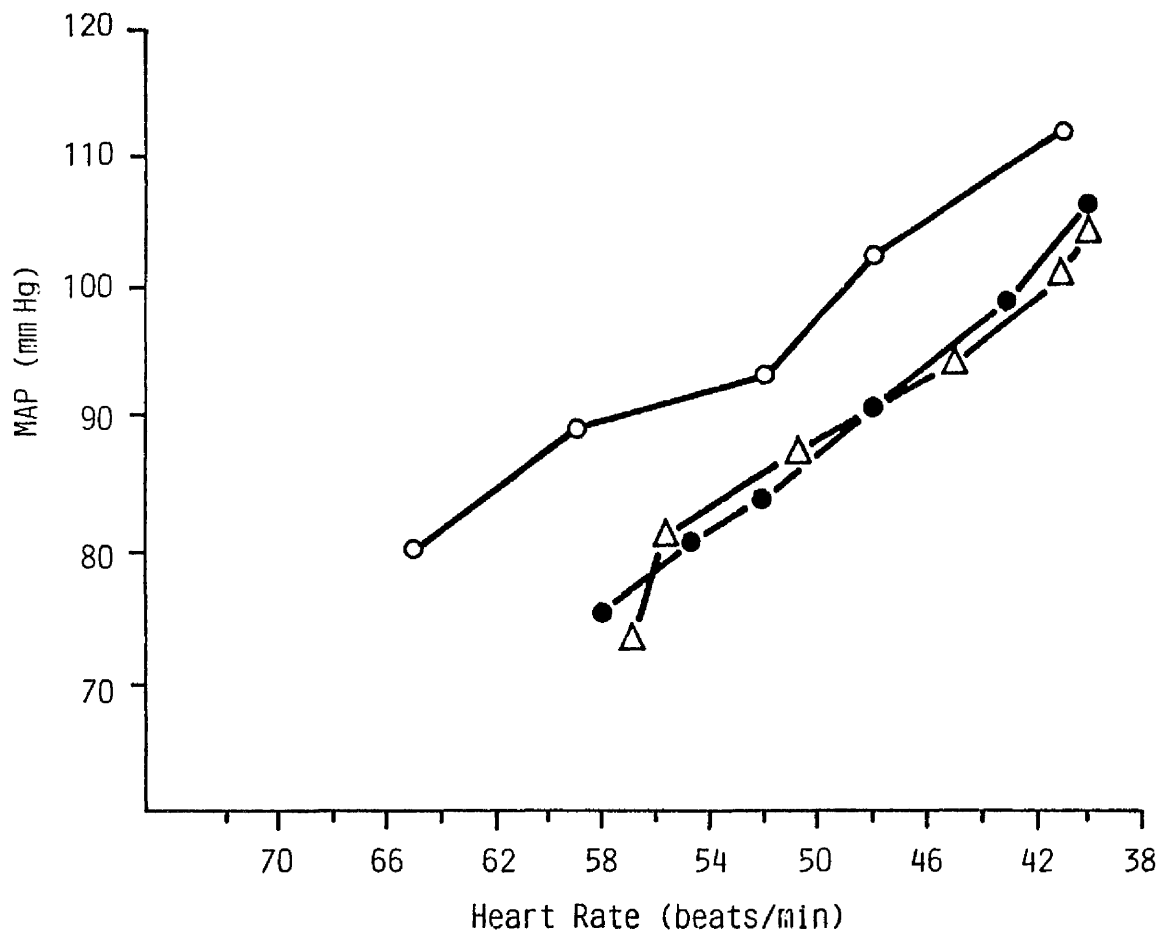


Figure 22.

• The relationship between mean arterial pressure and pulse rate in 6 subjects given graded infusions of phenylephrine. For clarity, mean values only are plotted.

Key. placebo O — O
 captopril Δ — Δ
 enalapril ● — ●

CHAPTER 13

DISCUSSION

I. THE FIRST DOSE HYPOTENSIVE EFFECT OF CAPTOPRIL

When captopril is first given there is a fall in arterial pressure which is precipitous in some severely hypertensive patients^(289,263,427,428). Although this phenomenon has been noted, the incidence of this potentially dangerous event is unknown, and insufficient information is available to the clinician to aid in its anticipation and, if necessary, treatment.

With the first dose of captopril there is an acute and highly variable fall in blood pressure which may be very different from the ultimate reduction in blood pressure achieved with long-term administration⁽⁴²⁹⁾. The magnitude of the acute fall is closely related both to the initial plasma concentrations of renin and angiotensin II and to the concomitant reduction of circulating angiotensin II concentration⁽⁴³⁰⁻⁴³¹⁾. Patients with renovascular hypertension often sustain greater acute falls in blood pressure, associated with a greater increase in plasma renin concentration, than patients with essential hypertension^(433,434). Removal of the direct vasoconstrictor effect of angiotensin II is probably a major, if not necessarily the sole^(435,436),

factor in the reduction of blood pressure in the first few hours after inhibition of converting enzyme.

A significant negative correlation between serum sodium and plasma renin concentrations has previously been shown in treated and untreated hypertensive patients and in cardiac failure^(299,309,437). Our results thus confirm the generally agreed relations between sodium, renin, and angiotensin II, although these may be modified by the effects of previous hypotensive treatment. Interestingly, however, in this series serum sodium concentration was not significantly correlated with the fall in blood pressure after captopril. The preceding dose of diuretic was significantly related to the fall in blood pressure in patients with secondary hypertension but not in those with essential hypertension. Greater falls in blood pressure occurred in those patients with secondary hypertension, and, as is the case with most hypotensive drugs, in those in whom initial pressures were highest. Renal function was not correlated with the fall in blood pressure.

The effect of the first dose carries a considerable risk in severely hypertensive patients, who are particularly vulnerable to sudden reductions in cerebral perfusion pressure, with the dangers of boundary zone cerebral infarction^(438,439). In this study 8% of patients sustained an acute reduction of mean

arterial pressure in excess of 50% within two hours of receiving captopril. Sudden suppression of the formation of angiotensin II is probably the most important factor, though other processes may also play a part. Possibly, in addition to arterial dilatation, venodilation and a fall in right atrial pressure may contribute to sudden circulatory collapse in patients with pre-existing volume depletion and poor cardiac reserve.

Unfortunately, this analysis shows that a severe first dose effect cannot be predicted consistently in individual patients. Nevertheless, the present findings suggest that caution should be observed particularly in patients with secondary hypertension, especially if they are already taking diuretics; patients with very high blood pressure; and patients with low or low normal serum sodium concentrations. If the pretreatment plasma renin or angiotensin II concentration is known greater precision of prediction is possible, with a high concentration indicating high risk.

As no single test or combination of tests in this study identified all patients at risk our policy is for all severely hypertensive patients, especially those receiving large doses of diuretics, to receive close medical supervision for at least three hours after the first dose of captopril; during this time the patient remains supine and blood pressure is checked at 10 minute intervals.

In the event of a profound fall in blood pressure we consider that the most appropriate and effective treatment is to restore immediately the substance whose loss led to the reduction in pressure. Thus we have found that graded infusion of angiotensin II (starting dose 0.5 ng/kg/min, range 0.5-8 ng/kg/min) is fully effective in immediately restoring and maintaining blood pressure. Care must be taken not to give an excessive dose and to raise blood pressure too far. By comparison, rapid infusion of 0.9% saline is a less effective and less prompt method of restoring blood pressure; moreover, it might prove dangerous in some patients with impaired cardiac function.

Controlled volume repletion with saline before administration of captopril should in theory reduce the risk of profound hypotension, as it will also reduce plasma renin and angiotensin II concentrations. It is, however, impractical to give an infusion to all patients to whom captopril is to be given, and patients at special risk cannot be consistently identified. Stopping diuretic treatment before captopril is given may also reduce the risk of hypotension, but care should be observed in some patients with very high blood pressure or borderline cardiac function.

After a profound fall in blood pressure has been corrected with an infusion of angiotensin II our usual

policy is to give at least 1 litre 0.9% saline over 24 hours before repeating the test dose of captopril. By infusing saline slowly we have not provoked cardiac failure. One patient required 4 l saline over four consecutive days to prevent excessive reduction in blood pressure after each daily dose of captopril.

In this study the use of lower doses of captopril (6.25 mg) conferred no protective effect. Smaller incremental dosage increases in the range 1-5 mg could, however, be explored in more detail as an approach to avoiding sudden, virtually complete suppression of generation of angiotensin II with its attendant fall in blood pressure.

Studies of the response to the first dose of enalapril in similar patients are awaited with interest. Suppression of angiotensin II is as complete but less rapidly achieved with enalapril than with captopril but is maintained for longer; initial hypotension might therefore be expected to be as profound, but more sustained, and less precipitous in its onset. I have observed such a reaction in a woman with severe resistant essential hypertension following enalapril 10 mg. Blood pressure fell slowly and progressively from 202/112 to 90/72 mmHg after 2 hours at which point she complained of marked tiredness, and physical weakness. An infusion of angiotensin II in the range

1-4 ng/kg/min was then required to maintain her blood pressure for the next 6 hours.

II. CAPTOPRIL IN SEVERE HYPERTENSION

Effects on blood pressure.

The mechanisms by which captopril lowers blood pressure have been discussed; most of the fall can be attributed to the suppression of the acute and chronic vasoconstrictor effects of angiotensin II. Thus, although effective in the treatment of renovascular hypertension, captopril would not be expected to have a marked hypotensive effect in essential hypertension, in which levels of circulating angiotensin II are generally unremarkable. Indeed, as discussed in Chapter 3, the moderate hypotensive effects are similar in magnitude to those of beta-blockers or thiazide diuretics^(270,271).

When diuretics are given to hypertensive patients, the natriuretic and hypotensive effects are limited by a compensatory rise in plasma angiotensin II and aldosterone. Captopril, by inhibiting this rise in angiotensin II, might therefore be expected to markedly potentiate the hypotensive effects of diuretic therapy. As we have seen⁽²⁷²⁻²⁷⁶⁾, this theoretically sound combination has been found in practice to be extremely potent, the combination having the same efficacy as

standard triple therapy of beta-blocker, diuretic and vasodilator.

The treatment of severe hypertension is often unsatisfactory, with good blood pressure control achieved only with the side-effects of drugs used in high doses. Minoxidil has proved particularly useful in such patients (440) but its value is limited by hirsutism (especially in women) and fluid retention.

Previous studies of captopril and diuretic in small numbers of patients (262-264, 455) have been encouraging. In this study captopril and diuretic controlled the blood pressure of patients in whom a wide range of anti-hypertensive drugs had been ineffective. Many had adverse features including renal disease and malignant phase hypertension. Although a substantial proportion of patients required a beta-blocker for tachycardia only 18% required the addition of a third antihypertensive agent (prazosin) to achieve target blood pressure, and none required a fourth drug. Although compliance was not directly assessed, it is known that compliance and tablet numbers prescribed are inversely related; in this study improved blood pressure control was associated with a reduction in tablets prescribed.

Adverse reactions.

The precipitous fall in blood pressure in some patients was disquieting although there were no permanent sequelae. It should be re-emphasised that only severely hypertensive patients appear vulnerable to this phenomenon. Patients with mild to moderate hypertension will be less likely to have volume depletion and high levels of circulating angiotensin II induced by their disease, or by previous therapy.

Drug induced tachycardia⁽²⁶³⁾ was a particular problem in this study. Tachycardia is recognised as an unusual (and unexplained) effect of captopril and, as we have seen previously, enalapril. Only one patient of this series developed tachycardia while taking captopril alone; fifteen required a beta-blocker to control tachycardia when diuretic therapy was introduced or increased to the limits of tolerance. It is therefore likely that the increased heart rate may be attributed largely, if not entirely, to diuretic induced sodium and volume depletion in the face of a blocked renin-angiotensin system.

The contribution of the beta-blockers to the overall antihypertensive effect in those with tachycardia cannot be assessed. Some studies, however, have shown that beta-blockers have little additional hypotensive effect when added to captopril alone, or captopril and

diuretic in combination^(441,442).

Renal function and serum electrolytes.

The renal function of the group did not change during long-term therapy although 3 patients had significant further impairment of renal function. In two patients there were prompt rises in serum creatinine when the dose of frusemide was rapidly increased; in both cases renal function returned to basal levels when the dose of frusemide was reduced. In one patient, previously described, deterioration of renal function occurred in association with the development of acute nephrotic syndrome. Both proteinuria and serum creatinine returned to pre-treatment values when captopril was stopped.

As will be discussed later, deterioration in renal function is a relatively uncommon event; it is most likely to occur when captopril and diuretic are used in the presence of a single kidney, or bilateral renal artery stenosis.

There was no significant change in serum sodium; there was, however, a minimal rise in serum potassium. As a consequence of aldosterone suppression, serum potassium may be expected to rise during treatment with converting enzyme inhibitors^(258,261,263,264), especially in the presence of renal impairment. Some cases of

hyperkalaemia have been reported⁽²⁶³⁾. In this study no patient developed hyperkalaemia, although the number of patients requiring potassium supplementation was reduced. Thus, even when large doses of diuretic are administered, potassium supplements or sparing agents should only be used in the presence of overt hypokalaemia; even under this circumstance caution should be observed.

The reduced requirement for potassium supplementation also contributes to the reduced daily tablet intake in many of these patients, with possible further improvement in treatment compliance.

Side-effects.

Captopril was withdrawn in one fifth of these patients because of toxic side-effects, which included taste loss, skin rash and nephrotic syndrome. These and their relationships with dose and renal function are discussed in the following section.

Despite these side-effects, captopril was well tolerated and numerous patients commented spontaneously on a feeling of well-being. This is also discussed in a later section.

III. SIDE EFFECTS OF CAPTOPRIL

We have seen that the widespread use of captopril has been limited by a variety of side-effects. Since captopril is excreted mainly in the urine⁽⁴⁰²⁾ an increased incidence of side-effects might be predicted in patients with impaired renal function. To my knowledge there have been no formal studies relating side-effects to dose and to renal function, as was performed in this study of one hundred patients.

The most serious side-effects of captopril are proteinuria and agranulocytosis, which are said to occur at a frequency of 1.2% and 0.3% respectively⁽⁴⁴³⁾, and to be more common in patients with underlying renal disease or renal failure⁽²⁷¹⁾. This study suggests that proteinuria is dose related, but that the minor side-effects, taste loss and skin rash, may not be. It should be noted nevertheless that significant proteinuria has been reported in a patient with normal renal function who received only 150 mg of captopril daily⁽⁴⁴⁴⁾.

Renal biopsies in patients who develop proteinuria while taking captopril have usually revealed an early membranous lesion⁽⁴⁴⁴⁻⁴⁴⁷⁾. This was true of one of these patients, although the findings in the second patient were inconclusive. Negative immunofluorescent tests in renovascular hypertension however, do not exclude the presence of an immune complex disease in

the other kidney⁽⁴⁴⁸⁾. The commonest cause of a drug-induced nephrotic syndrome is currently penicillamine⁽⁴⁴⁹⁾, and many similarities exist between this and captopril. Both may cause a membranous glomerulonephritis, taste disturbance and bone marrow depression, and the risk of proteinuria in patients taking penicillamine may also be dose related⁽⁴⁵⁰⁾. Since captopril and penicillamine share a reactive sulphhydryl group it seems possible that the pathogenesis of the membranous lesion is the same for both drugs. An allergic phenomenon leading to formation and deposition of immune complexes in the kidney has been proposed⁽⁴⁴⁹⁾.

The optimum daily dose of captopril has yet to be defined. Our current practice is to use a maximum of 100 mg captopril daily in patients with normal renal function and to reduce the dose further in renal failure. Low-dose captopril (<150 mg daily) has already been shown to be effective and free from serious side-effects in the treatment of mild to moderate hypertension⁽⁴⁵¹⁻⁴⁵³⁾. Smith et al.⁽⁴⁵⁴⁾ suggest that the same may be true also for patients with resistant hypertension. The present study confirms that serious side-effects are unlikely to occur with low doses of captopril but indicates that occasional patients with captopril induced taste loss and skin rash may still be seen.

IV. CAPTOPRIL AND MOOD ENHANCEMENT

There are several anecdotal reports of spontaneous comments by patients of enhanced sense of well-being when captopril is begun, usually in hypertensives subjected previously to complex drug regimes (259,273,262,455,456). This was also strikingly apparent in the patients previously described here.

While a greater feeling of well-being is an advantage, if captopril had a distinct euphoriant action this might impose limitations to its use (for example in airline captains). Thus quantitative assessment is needed to detect mood changes of any nature which might be caused by antihypertensive drugs.

This small study has provided no evidence of a euphoriant effect of captopril. Indeed, the Goldberg questionnaire revealed a slight but significant lowering of mood when captopril was added to atenolol and bendrofluazide in moderately severe hypertension. Moreover, this pattern was seen with subscales of questions addressing somatic symptoms, anxiety and insomnia, and social dysfunction, but not depression. The paced auditory serial addition task did not discriminate between placebo and captopril; nor did captopril detectably affect the mania rating scale.

Compliance in the trial, assessed by tablet counts, blood pressure reduction, elevation of plasma renin and lowering of angiotensin II, was satisfactory.

Thus, at least in these circumstances, there seems no cause for concern about a euphoriant action of captopril, despite evidence from animal experiments (discussed in Chapter 3) that the drug may inhibit the degradation of metenkephalin, substance P and kinins⁽⁴⁵⁷⁻⁴⁶⁰⁾.

There seems to be a lack of systematic prospective studies of the psychological effects of antihypertensives⁽⁴⁶¹⁾, although the need for such trials is becoming increasingly apparent. Mann⁽⁴²⁰⁾, using the Goldberg questionnaire of the present study, found that entry to a treatment trial for mild hypertension was accompanied by a lessening of psychiatric symptoms. Snaith and McCoubrie⁽⁴⁶²⁾ found no evidence of a relationship between antihypertensive medication and depression using a cross-sectional study of a large patient group. Bulpitt, Hoffbrand and Dollery administered to a large number of hypertensive outpatients the Middlesex Hospital Questionnaire⁽⁴⁶³⁾. The latter was initially developed for the detection of neurotic disorder in psychiatric outpatients. It contains questions which refer to long-standing personality traits as well as neurotic symptoms and is perhaps less

sensitive than the Goldberg questionnaire as an indicator of change in mental state related to drug treatment.

Despite the small numbers in this study the Goldberg questionnaire was able to detect significant differences between placebo and captopril despite being applied against a background of other antihypertensive therapy. Captopril is currently restricted in its use in the U.K. to resistant hypertension and that associated with renovascular disease. The present trial has provided evidence that similar quantitative psychological assessment could usefully be made when small doses of captopril are given alone in mild hypertension.

V. ENALAPRIL AND LYSINE ANALOGUE IN NORMAL SUBJECTS

As outlined in Chapter 4, the pharmacology of these two compounds has been determined in single dose studies, which have shown them to be potent inhibitors with a long duration of action. This study has compared both their immediate and long-term effects on blood pressure, electrolyte excretion and the renin-angiotensin system in normal subjects.

Blood pressure.

In this study, blood pressure was significantly lowered by both active drugs and this effect was sustained after 24 hours and with long-term treatment.

Other placebo-controlled, single dose studies have suggested similar findings in normal subjects given captopril, MK421^(220,464,375) and MK521⁽³⁷⁵⁾. As has been discussed, while the hypotensive effects of these drugs is possibly multifactorial, these results do suggest an important role for the renin-angiotensin system in the maintenance of blood pressure in sodium replete man. Studies in small numbers of hypertensive patients have shown MK421 to be an effective agent in essential hypertension⁽³⁸⁰⁾ and hypertension associated with renovascular disease, as was seen in Chapter 10.

Despite the fall in blood pressure with both MK421 and MK521, there were no differences in supine or standing pulse rates when compared with placebo. This finding is consistent with the findings in Chapter 12 which showed baroreceptor resetting, but not impairment, during treatment with captopril and MK421.

Renin-angiotensin system.

Plasma converting enzyme activity was distinctly suppressed by both MK421 and MK521, although the effects were more prolonged with MK521. The expected rise in plasma renin and angiotensin I was seen and was greater with MK521. Plasma concentrations of aldosterone and angiotensin II were significantly reduced after 6 hours but had returned to basal values after 24 hours. The same patterns of diurnal variation in all the above

measurements were seen after 8 days of continuous treatment.

At 24 hours after the last dose of each drug the concentration of plasma renin was significantly higher on day 8 than on day 1, although angiotensin I levels did not increase in proportion. This apparent dissociation between renin and angiotensin I during long-term treatment presumably reflects the fall in renin-substrate which is a feature of prolonged inhibition of converting enzyme⁽⁴⁶⁶⁾. A similar lack of parallelism has been observed between the increase in plasma renin and blood angiotensin I concentrations during long-term treatment with captopril⁽⁴²⁹⁾ and enalapril in renovascular hypertension (see Chapter 11).

Despite the very high circulating levels of renin and angiotensin I, there is no evidence of the converting enzyme inhibition being overcome during long-term drug administration and angiotensin II remains low.

Des-Asp¹-angiotensin II (angiotensin III) accounts for less than 10% of the total angiotensin II immunoreactivity in plasma from man⁽⁴⁶⁷⁾. Consequently, although it is considered to be a potent stimulus to aldosterone secretion, its low concentration in human plasma would suggest a relatively unimportant role. The possibility that the residual concentration of AII

immunoreactive material detected following ACE inhibition with MK421 or MK521 may contain a higher concentration of angiotensin III than normal, with a consequently increased effect on aldosterone secretion, seems unlikely. Angiotensin III is formed primarily by the action of aminopeptidase A on angiotensin II, consequently any suppression of plasma angiotensin II following converting enzyme inhibition will automatically result in a proportional fall in angiotensin III.

The possibility that angiotensin III may be formed by an alternative pathway such as the action of converting enzyme on des-Asp¹-angiotensin I is also unlikely, as animal studies have shown that converting enzyme inhibitors are very effective in blocking this reaction (468), even at very high concentrations of des-Asp-angiotensin I.

Duration of action.

Although plasma concentrations of angiotensin II and aldosterone had returned to basal levels after 24 hours, with sustained elevation of renin and angiotensin I, blood pressure did not rise with either drug at 24 hours after the last dose. There was a similar diurnal change in components of the renin-angiotensin system in patients with renal artery stenosis (Chapter 11) and this again was not associated with loss of blood pressure control.

We have also shown that by using doses of MK421 in excess of 10 mg, more prolonged, though not necessarily more profound, suppression of converting enzyme activity and angiotensin II is induced.

Sodium excretion.

Previous studies in subjects on free dietary intake (371,375) have suggested a natriuresis following a single dose of both MK421 and MK521. This study, in which the sodium intake was fixed, confirms a significant natriuresis, as has been observed with captopril⁽⁴²⁹⁾. The natriuretic effects of MK521 appeared later in keeping with the known pattern of drug absorption. After 8 days of continuous therapy the natriuretic effect was no longer apparent, despite continued suppression of plasma aldosterone and angiotensin II. Cumulative measurements of urinary sodium excretion suggest a net sodium loss of up to 50 mEq in excess of placebo over the 8 day period. It is thus unlikely that sodium excretion contributed significantly to the hypotensive effects of either drug in this study. It should however be remembered that in the patients with renal artery stenosis there was a highly significant mean fall in exchangeable sodium of 140 mEq over a 3 month period with MK421. This may have contributed in a distinct way to the antihypertensive effects of the drug.

The potency of each drug in a dose of 10 mg appeared comparable, although the effects on components of the renin-angiotensin system appeared more prolonged with MK521. These differences were still apparent after 8 days of continuous treatment, but there was no evidence that this affected blood pressure control. Thus, MK421 and MK521 are potent inhibitors with a prolonged duration of action. They are well tolerated, although further testing is required to exclude significant toxicity.

VI. ENALAPRIL AND RENAL ARTERY STENOSIS

Workers in this department⁽⁴²⁹⁾ have reported the use of captopril in the pre-operative treatment of hypertension associated with unilateral renal artery stenosis. Long-term captopril effectively controlled hypertension in the majority of such patients. Moreover, it promised well as a predictor of the blood pressure response to operation, and this latter aspect has since been confirmed⁽⁴⁶⁸⁾. Oral captopril in a dose of 150 mg three times daily achieves sustained suppression of peripheral plasma angiotensin II concentration throughout 24 hours⁽⁴⁶⁹⁾; however, as seen in Section III of this chapter, the efficacy in this respect of the lower doses of captopril currently recommended⁽⁴⁷⁰⁾ remains to be established. This study has examined the use of long-term oral enalapril in a single daily dose in 20

patients with hypertension and renal artery stenosis.

Converting enzyme inhibition: effects on renin, angiotensins I, II and III, and aldosterone.

The present study has shown that enalapril in the range 1.25 to 40 mg daily produces distinct and dose-related falls in plasma converting enzyme activity and in angiotensin II, which are greater at 6 hours than at 2 hours and 24 hours from dosing, and are sustained during continued treatment. After 3 months of therapy, and 24 hours from the last dose of enalapril, plasma converting enzyme activity and angiotensin II remain below pretreatment values, but are further reduced 6 hours after the morning dose of drug. Sustained inhibition of converting enzyme 24 hours from the last dose is emphasised by the low levels of angiotensin II despite some fivefold elevation of angiotensin I in the same blood sample (Fig. 15).

Plasma active renin concentration, and blood angiotensin I, rise as angiotensin II falls with the first dose of enalapril, and increase progressively over the first 6 days of therapy, presumably because the secretion of renin continues to rise with sustained reduction of angiotensin II. Mean plasma active renin concentration is even higher at the third month of pretreatment, although angiotensin I levels do not increase in proportion. This apparent dissociation between renin

and angiotensin I during long-term treatment presumably reflects the fall in renin-substrate which is a feature of prolonged inhibition of converting enzyme⁽⁴⁷¹⁾. We have observed a similar lack of parallelism between the increase in plasma renin and blood angiotensin I concentrations during long-term treatment with captopril in renovascular hypertension⁽⁴²⁹⁾ and, as seen previously, with both enalapril and MK521 in normal volunteers.

Despite the very high circulating levels of renin and angiotensin I, there is no evidence of the converting enzyme inhibition being overcome during prolonged therapy, and angiotensin II remains low. Moreover, as the antibody used by us for the assay of angiotensin II cross-reacts with angiotensin III, these low values for angiotensin II on immunoassay mean that angiotensin III formation also is effectively inhibited. This latter finding confirms our previous results with captopril, in which angiotensin III was measured after separation from angiotensin II by chromatography⁽¹⁸³⁾.

In this series, mean plasma aldosterone was not high before treatment, and the reduction with enalapril was not statistically significant. This probably reflects the observation that plasma angiotensin II, although reduced, was not fully suppressed over 24 hours. While aldosterone secretion does appear to require the presence of at least some angiotensin II^(35,66,472),

once this obligatory requirement has been met, other influences, such as corticotrophin (ACTH) and potassium, can and do regulate its secretion^(35,66,472), and presumably did so here. In this study, with once daily enalapril, both plasma angiotensin II and aldosterone were demonstrably less completely suppressed over 24 hours than in our earlier trial of captopril 150 mg three times daily⁽⁴⁶⁹⁾.

Effects on blood pressure.

Of the 10 patients of Group 1, in no case was pre-treatment plasma renin or angiotensin II particularly high; indeed the mean plasma renin concentration for the group was just above the normal upper limit, while mean plasma angiotensin II was in the upper part of the normal range (Fig. 15). Thus while the fall in arterial pressure 6 hours from the first dose of enalapril was (as previously found with captopril⁽⁴²⁹⁾), proportional to the concurrent fall in plasma angiotensin II, in no instance was there profound hypotension, and there were no associated problems (see Chapter 6). Although the onset of action of enalapril is slower than that of captopril, care is nevertheless advisable in introducing the drug in a patient known or suspected of having high plasma renin values, or otherwise at risk of severe first-dose hypotension.

Highly significant reduction in arterial pressure, without marked postural effect, was seen at the 6th day of therapy (Figs. 15 and 17). At 3 months, although mean plasma angiotensin II concentration was no different from that at 6 days, a further highly significant fall in blood pressure had taken place; possible mechanisms in this slow fall in pressure have been discussed elsewhere. Despite the distinct, but minor, variations in angiotensin II over 24 hours, blood pressure control remained good through the day.

The fall in blood pressure 6 hours after the first dose of enalapril was significantly related to the pre-enalapril plasma renin and angiotensin II concentrations, and to the concurrent fall in plasma angiotensin II. However, by the third month of treatment, these relationships were much less close, and were not statistically significant with the numbers of patients studied. Thus these measurements, while good guides to the initial response to converting enzyme inhibition, are less reliable indicators of the long-term blood pressure fall.

The effectiveness of converting enzyme inhibition in controlling hypertension even in patients whose pre-treatment renin and angiotensin II levels are not high, deserves emphasis (429, 261, 473).

Blood pressure reduction by enalapril was similarly impressive in the more severely hypertensive and more complicated patients of Group 2, and additional therapy was largely unnecessary.

Tachycardia was only an occasional problem with enalapril, as with captopril previously. In one patient in Group 1, it subsided spontaneously with continued treatment. Nevertheless, two patients, both in Group 2, needed the addition of beta-blockade for its control.

Electrolyte changes.

The most striking change was the consistent reduction in exchangeable sodium with long-term enalapril. This finding contrasts with the previous work done in this department on captopril in renovascular hypertension (429); however, that was a more heterogeneous series, including patients with the hyponatraemic syndrome and marked sodium depletion, which was corrected as the blood pressure came under control (429). In the present series, there were no initial electrolyte abnormalities and mean pre-treatment exchangeable sodium was close to the expected normal value. In this study the sodium loss took place despite the fall in arterial pressure, and hence in diminution of pressure-natriuresis. The fall in plasma aldosterone seems too minor and inconsistent to provide a full explanation. Perhaps more plausible is the elimination by converting enzyme inhibition of

direct actions of angiotensin II upon the kidneys^(429,474). Furthermore, although previous therapy was stopped 14 days before enalapril was begun, we cannot exclude the possibility of initial minor sodium retention resulting from earlier treatment. Nevertheless, reduction of exchangeable sodium with enalapril was also seen in two patients of the series who had never received other agents.

The slight but significant increase in serum potassium is well known with converting enzyme inhibition. In the present series it probably resulted in part from loss of sodium in the absence of a rise in aldosterone.

Renal function.

The slight but consistent increases in serum creatinine and urea in Group 1 are noteworthy; these were not found in our earlier study of captopril⁽⁴²⁹⁾. In the present trial these changes could well reflect the fall in exchangeable sodium.

There are several reports of worsening of renal impairment with converting enzyme inhibition particularly in patients with bilateral renal artery stenosis or with stenosis of the artery to a sole remaining kidney⁽⁴⁷⁵⁻⁴⁷⁷⁾. To what extent this results from a fall in systemic arterial pressure or from loss of an intrarenal action of angiotensin II is at present uncertain. Bilateral renal

artery lesions were present in 5 patients of Group 2 in the present series. Blood pressure was effectively lowered in all by enalapril without appreciably raising serum urea or creatinine. However, most of the accounts of renal failure induced in such circumstances have mentioned concurrent use of loop diuretics. These were not employed in any of our 5 patients and this could have been the explanation for their sustained renal function. Indeed, in the whole of Group 2, mean values of serum creatinine did not change, and control of the arterial pressure undoubtedly was important in maintaining renal function, notably in the patients with malignant phase hypertension.

Both in Group 1 and Group 2, proteinuria moderated or cleared in all patients in whom it was present before enalapril was started.

One patient in Group 1 occluded an already severely narrowed renal artery during enalapril therapy, although his overall renal function did not deteriorate appreciably. Many such patients progress to renal artery occlusion irrespective of treatment, and possibly blood pressure reduction can heighten this risk. Concern has been expressed that converting enzyme inhibitors carry an especial danger in this regard⁽⁴⁷⁸⁾, and there is theoretical support for such a notion⁽⁴⁷⁹⁾. However, the occurrence seems not to be unduly frequent, at least with captopril⁽⁴⁸⁰⁾.

Enalapril as specific therapy in renal artery stenosis.

Only one patient of the present 20 has subsequently had surgical relief of renal artery stenosis; although postoperative blood pressures related well to those obtained during long-term enalapril obviously little light is shed on the question of usage of enalapril as a predictor of surgical outcome.

It further follows that we are uncertain whether the renal artery lesions were responsible for the hypertension in the majority of this series (although the diagnosis was supported by tests additional to renal arteriography). Certainly enalapril controlled blood pressure in many patients whose pre-treatment renin and angiotensin II values were normal; normal peripheral renin levels are however not uncommon in true renovascular hypertension and do not preclude a good surgical response.

Side-effects.

Side-effects were trivial in this series. Several patients commented on an enhanced feeling of well-being on enalapril in comparison with their earlier experience of other antihypertensive drugs, and this was an important factor in limiting their enthusiasm for surgical intervention. As we have seen with captopril, probably the

sense of well-being is the result of elimination of side-effects of other drugs.

There can be little doubt from the present trial that enalapril is an effective and well-tolerated agent given once daily in hypertension with associated renovascular disease.

VII. ALPHA-ADRENORECEPTOR AND BAROREFLEX FUNCTION DURING CONVERTING ENZYME INHIBITION.

When converting enzyme inhibitors are given to animals, normal subjects, or to patients with hypertension or congestive cardiac failure there is a reduction in blood pressure and afterload without evidence of reflex tachycardia^(240-243,303-306,375). As we have seen, the fall in blood pressure is proportional to the fall in circulating angiotensin II but there is evidence that other mechanisms contribute to the fall in blood pressure; in particular, it has been suggested that there is modification of sympathetic nervous system function.

It has been known for some time that alpha-adreno-receptor function is dependent on facilitation by subpressor concentrations of angiotensin II⁽⁴⁸¹⁾. Recently it has been shown that converting enzyme inhibitors can modify vascular responses to infused noradrenaline and sympathetic nerve stimulation in animal preparations,

and this may be angiotensin II dependent (243-247).

Vasoconstrictor tone is thought to be modulated mainly via the α_1 -adrenoreceptor (482-484), and α_1 -receptor antagonists have been widely used in the treatment of both hypertension and congestive cardiac failure (321). This experiment assessed and compared the effects on α_1 -receptor function of enalapril (MK421) and captopril, in normal therapeutic doses in normal subjects.

Post-synaptic alpha-adrenoreceptors may be divided into α_1 and α_2 subtypes (485,486), based on receptor demand between subtypes for agonists and antagonists. In vascular smooth muscle, the stimulation of either α -receptor subtype with an appropriate agonist causes vasoconstriction in resistance vessels and hence a rise in arterial pressure. The rise in blood pressure occurs much more rapidly when post-synaptic α_1 -receptors are stimulated selectively with cirazoline, phenylephrine or methoxamine, than when stimulated with the α_2 -adrenoreceptor agonists B-HT 920, azepexole or B-HT 933 (483). Furthermore, the magnitude of the pressor effect achieved by α_1 -adrenoreceptor stimulation is greater than that of α_2 -adrenoreceptor excitation with selective agonists (483,484).

In addition, alpha-receptor function is modified by angiotensin II, with increased noradrenaline release

and facilitation of its post-junctional effects, although the levels of angiotensin II required to potentiate adrenergic vasoconstrictor in vivo are below those require to exert a direct vasoconstrictor effect^(481, 487).

In the pithed rat^(489,491), isolated mesenteric artery⁽⁴⁹²⁾, and isolated, perfused rat and rabbit kidney^(493,494), captopril has been shown to attenuate, at pre- or post-junctional levels, the pressor effects of infused noradrenaline or sympathetic nerve stimulation. However, in intact animals this effect may not be seen.

In normal subjects, captopril has been shown to attenuate the pressor response to infused noradrenaline but this effect is abolished if sub-pressor doses of angiotensin II are also infused⁽⁴⁹⁵⁾. Interestingly, in a study on the vascular contraction induced by norepinephrine in the perfused rat mesenteric vascular bed, captopril has been shown to attenuate the vascular contraction induced by norepinephrine, but teprotide and SA 446 revealed no effect on vascular reactivity. In this experiment, the effect of captopril was not altered by the presence of bradykinin or angiotensin II in the perfusate⁽⁴⁹⁶⁾. Similarly, in another comparative experiment in the pithed spontaneously hypertensive rat, captopril, but not MK421, has been shown to inhibit pressor responses to sympathetic nerve stimulation and noradrenaline infusion⁽⁴⁹⁷⁾.

In most studies of captopril in human hypertension there is no change in the levels of circulating catecholamines⁽⁴⁹⁸⁻⁵⁰¹⁾, although in some severely hypertensive patients with high circulating catecholamines there is a fall after captopril is given⁽⁵⁰²⁾. Studies in heart failure have generally shown a fall in catecholamine concentrations when captopril is given⁽⁵⁰³⁻⁵⁰⁵⁾ but this effect is probably non-specific, occurring equally when nitroprusside is infused⁽⁵⁰⁵⁾.

Thus, while there is general agreement that captopril has a demonstrable effect on adrenoreceptor function, there is debate as to whether this effect is pre- or post-synaptic, dependent upon the presence of circulating angiotensin II, or peculiar to captopril rather than being a shared property of all converting enzyme inhibitors.

The results of this study suggest that in normal clinical doses, neither captopril nor MK421 reduce blood pressure and afterload by effects on the α_1 -adrenoreceptor. Thus, while captopril and prazosin may induce similar haemodynamic effects, the mechanisms of action are distinct. These findings do not preclude effects on α_2 -receptor function although such effects in normal physiological circumstances would play a relatively minor role in the modification of vasoconstrictor tone.

Captopril reduces blood pressure without increasing heart rate and this suggests that the drug may effect baroreflex mechanisms. A previous study⁽⁵⁰⁶⁾ with bolus injections of phenylephrine and nitroprusside has suggested that baroreceptor sensitivity to stimulation with phenylephrine is maintained unchanged, although the response curve is reset. Baroreceptor deactivation with nitroprusside, however, produced augmented responses of the pulse rate and mean arterial pressure, suggesting increased baroreceptor sensitivity.

This study shows similar resetting to baroreceptor function during stimulation with phenylephrine, and this resetting is exactly similar for both captopril and MK421.

VIII. CONCLUSIONS.

These, and other studies, have shown that converting enzyme inhibitors, particularly when combined with a diuretic, have the antihypertensive potency to be used effectively in most forms of hypertension of whatever severity. Their use is not associated with orthostatic hypotension or a rise in heart rate.

Occasional patients experience adverse reactions to their use which appear to be due to converting enzyme inhibition per se rather than to direct drug side-effects.

These include hypotension with the first dose, tachycardia, Raynaud's phenomenon, hyperkalaemia and deterioration of renal function. These effects are uncommon, rarely serious and may possibly be minimised with improved drug formulations and dosage regimes.

Side-effects have proved a particular problem with captopril. Dosage reduction may avoid the more serious effects, but the minor side-effects of skin rash and taste loss may still occur. Enalapril and MK521 were designed without a sulphhydryl group and to date there have been no reports of captopril-like side-effects.

To enhance compliance with therapy, dosage intervals should in general be longer rather than shorter. In this respect, captopril, in twice daily doses, and enalapril, in a once daily dose, compare favourably with other agents.

Many, if not most, treated hypertensive patients are physically, mentally and sexually active. Tolerability is therefore a fundamental requirement of any antihypertensive drug. Although there is no evidence to suggest a euphoriant effect, the converting enzyme inhibitors are clearly well accepted and compare favourably with previous drug therapy.

In summary, captopril and the new converting enzyme inhibitors are potent, well-tolerated antihypertensive agents. Enalapril and its lysine analogue appear relatively free from toxic side-effects although large scale studies are required to confirm this. In this event, these and other inhibitors will undoubtedly be of lasting value in the future of cardiovascular therapeutics.

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