STUDIES ON

ANGIOTENSIN CONVERTING ENZYME INHIBITORS.

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

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CONTENTS

CHAPTER 7:STUDIES OF ACE INHIBITION IN NORMAL AND PATIENT VOLUNTEERS

7.1 TRANSPULMONARY PHARMACOKINETICS OF PERINDOPRILAT IN MAN

7.2. PERINDOPRILAT INFUSION STUDIES IN SALT REPLETE MAN

Index of Figures

Chapter 7

Index of Tables **Following page**

Chapter 2

Chapter 3

Chapter 4

Chapter 6

Chapter 7

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SUMMARY

A range of studies are described examining the responses to blockade of the renin angiotensin system with angiotensin converting enzyme inhibition in patients with heart failure and normal volunteers. The common theme of the studies is the definition of response at low doses of drug and differentiation of effects between agents or over time on first administration.

First dose response to ACE Inhibitors in heart failure

(a) Oral doses of placebo, captopril 6.25mg, enalapril 2.5mg and perindopril 2mg were given in a randomised double blind parallel group study of 48 elderly patients with stable heart failure. Despite diuretic withdrawal considerable falls in supine blood pressure and heart rate were noted with captopril and enalapril. Compared to placebo perindopril produced no significant fall in pressure. Enalapril and perindopril produced similar plasma ACE inhibition. No patient experienced adverse symptoms.

(b) Intravenous doses, by slow (6hr) constant rate infusion, of placebo (saline), enalaprilat (1.5mg) or perindoprilat (1mg) were given in a randomised, double blind, parallel group study of 36 elderly patients with stable heart failure. Both diacid ACE inhibitors caused falls in blood pressure and similar though greater inhibition of plasma ACE activity compared to the oral treatments. Five infusions were terminated on a priori criteria because of the degree of blood pressure fall. Termination of infusion effectively controlled the blood pressure fall. Two patients on active treatment had asymptomatic deterioration in biochemical indices of renal function necessitating withdrawal of maintenance ACE inhibitor treatment.

Studies of the response to ACE inhibition in volunteers

(a) The transpulmonary extraction of perindoprilat (1mg; by constant rate infusion into a

peripheral vein over 20minutes) was examined in a novel study in 10 patients undergoing diagnostic cardiac catheterisation predominantly for symptoms suggestive of ischaemic heart disease. A co-infusion strategy employing concurrent indocyanine green as an intravascular marker was used. Concurrent sampling in the ascending aorta and the pulmonary trunk failed to show any significant first pass uptake of the drug into the pulmonary circulation. Pharmacokinetic models designed to incorporate elements describing simple nonlinear kinetics based on "tissue" distribution were not found to be applicable. A variety of procedural problems may have been responsible for the failure of this study to document tissue distribution.

(b) Protracted low dose constant rate infusion of perindoprilat (1mg) over 1, 3 or 6hrs or placebo (saline over 3hr) was conducted in a single (subject) blinded randomised study in 10 healthy male volunteers. This predominantly pharmacokinetic study confirmed a blood pressure fall despite the low doses employed in normal volunteers. Despite higher peak drug concentrations the plasma ACE inhibition profile summarised by the area under the curve was greatest following the 6hr infusion where peak drug concentration was lowest. Drug concentration time profiles showed a clear sigmoid accumulation profile during drug infusion. Pharmacokinetic modelling of individual profiles confirmed that from a hierarchy of systems a non linear model with terms to represent tissue and plasma binding of drug provided the best fit to the observed data.

(c) A model of activation of the renin angiotensin system was outlined in a double blind randomised study of low dose oral enalapril (5mg) in eight healthy salt depleted normal volunteers. A system of modest dietary salt restriction (40mmol per day) and diuretic therapy (40mg Frusemide BDS) over three prestudy days produced a reliable activation of the renin angiotensin system. Baseline and reactive elevation of renin activity was recorded in response to enalapril with an associated significant fall in supine and erect blood pressure.

Conclusions

Differential effects on blood pressure are evident between similar ACE inhibitors (enalapril and perindopril) but not with their respective diacid metabolites despite similar circulating enzyme inhibition in controlled circumstances. This may relate to a differential interaction with the tissue based elements of the renin angiotensin system. Although the transpulmonary study did not reveal significant extraction of the diacid ACE inhibitor perindoprilat into the lungs, the subsequent pharmacokinetic studies with low dose infusions of the same drug indicate that an indirect index of the tissue based system may be possible. Studies in heart failure patients may be usefully extended in volunteers whose renin system is activated using the salt depletion protocol as described.

CHAPTER 1

INTRODUCTION

Developments in the drug therapy of disease have proceeded with astounding speed in the 20th Century. During my undergraduate training in pharmacy in the 1970's it was common to be told that the pace of development had been so rapid, on all fronts, that there would undoubtedly be a hiatus in progress during the latter part of the 20th century. Perhaps not surprisingly this has not been the case. During this period, at least in the realm of cardiovascular drugs, there have been continued developments. There are few agents introduced into clinical practice in recent years which might reasonably be compared to the group of drugs known as the angiotensin converting enzyme inhibitors.

The origins of the renin angiotensin aldosterone system, one of the key physiological systems regulating blood pressure and sodium homeostasis, date back to experiments in the laboratory of Dr Robert Tigerstedt at the end of the 19th Century in Finland. It took almost 60 years to analyse the functions of this system and develop inhibitors. Useful drugs appeared in clinical research studies in the mid to late 1970's. Since that stage their initial use in hypertension has been expanded and refined in general medical application with the subsequent development of large series of compounds sharing the property of inhibiting the non rate limiting step catalysed by the non specific metalloprotease angiotensin converting enzyme (ACE). In last ten years ACE inhibitors have continued to develop in their clinical uses. For the first time they provided a therapy for the effective management of chronic heart failure capable of making a positive step in reducing the symptoms and mortality of this disease. Developments continue with extension of the assessment of these drugs into different populations of cardiovascular

patients, and new insights of the role of the renin angiotensin system in a variety of pathological states.

This thesis comprises two sections firstly dealing with ACE inhibitor studies in heart failure and subsequently a series of investigations in normal human or patient volunteers. This is preceded by a detailed description of ACE inhibitor pharmacology. An example is made of the properties of the long acting prodrug ACE inhibitor perindopril, which is employed in a number of the subsequent studies. In addition the renin angiotensin system, particularly the more recent work concentrating on the relevance of an extra-plasmatic site mediating drug effects is also described in detail. The practical and pharmacological management of heart failure including the role of ACE inhibition is also described by way of introduction to the experimental section.

The first series of studies are designed to assess and refine the application of ACE inhibitor drugs, more specifically to allow better understanding of the relationship between dose, concentration and (predominantly haemodynamic) effects in heart failure patients. Particular attention is paid to the response to the introduction of the drugs in heart failure where, for a variety of reasons, the endogenous renin angiotensin system is activated and a marked haemodynamic response might be anticipated. Despite many years of use the exact dose required to produce a given effect, be that chemical, pressure or flow related or more importantly a symptomatic benefit in the individual patient has remained obscure. This is not unusual in cardiovascular therapy. First dose studies are of interest as they allow the lower end of the concentration response relationship to be explored. This has been a notable failure in the development of the ACE inhibitor drugs whose "effective" doses have fallen after introduction into clinical practice and experience. In addition comparative studies, commercially unpopular but

pharmacologically essential, are described which portray novel findings in these patients.

To complement this series of observations in heart failure are a range of studies in the normal human volunteer are presented where control of the variables affecting the response can be more precise. Controlled low dose infusion strategies are used in an attempt to characterise the use of a pharmacokinetic model as an index of tissue distribution of drug at low doses relevant to heart failure therapy, at least during the introduction of treatment. An attempt to define the transpulmonary handling of an intravenous ACE inhibitor is described. This is again based on the plasma pharmacokinetic profile and looking at its potential as an index of drug distribution into tissue based sites, of which the lungs has been highlighted as a key area of ACE activity. The studies are complemented by a series of preliminary observations involving activation of the renin angiotensin system based on controlled salt depletion and the subsequent response to low dose ACE inhibition.

CHAPTER 2

ANGIOTENSIN CONVERTING ENZYME INHIBITION: PERINDOPRIL

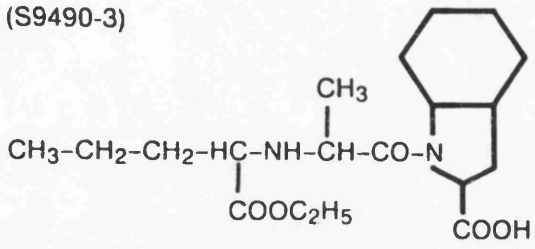
2.1. GENERAL INTRODUCTION

Perindopril is a long-acting non-thiol, prodrug inhibitor of angiotensin converting enzyme (ACE). The compound has been undergoing evaluation since the early 1980s and is at present available for clinical use in several countries throughout the world (Todd & Fitton,1991). An overview of its chemistry, pharmacodynamic and pharmacokinetic properties is provided here for this drug as representative of its class and as the subject of a number of the following research studies which form the first part of the thesis. As a relatively less popular agent, in terms of its post marketing use, full appreciation of the specific advantages or disadvantages of this agent will emerge only after widespread, post-marketing surveillance and further clinical research (Editorial, 1988). Perindopril is available alongside the current agents in this class, captopril (Brogen et al, 1988), enalapril (Todd & Heel, 1986; Todd & Goa, 1989), lisinopril (Lancaster & Todd, 1988) ramipril (Todd & Brogen, 1989), quinapril (Frank et al,1990), cilazapril and fosinopril, all of which are licensed for use in the United Kingdom at the time of writing.

2.1.1. Origin and Chemistry

The synthesis of perindopril was first described by Vincent and colleagues (1982) from Servier Laboratories. Orally active, potent, long-lasting inhibition of ACE was confirmed as the primary pharmacological response. This compound is a third generation ACE inhibitor, the perhydroindole 2-carboxylic acid derivative of an N-carbethoxybutyl alanine. The diastereoisomer (s,s) perindopril is prepared as a tertiary

(S9490-3)



(S9780)

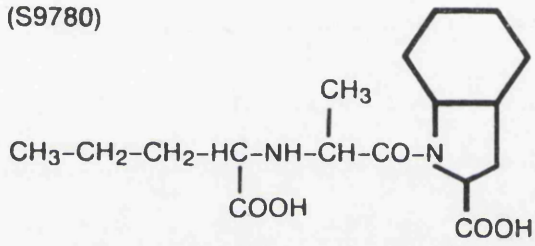


Figure 2.1: Structural formulae of perindopril (S-9490-3) as butylamine salt, and perindoprilat (S-9780).

perindoprilat (S9780) (Figure 2.1). The parent molecule resists hydrolysis of the amide bond due to the stabilising influence of the bicyclic ring (Drummer et al, 1987).

Metabolism is by esterases in the liver, plasma and possibly also in the gut wall to the active diacid perindoprilat. A desprolyl metabolite similar to that noted with enalapril (Drummer & Kourtis, 1987a) is not produced but a glucuronide conjugate has been identified which cross-reacts with antisera to perindoprilat (Drummer & Kourtis, 1987b; Drummer et al, 1987;1988). This conjugate can be excreted or is available for conversion back to a form capable of inhibiting ACE. The conjugate per se is biologically inactive.

2.1.2. Analysis

Perindopril and perindoprilat can be assayed in plasma and tissue extracts by a variety of techniques. An enzyme inhibition assay, similar to that employed by Tocco and colleagues (1982) for enalapril, has been employed using the inhibition of exogenous ACE generation of a chromophobe from a variety of synthetic substrates (see Chapter 5). Alternatively, radio- inhibitor binding and displacement principles have been applied employing a radio-iodinated tyrosyl derivative of lisinopril, ^{125}I-351A (Johnston et al, 1987). The principles of competitive protein binding are adapted where the substrate is the radioligand and the drug and the ACE interaction is time, concentration, pH, chloride and zinc dependent (Shapiro & Riordan, 1984; Jackson, Cubela & Johnston, 1986; Johnston et al, 1988). Specific and sensitive immunoassay of ACE inhibitors are also available (Hichens et al, 1981) but such radioimmunoassay methods for perindopril have not been published. Analysis of both perindopril and perindoprilat by combined high pressure liquid chromatography and mass spectrometry has been described (Tsaconas et al, 1989).

2.2. PHARMACOLOGICAL PROPERTIES

The interaction of inhibitors of ACE in a variety of physiological and pathophysiological states is more complex than initially appreciated. Initially characterised in horse plasma by Skeggs and colleagues (1956), ACE is now known to be widely distributed throughout many tissues. The specific evidence for this differentiation and its implications for the results of treatment with ACE inhibitor drugs is considered in the following chapter (see chapter 3). Although ACE has a variety of neurohormonal substrates it is generally felt that the predominant effects of enzyme inhibition are mediated through a reduction in the generation of angiotensin II from angiotensin I. The central effect of this hormone is pressure and volume homeostasis. Secondary elevation of renin, angiotensinogen and angiotensin I by negative feedback and falls in aldosterone and angiotensin II are observed after acute treatment. Enzyme inhibition is known to be competitive for a number of inhibitors (Ondetti & Cushman, 1982) with at least two binding sites on the enzyme itself (Perich et al,1991). This is also likely to be the case for perindopril (Laubie et al, 1984).

It should be noted that recent studies with sensitive and specific assays of angiotensin peptides have suggested that persistent ACE inhibition can be accompanied by resetting of the renin angiotensin system and the reappearance of angiotensin II in plasma, which is not reflected by ACE inhibition determined in vitro (Nussberger et al, 1988, 1989).

2.2.1. Inhibition of ACE and hormonal effects

A. Circulating ACE

Laubie and colleagues (1984) demonstrated ACE inhibition by perindopril, attenuation of the pressor dose response curve to angiotensin I infusion and a potentiation of the vasodilatation with exogenous bradykinin in a variety of animal

species. In these studies the in vitro potency of perindoprilat was approximately **1,000 fold** greater than that of the parent compound against serum ACE. Both drug and metabolite exhibited competitive antagonism of the enzyme. Intravenous administration of perindopril was followed by de-esterification to perindoprilat, which appeared to be more efficient in rats and rabbits than in cats, guinea pigs and dogs. This was reflected by the ED_{50} of circulating ACE and in the ID_{50} of the pressor response to angiotensin I in anaesthetised animals (rabbits > rats >> cats >> guinea pig >> dog). Unger and associates (1986) using inhibition of rat plasma ACE showed that the curves for ramiprilat and perindoprilat were almost identical and both were 50-100 fold more potent than enalaprilat or captopril respectively. Using human plasma ACE, Johnston and colleagues (1988) found that the rank order of potency of a variety of ACE inhibitors including both perindopril and perindoprilat was comparable whether expressed in terms of IC_{50} or by radio-inhibitor displacement. They reaffirmed the approximately 1,000 fold difference between perindopril and perindoprilat.

It should be noted that the source of circulating plasma ACE has not been clearly established (Erdos, 1976). Although lung, kidney and vascular endothelium in culture can release large amounts of the enzyme with similar kinetic and inhibitory characteristics between different species (Takada et al, 1982; Miyazaki et al, 1984), evidence is emerging that this may not be the case for circulating serum or plasma ACE (Ibarra-Bubio et al, 1989; Harrigan, Hughes & Meredith, 1989). Thus data on the tissue assimilation of specific agents for example perindopril, should probably be assessed in human tissue and circulating ACE.

ACE inhibition, as assessed by synthetic substrate in vitro, has repeatedly been shown to be poorly correlated to the haemodynamic effects (Waeber et al, 1980; Unger et al, 1986). Persistent and unquantified tissue ACE inhibition may be one explanation for this phenomenon but Nussberger et al (1989) have shown that the definition of ACE

inhibition in vivo using sensitive analysis of angiotensin I and angiotensin II may clarify the relationship (see section 3.2.1). As yet the effect of perindopril or perindoprilat on ACE has only been defined using in vitro techniques. There may be a question mark over the reliability of these assessments. The comparative potency of perindopril and perindoprilat on the IC_{50} of human plasma ACE is summarised in Table 1.

The onset of circulating ACE inhibition after oral perindopril is slower than that seen with other ACE inhibitors (Brunner, Nussberger & Waeber, 1985) taking approximately four hours to achieve greater than 90% inhibition in studies conducted in normal volunteers (Bussien et al, 1986; Richer et al, 1987; Lees & Reid, 1987). Bussien and colleagues (1986) found that doses of greater than 4 mg provided greater than 70% inhibition of plasma ACE after a single dose with a maximum response of 79% inhibition after 16 mg. Lees and Reid (1987) found comparable inhibition 24 hours after a dose of 8 mg or greater.

Chronic treatment with oral perindopril in doses of 1- 16 mg daily has also been reported in normotensive, male volunteers. In two studies the level of ACE inhibition 24 hours after dosage remained at approximately 60-70% of baseline, dose related, with a slow return towards normal in the days after discontinuation. Neither of these studies recorded the return to baseline activity during monitoring (Bussien et al, 1986; Lees & Reid, 1987).

B. Neurohormonal changes

It would be expected that inhibition of circulating ACE would cause falls in angiotensin II and aldosterone with elevation of plasma renin activity and angiotensin I. Oral perindopril in acute and chronic settings has been shown to produce these trends four hours after oral doses of 4 and 8 mg (Bussien et al, 1986). In this study, although

Table 2.1: Plasma concentrations (EC_{50}) of a variety of angiotensin converting enzyme inhibitors corresponding to 50% inhibition of maximal ACE activity (human plasma) (after Johnston, 1988).

DRUG	EC_{50}(M)	DRUG	EC_{50}(M)
Perindopril	0.1×10^{-4}	Perindoprilat	0.18×10^{-8}
Enalapril	0.24×10^{-4}	Enalaprilat	0.25×10^{-8}
Ramipril	0.8×10^{-5}	Ramiprilat	0.19×10^{-8}
Cilazapril	0.28×10^{-6}	Cilazaprilat	0.8×10^{-9}
Lisinopril	0.45×10^{-8}		
Captopril	0.22×10^{-7}		

aldosterone was seen four hours post dosing, at 24 hours the hormones had returned to baseline values. This is in contrast to the first dose response where angiotensin II and aldosterone remained low at 24 hours. Lees and Reid (1987) confirmed dose related elevation of plasma renin activity after oral doses of 1-16 mg, however the effects on plasma aldosterone were variable. Chronic treatment for 7 days produced further elevation of plasma renin activity. Circulating levels of catecholamines were unaltered.

Richer et al (1987) conducted more detailed temporal sampling of plasma renin activity and aldosterone and found with doses of 4, 8 and 16 mg that there was a progressive elevation of plasma renin activity peaking at 6 hours post dosage though once again plasma aldosterone was not significantly altered. These workers suggested that this was due to the low baseline values associated with supine sampling.

2.2.2. Haemodynamic Effects

A. Normal Volunteers

The effects of ACE inhibitors on arterial pressure, organ perfusion and blood flow in normal volunteers and cardiovascular diseases have been extensively investigated. In normal salt replete volunteers the effects of acute inhibition on supine and erect blood pressure are controversial. Carefully controlled studies with appropriate doses and placebo usually reveal small but significant falls in blood pressure in animals. The acute effects of ACE inhibitors are suggested to be mediated in part by reductions in angiotensin II and are dependent on the prevailing activation of the circulating renin angiotensin system and dietary salt depletion where there is renin activation (Lever, 1989). However, ACE inhibition will also produce a hypotensive response on long-term treatment in low renin states such as is the case in the majority of essential hypertension (Brown et al, 1965). This has been taken to reflect modification of the slow pressor element of hypertension which may be mediated through renin, angiotensin II or

aldosterone (Lever, 1986, 1989).

In the conscious dog, Laubie et al (1984) demonstrated an inhibition of the pressor response to angiotensin I using both intravenous and oral perindopril and intravenous perindoprilat in cumulative dosage. Vasodilatation mediated by exogenous bradykinin was seen to be potentiated. Conscious animals did not show a hypotensive response until sodium restriction was employed although this was not required in anaesthetised animals.

Lees and Reid (1987b,c) observed the response to oral perindopril or intravenous perindoprilat in salt replete normal volunteers. They noted a fall in erect systolic blood pressure with an oral dose of 16 mg of perindopril but not with lower doses. This was maintained over seven days chronic therapy and was present without alterations in heart rate. Intravenous perindoprilat given as a bolus of 4 mg caused a fall in erect diastolic blood pressure over 8 hours and a similar but not significant trend was noted in erect systolic blood pressure. Ajayi et al (1986) studying ten normotensive salt replete volunteers found a modest but significant fall in supine systolic and diastolic blood pressure when giving 8 mg oral perindopril in a double-blind, crossover assessment of autonomic function. There was no effect on heart rate.

In contrast Bideville et al (1987) studied five of 16 healthy, male volunteers treated with 8 mg perindopril daily using semi- automatic, ambulatory blood pressure monitoring. Neither this treatment nor cilazapril (5mg per day) nor CGS 12824A (10 mg) produced any consistent effect on blood pressure either acutely or over eight days chronic therapy. Similarly Richer et al (1987) in a double-blind, crossover study in six healthy volunteers found no effect on blood pressure or heart rate after single doses of 4, 8 and 16 mg perindopril despite dose dependent augmentation of brachial and carotid artery flow for up to ten hours after drug treatment and the observation of appropriate neurohormonal changes in circulating ACE, plasma renin activity and aldosterone.

Dollery and colleagues (1984) have described initial dose finding studies in normal volunteers employing individual angiotensin I pressor responses in five subjects. Perindopril (2.5, 5 and 10 mg orally) inhibited responses by between 30 - 90%. Maximal inhibition of angiotensin II pressor response, set at 25 mmHg for each individual, occurred approximately 4-6 hours after treatment with maximal rises of 17.2, 9.5 and 4.6 mmHg with the three doses employed. At 24 hours after a dose of 10 mg a pressor response of only 16.6 mmHg was noted, which was associated with persistent ACE inhibition and elevation of plasma renin activity.

Boussien et al (1986) assessed the individual pressor response in three subjects to intravenous angiotensin I with concurrent oral perindopril in doses of 2, 4 and 8 mg on different days. Only with 8 mg was a consistent attenuation of the pressor responses noted within 1.5 hours of dosing. Eight days chronic therapy with 4 mg oral perindopril in twelve subjects produced no changes in semi- recumbent blood pressure or heart rate.

B. Essential Hypertension

Although initially restricted to the treatment of severe hypertension where other agents had failed, it is now clear that ACE inhibitors are effective in any stage of hypertension regardless of the prevailing renin activity (Williams, 1988). Moreover the United States Joint National Committee on the detection, evaluation and treatment of high blood pressure (Chobanion et al, 1988) recommends that they can be considered as first line agents similar in standing to diuretic therapy or beta blockade. In the UK all recent ACE inhibitors are licensed for first line use in hypertension.

In experimental hypertension perindopril has been shown to lower blood pressure in several strains of spontaneously hypertensive rat (DiNicolantonio & Doyle, 1985; Unger et al, 1986) and in experimental Goldblatt 2 kidney 1 clip hypertension (Michel et al, 1986). In acute studies with or without salt depletion the response appears to be unrelated to pretreatment plasma renin activity or the degree of circulating ACE

inhibition and is dissociated in onset and duration from the hormonal changes (Di Nicolantonio & Doyle, 1985). The initial response to ACE inhibition is thought to be mediated mainly by a decreased peripheral vascular resistance in the absence of changes in mean arterial pressure. This has been shown in normal, human volunteers by Thiullez et al (1988) and Webb and colleagues (1988) using pulsed Doppler techniques and forearm venous occlusion plethysmography.

The haemodynamic efficacy of oral perindopril in human essential hypertension has been documented by several groups. Lees and Reid (1987d) showed an average reduction of arterial pressure from 164/93 on placebo to 142/82 in a group of seven salt replete essential hypertensive patients treated with 4 mg perindopril daily for one month in a single-blind study. No first dose accentuation of the antihypertensive effect was noted and symptomatic hypotension was absent. Blood levels of perindoprilat on chronic therapy revealed high peak concentrations but comparable circulating ACE inhibition although the latter tended to be less pronounced. Plasma renin activity remained elevated on chronic therapy whereas aldosterone was unchanged. These patients did not have high renin hypertension.

Luccioni and colleagues (1988) demonstrated a dose- related antihypertensive effect of oral perindopril in forty patients with essential hypertension treated in a double-blind assessment. Doses of 2, 4 and 8 mg oral perindopril or placebo given once daily after a two week placebo run-in produced dose related falls using automatic sphygmomanometry.

Morgan and colleagues (1987) in 32 patients with essential hypertension found that the falls in erect and supine blood pressure noted after dose titration with 2 - 8 mg of oral perindopril given once daily were unrelated to the sodium excretion or the plasma renin activity in this group.

Safar and colleagues have studied the effect of perindopril on arteriolar dilatation

using a pulsed Doppler flow meter in essential hypertension (Asmar et al, 1987; Asmar et al, 1988a,b). In 16 patients with essential hypertension defined as diastolic blood pressure greater than 100 mmHg, three months treatment with oral perindopril 2 - 8 mg per day significantly reduced blood pressure and this was associated with increased brachial artery flow, flow velocity and arterial diameter. This was not thought to be due to a simple flow dependent dilatation as occlusion produced a greater fall in flow velocity in perindopril treated patients with an equivalent fall in arterial diameter. Treatment was found to be associated with increased arterial compliance and reduced pulse wave velocity. In this same series of patient studies echocardiographic cardiac mass was also reduced by perindopril treatment and this beneficial effect persisted after discontinuation of therapy despite the return of arterial parameters towards pretreatment baseline values. Structural changes were therefore dissociated from simple pressure reduction at a level beyond first order windkessel vessels and these clinical studies were in support of experimental work by the same group in Goldblatt renovascular hypertension (Levy et al, 1988). Not all experimental studies however ,support such close association between continued antihypertensive effect after perindopril withdrawal and the vessel structure (Christensen et al, 1988).

The regression of left ventricular hypertrophy on chronic therapy with 4 - 8 mg oral perindopril daily was also found by Grandee et al (1988) in a study of 15 patients with essential hypertension. Echocardiographic left ventricular mass was decreased without an alteration of left ventricular diastolic diameter or peak systolic shortening though diastolic relaxation rate was increased. A cold pressor test was employed to assess the response to acute increases in afterload and this was unimpaired by the changes mediated by perindopril therapy.

In animal models of hypertension reduction of left ventricular hypertrophy has been noted on chronic perindopril treatment by several studies (Barres et al, 1985;

Cadilhac & Giudicelli, 1986). However Michel et al (1988a) in 1 clip 2 kidney renovascular hypertensive rats or deoxycorticosterone salt hypertension failed to show reversal of structural changes in the myocardium with chronic perindopril therapy though the collagen content was considerably reduced.

West and colleagues (1989) studied eight patients with essential hypertension using 24 hour intra-arterial blood pressure monitoring. During six weeks therapy with 8 mg perindopril daily in a double-blind, placebo controlled, crossover study they noted reduction of average hourly blood pressure throughout the 24 hour period with maintenance of the normal circadian pattern. Forearm blood flow assessed by venous occlusion plethysmography was increased. The haemodynamic response to Valsalva's manoeuvre, tilt, isometric exercise and cold pressor testing was unaffected by perindopril but the sino-aortic baroreceptor heart rate reflex was reset within two hours of the first dose. This remained altered throughout chronic therapy and the heart rate remained unchanged. Chronic therapy with perindopril was associated with a persistent increase in parasympathetic tone. Enhanced parasympathetic tone has been found in normotensive volunteers in the studies of Ajayi et al (1986) using oral perindopril and has been noted with other ACE inhibitors (Sturani et al, 1982; Campbell, et al, 1985; Ajayi et al, 1985).

In a study of 21 patients monitored by non-invasive 24 hour ambulatory blood pressure recording Asmar and colleagues (1988c) found that 4 - 8 mg of perindopril once daily affected systolic pressure greater than diastolic pressure. This conclusion was derived from the relationship between systolic and diastolic pressures before and after three months therapy. This is also a feature of other ACE inhibitors (Webster et al, 1986).

The place of perindopril as an antihypertensive agent in clinical practice has been studied in three multicentre trials of similar design described by Zanchetti and Desche

(1989) and Lees and colleagues (1989). Oral perindopril (4 mg) was compared to captopril 25 mg twice daily (165 patients); atenolol 50 mg (173 patients) or to a diuretic regimen of amiloride and hydrochlorthiazide (165 patients) in double-blind, placebo controlled studies in mild to moderate essential hypertension. After single blind placebo washout, treatment was designed to obtain a diastolic blood pressure less than or equal to 90 mmHg. If this was not achieved at monthly review then the daily dose was initially doubled and if required subsequent treatment was supplemented by hydrochlorthiazide 50 mg (captopril or atenolol comparisons) or atenolol 50 - 100 mg daily (diuretic comparison).

In the study comparing perindopril to captopril (Lees et al, 1989) monotherapy was equally effective in each group (49% vs 49%) though the overall fall in diastolic blood pressure after three months therapy was greater with perindopril (26.9 $\pm$ 1.9 vs 18.9±1.9 mmHg). This probably reflects the higher initial pretreatment diastolic blood pressure in this group (105.4±0.8 vs 102.3±0.6). With dose titration, control was achieved more readily with perindopril monotherapy than captopril (75% vs 57%). In an extension of this trial Herpin et al (1989) using 24 hour ambulatory monitoring suggested that diastolic blood pressure was controlled for a significantly greater part of 24 hours by perindopril once daily than by captopril twice daily

In the series comparing perindopril to atenolol, after three months therapy the reduction in erect systolic but not diastolic blood pressure was greater with perindopril. The mean reduction in supine blood pressure (26.5 mmHg) was greater than that achieved with atenolol (20.6 mmHg) and in this, as in the diuretic arm of the study, the pretreatment blood pressures were equivalent.

Diuretic therapy was compared to perindopril 4 mg daily in the preceding format with dosage doubling and the addition of beta blockade as stepped therapy. After three months treatment monotherapy was equally effective in the perindopril and diuretic

treated groups (72% vs 72%) and in total the percentage of patients responding with monotherapy or combination was not significantly different between perindopril therapy (78%) or diuretic based treatment (84%). Although the reductions in supine blood pressure were similar, erect mean blood pressure was reduced to a greater extent by diuretic therapy (-31.1 mmHg) and perindopril (-24.6 mmHg).

Backhouse and colleagues (1989) in forty patients with essential hypertension has reaffirmed the antihypertensive efficacy of perindopril. With the combination of perindopril and a thiazide diuretic a synergistic response was noted in antihypertensive effect and in the elevation of plasma renin activity in this trial conducted over one month using double-blind therapy.

C. Cardiac Failure

It is now generally accepted that ACE inhibitors are the major adjuncts to diuretic therapy in the management of chronic cardiac failure (Packer, 1989). Efficacy has been proven in terms of alleviating symptoms increasing exercise capacity and producing a significant reduction in long-term mortality (Cleland et al, 1984; Consensus Trial Study Group, 1987; Captopril Digoxin Multicentre Research Group, 1988). While efficacy is accepted in severe chronic heart failure there is also evidence to support the use of ACEI in less serious degrees of cardiac failure (Kleber et al,1992; SOLVD Investigators 1991;1992). These issues are dealt with fully in chapter 4 (section 4.4).

2.2.3. Effects on renal function and renal blood flow

In a double blind study in eleven normotensive volunteers on controlled sodium intake Reyes et al (1988) showed that single doses of 4 and 8 mg perindopril produced dose dependent increases in 24 hour urinary output and instantaneous renal clearance of sodium and chloride without affecting urinary water production. Other solutes were

unaffected by treatment though urate excretion tended to increase.

Morgan and colleagues (1987) in a study of 32 hypertensive patients without renal failure stratified the group in accordance with 24 hour sodium excretion. No effect of perindopril treatment (2 - 8 mg daily) was found on glomerular filtration rate, 24 hour urinary protein or plasma urea or creatinine with titrated antihypertensive therapy.

In ten patients with hypertensive nephropathy Rondeau et al (1988) reported that 15 days therapy with oral perindopril did not alter proximal or distal tubular sodium re-absorption as reflected by renal sodium or lithium clearance.

The effects of ACE inhibition on renal blood flow and sodium handling are complex and related in most instances to the modulating effect of angiotensin II on vascular resistance at a variety of pre and post glomerular sites (Hollenberg & Williams, 1988). Renal arteriolar vasoconstriction and reduced renal blood flow are common in essential hypertension and thus improved flow with ACE inhibitor therapy is an important component of antihypertensive effect. This response has been documented for other ACE inhibitors (Mimram et al, 1979; Hollenberg & Williams, 1988).

Experimental studies have been conducted in anaesthetised dogs using local bolus injection of perindoprilat into the renal arteries (Schmidt et al, 1989). Increased renal blood flow was demonstrated after 48 hours sodium restriction associated with a decreased filtration fraction. Renal vascular resistance was reduced in association with effects predominantly on the efferent side of the glomerulus, the preferential site of action of angiotensin II. Increased fractional elimination of electrolytes was noted due to altered sodium and water reabsorption in the proximal tubule secondary to the decrease in filtration fraction. In general the effects were much less prominent in sodium replete animals as would be expected (Hollenberg & Williams, 1988).

In eight essential hypertensive patients without nephropathy on a standard sodium intake Chaignon and colleagues (1988) saw only marginal increases in renal blood flow

in an open study using four days therapy with perindopril (8 mg). Calculated renal vascular resistance fell with initial therapy and remained depressed on day 5. Glomerular filtration rate was unchanged and filtration fraction decreased significantly from 0.27 to 0.25 after four days treatment. An acute and chronic natriuresis was evident in this study.

In the last few years a number of studies in mild diabetic nephropathy associated with persistent or exercise related microalbuminuria have suggested a beneficial effect of converting enzyme inhibition in the absence of hypertension and without a fall in mean arterial pressure (Taguma et al, 1985; Marre et al, 1987; Romanelli et al, 1989). These observations were recorded in patients with equivalent diabetic control. Similar benefit has been recorded in patients with proteinuria due to hypertensive renal disease (Ikeda et al, 1989). Preliminary reports have suggested that this beneficial effect on microalbuminuria in hypertension complicated by diabetes is also evident on long-term perindopril therapy (Brichard et al, 1988). Other studies suggest that this property is at least shared by calcium channel blocking drugs (Melbourne Diabetic Nephropathy Study Group,1991)

2.2.4. Cardiac ischaemia and arrhythmia

The investigation of the role of ACE inhibition in ischaemic heart disease and in arrhythmia control is a rapidly developing field. Myocardial infarction is known to be associated with activation of the renin angiotensin system and linked alterations in sodium and potassium balance may generate an arrhythmogenic state (Dargie et al, 1987; Michorowski & Ceremuzynski, 1983). Interactions with the sympathetic nervous system, myocardial perfusion and possibly antiarrhythmic effects may underlie some of the beneficial effects of ACE inhibition in ischaemic heart disease and infarction (Ertl, 1988).

Ribuot and Rochette (1987) have presented experimental evidence in ischaemia induced arrhythmias which suggests that captopril, enalapril, or perindopril all reduce the incidence of arrhythmia and death to an equal extent in comparison with untreated control animals. Using a similar model in rats Howes et al (1989,1991) demonstrated that chronic perindopril therapy post infarction attenuates the development of cardiomegaly seen in control animals. Tissue concentrations of 3,4 dihydroxyphenylethylene glycol were also noted to be reduced suggesting an attenuation of the chronic increase in cardiac sympathetic activity. Michel et al (1988b) also using a rat infarction model, found treatment with perindopril produced a hypotensive response, attenuation of the infarct related rise in atrial natriuretic peptide and reduced atrial and right ventricular mass. The reduced ventricular mass seen on therapy was accompanied by a reversal of infarct related changes in isomyosin profile and a reduction in the volume density of collagen. As yet there are no clinical studies assessing the effects of perindopril or perindoprilat in human ischaemic heart disease.

2.3. PHARMACOKINETIC PROPERTIES

The pharmacokinetics of ACE inhibitors are non linear and model independent methods are the most appropriate techniques to assess this group of drugs (Till et al, 1984).

2.3.1. Protein binding and tissue distribution

The protein binding of ACE inhibitors is variable between agents and this influences the distribution and availability of the drugs to active sites. The binding of carbon 14 labelled perindopril and perindoprilat to plasma proteins has been studied using membrane dialysis techniques to steady state. The data are summarised in Table 2.2. In general the binding of perindopril is higher than that for perindoprilat, the predominant fraction being bound to albumin *in vitro*. Limited in vivo studies in

humans showed a decrease in plasma protein binding with time after administration of perindopril which was thought to be related to the metabolic conversion to perindoprilat.

Limited information on the tissue distribution of the drug in experimental animals is available. Using tritiated perindopril in tracer doses with our without the addition of a 10 mg/kg bolus intravenous dose, Borge et al (1987) showed a rapid and extensive distribution to the lung within three hours of administration. Distribution to the kidney lagged behind that of the lung but was also extensive at 24 hours and in general all binding was less marked after larger doses.

3.2. Pharmacokinetics in normal volunteers and essential hypertension

Lees and Reid (1987b) have defined the pharmacokinetics of intravenous perindoprilat in eight normotensive volunteers using 1, 2 or 4 mg bolus doses (Figure 2.2). Plasma concentration time profile was fitted best by a three compartment model in this study with half lives for the alpha, beta and gamma phases of 0.2±0.04 hours, 1.24±0.2 hours, and 31±17 hours respectively. The respective area under the curve (AUC) for the three doses was 206±31 hr/ng/ml, 411±63 hr/ng/ml and 822±125 hr/ng/ml giving an estimated clearance value of approximately 4.9 l/hr.

After similar studies with oral doses of perindopril 4, 8 or 16 mg daily for seven days estimated clearance was similar after the first and seventh doses (45.9±13.2 l/hr vs 40.9±8.6 l/hr) as was the volume of distribution (305±12.8 litres vs 311±14.4 litres). Time to the peak plasma concentration of perindoprilat was dose dependent, being 0.4±0.6 hours after 4 mg, 2.1±0.9 hours after 8 mg and 2.9±0.6 hours after 16 mg of oral perindopril. Again no changes were found in the pharmacokinetics after one week's chronic therapy.

Table 2.2: Plasma protein binding of ^{19}C-perindopril and ^{14}C-perindoprilat studied using membrane dialysis to steady state in vitro.

PROTEIN	PERCENT BINDING AT STEADY STATE (mean ± SD)					
	PERINDOPRIL (ng/g)			PERINDOPRILAT (ng/g)		
	10	100	500	10	100	500
Human plasma (n=5)	59.9±0.9	61.0±12.2	59.4±0.3	18.2±0.4	10.2±0.9	9.3±0.5
α-1, glycoprotein (0.7g/l)(n=10)	6.8±2.9	0.8±2.4	0.2±0.2			
Albumin (n=10)	42.4±1.1	8.1±2.0	7.2±0.3			
Albumin/fatty acids (n=9,10)	63.8±0.8	39.0±1.2	38.6±1.2			

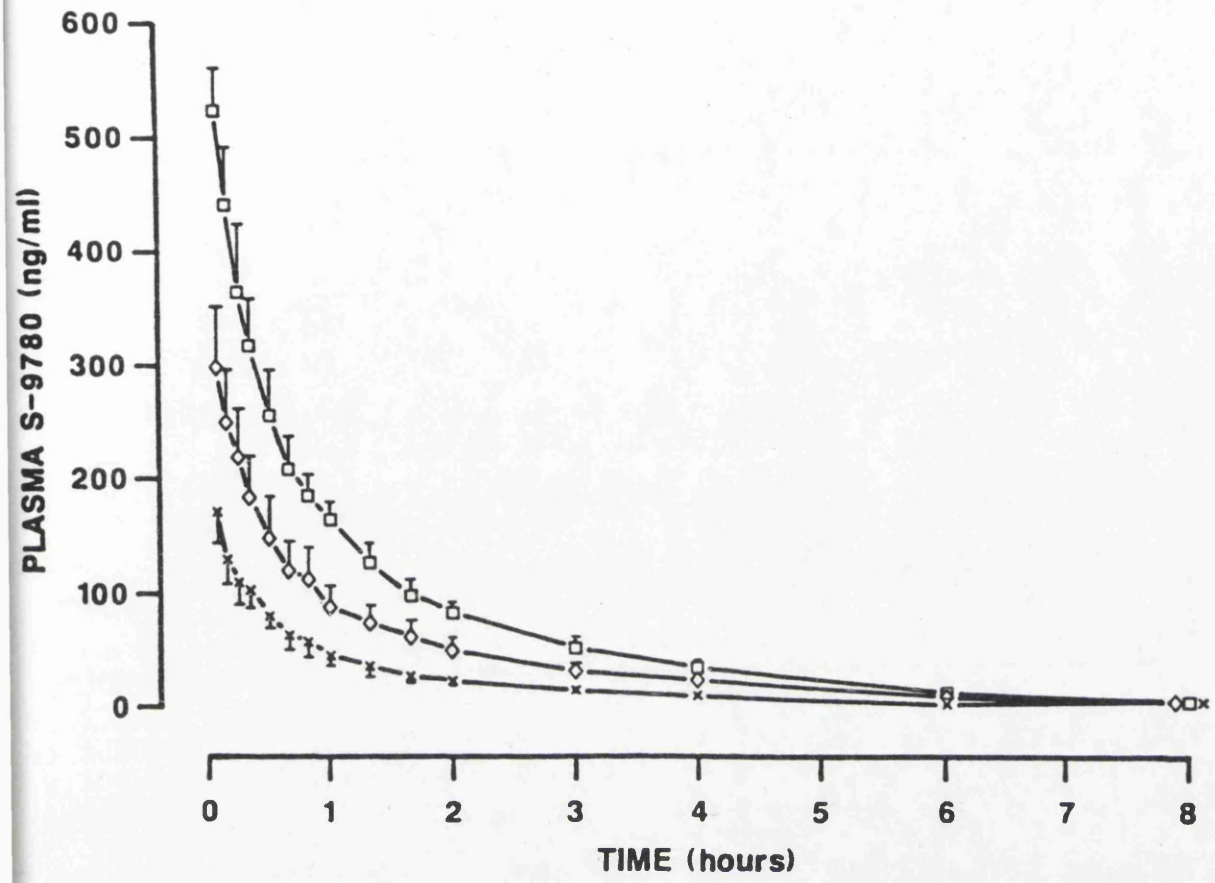


Figure 2.2: Plasma perindoprilat concentrations following acute bolus intravenous administration of 1, 2 and 4 mg perindoprilat (n=8). Data from Lees & Reid (1987b).

Drummer et al, (1987c) studied 24 essential hypertensive patients treated with 2, 4 or 8 mg oral perindopril for four weeks. Acute studies revealed a dose dependent increase in plasma perindoprilat with a T_{max} of approximately 6.5 hours and peak concentrations of 6.7, 19 and 58 ng/ml respectively. Four weeks therapy produced higher peak concentrations and a significant reduction in the time to peak to approximately 3.2 hours. The area under the curve (AUC) at all three doses was unchanged by chronic therapy. The glucuronide conjugate of perindopril was readily detectable after the first dose in this study but declined rapidly with a half life of approximately 2.9 hours. During chronic therapy a smaller amount was found. Urinary excretion studies after a single oral dose of 4 mg revealed 2.6% excreted as perindopril, 6.4% as perindoprilat and 6.5% as the glucuronide which on chronic therapy increased to 4.2% for the parent drug and 13.2% for the diacid but that excreted as glucuronide was reduced. Predictably the urinary recovery for oral perindopril was much less than that seen after intravenous treatment found by Lees, Green and Reid (1988) of 70±16% in normotensive volunteers studied up to 120 hours.

The excretion of perindopril is characterised by an extended terminal elimination phase close to the limit of assay detection. This has been observed with other ACE inhibitors (Till et al, 1984; Francis et al, 1987). This phase does not predict the accumulation profile during repeated dosing and is thought to relate to saturable protein binding to ACE in blood and possibly at tissue sites. Lees et al (1989) have studied their previously collected data with intravenous perindoprilat to assess the applicability of a non linear model incorporating zero order saturable tissue and plasma ACE binding. On comparison to standard 1, 2 and 3 compartment models the non linear binding model gave the best fit to the observed data.

2.3.3. Age and other disease states

A. Age

The influence of increased age on the pharmacokinetics of perindopril have been studied by Lees, Green and Reid (1988). In a double-blind crossover design using oral perindopril (8 mg) with saline intravenously or intravenous perindoprilat (1 mg) with oral placebo, the plasma profile and renal elimination was examined in eight healthy elderly volunteers in comparison to eight young volunteers. The systemic availability of perindopril after oral perindopril was increased in the elderly (35±17% vs 19±7%) primarily due to an increased conversion of perindopril to perindoprilat (61±20% vs 32±15%). Renal clearance of the drug estimated from the cumulative urinary recovery divided by the AUC from 0 - 120 hours was reduced in the elderly in comparison to the young (57±25% vs 70±16%) although this was not statistically significantly different. Increased availability of the drug in this group with oral therapy may require dose reduction to prevent excess blood pressure falls. The changes are similar to those previously observed with other ACE inhibitors (Lees & Reid, 1987a; Hockings et al, 1986).

B. Hepatic impairment

Tsai and colleagues (1989) have conducted pharmacokinetic studies in patients with hepatic cirrhosis. In eight patients with stable, biopsy proven, predominantly alcohol mediated cirrhosis (7/8) oral perindopril (8 mg) or intravenous perindoprilat (2 mg) was administered in a placebo controlled, double-blind, crossover study. The calculated systemic availability of perindoprilat in molar terms after oral perindopril was not reduced, indeed it tended to be greater than in similar studies in normal volunteers (30±6% vs 19±7%; Lees and Reid, 1989). The patients in this study were middle aged (59±9 years) and this was suggested to be a factor in explaining the apparent increase in

drug availability.

C. Renal impairment and congestive cardiac failure

Lees et al (1988) in their study of the influence of age on the elimination of perindopril found that renal clearance of the drug was reduced in proportion to the lower creatinine clearance in the elderly group. Previous studies with other ACE inhibitors have shown an elevation of serum concentration, decreased excretion and urinary recovery (Lancaster & Todd, 1988; Duchin et al, 1988; Todd & Goa, 1989).

Sennesael and colleagues (1992) studied 23 patients with stable chronc renal impairment secondary to hypertension given 2 to 4mg perindopril for 15days. Steady state was reached in three days and no decline in renal function was noted. Pharmacokinetics of perindopril were unaffected by renal dysfunction, however the renal clearance of perindoprilat closely correlated with creatinine clearance. Though the direct implications of this for toxicity are unclear, dosage reduction or decreased frequency of administration is generally recommended.

Similarly the effects of chronic cardiac failure on the pharmacokinetics of perindopril have not yet been defined. Evidence from studies with other ACE inhibitors are contradictory in that in some cases pharmacokinetic parameters are altered (Till et al, 1989; Duchin et al, 1988) whereas in other studies no changes have been observed (Dickstein et al, 1987).

4. CLINICAL USE AND SIDE EFFECTS

An adequate description of the side effect profile of any new drug awaits widespread clinical use with appropriate and detailed post marketing surveillance. Such data is not yet available for perindopril given its limited general release at the time of preparation of this review. The adverse reactions to this group of drugs are often

divided into those which are class related and those which are seen only with individual agents (Williams, 1988; Di Bianco, 1986). Hypotension, renal haemodynamic dysfunction, hyperkalaemia, cough and, rarely, angioedema are regarded as present with all ACE inhibitors and in general associated with concurrent potassium sparing diuretic use, low salt intake, renal artery stenosis, solitary kidney or uncontrolled cardiac failure. In this respect patients who would be regarded as at high risk of class related adverse reactions include those with collagen vascular disease, renal disease including renal artery stenosis (regardless of the serum creatinine) or essential hypertension/refractory cardiac failure with a serum creatinine greater than 1.7 mg/dl (1.5 $mmol.l^{-1}$) prior to treatment (Rucinska et al, 1989). Non productive cough is an often quoted side effect yet has proved difficult to quantify. It has been reported relatively infrequently (2- 4%) in clinical trials. However its true incidence in clinical practice may be nearer 10-15%. Idiosyncratic dermatological reactions seen with captopril have proven to be dose related (Irvin & Viau, 1986) and cross-reactivity between various agents is invariably limited when proteinuria, skin rash and taste disturbance are considered (Jackson et al, 1988c).

In the largest clinical studies with perindopril so far published, Zanchetti and Desche (1989) recorded patient withdrawal in 14 of 249 patients treated with perindopril compared with 18 of 254 patients treated with alternative therapies excluding placebo. These figures include non compliance and personal reasons for withdrawal and all recorded symptomatic withdrawals were minor and infrequent. A summary of the limited side effect data available from this experience is given in Table 2.3.

Table 2.3: Incidence of side effects in a multicentre trial of oral perindopril in patients with essential hypertension (after Zanchetti & Desche, 1989).

SIDE EFFECT	NUMBER OF PATIENTS EXPERIENCING SIDE EFFECT SUFFICIENT TO CAUSE DRUG WITHDRAWAL			
	PERINDOPRIL (n=249)	CAPTOPRIL (n=83)	ATENOLOL (n=88)	CO-AMILOZIDE (n=83)
Rash	2	1	-	-
Dizziness	1	1	1	2
Headache	1	-	1	-
Flushing	1	-	-	-
Nausea	-	1	1	-
Hypotension	-	-	-	2
Aggravation of IHD/CCF	1	1 (MI)	-	1 (CCF)
Electrolyte disturbances	-	-	-	1
Overall S/E(%)	3.6	3.6	4.5	4.8

CHAPTER 3

THE TISSUE AND PLASMA BASED RENIN ANGIOTENSIN SYSTEM

Angiotensin converting enzyme (ACE; kininase II; EC 3.4.15.1) is a zinc based metalloprotease closely related in structure and function to pancreatic carboxipeptidase A. Inhibitors of the ACE enzyme developed from the mechanistically related zinc metalloprotease bovine pancreatic carboxypeptidase A (Martin et al,1989). ACE has been extensively studied since it was first described by Skeggs and colleagues in horse plasma in 1956 (Skeggs et al, 1956). The enzyme is known to circulate in soluble form and can also be found anchored by carbohydrate residues to a variety of membrane structures. It operates primarily as an exopeptidase but also has recently described endopeptidase functions (Ehlers & Riordan, 1989). The molecular structure of the enzyme is known to include two identical putative active sites. Probably only one functional site is involved in catalysis. This high affinity binding site incorporates a single zinc atom providing competitive interaction with a variety of substrates, primarily angiotensin I or ACE inhibitors (Shapiro & Riordon, 1984; Strittmatter & Snyder, 1985; Martin et al, 1989).

In phylogenetic terms the enzyme is widely distributed among invertebrate and vertebrate species as a tissue based system with the addition of a circulating renin angiotensin system (RAS) appearing in species more advanced than the chondrichthian fish (Nishimura et al, 1973). In general terms the enzyme is non specific interacting with a range of peptide substrates in addition to its major role in the generation of angiotensin II (AII) from angiotensin I (AI) and to a lesser extent in the degradation of bradykinin. Alternative routes for the generation of angiotensin II from angiotensin I have been demonstrated both in plasma and tissues (de Silva et al, 1988; Jacobsen et al,

1988; Johnson & Drummer, 1988; Okamura et al, 1990).

Specific inhibitors of ACE activity were initially purified from the venom of the snake Bothrops Jararaca which led to the peptide intravenous ACE inhibitor, teprotide (Ondetti et al, 1971) and subsequently to studies with the first oral derivative, captopril, in man some years later (Ondetti, Rubin & Cushman, 1977). Since that time numerous developments in ACE inhibitor structure have led to the production of prodrug and non prodrug molecules, further sulphydril group containing inhibitors, the availability of intravenous diacids and a variety of hepatic and renal routes of elimination to the extent that recent estimates suggest that some 50 compounds are undergoing various stages of clinical development or clinical use (Brunner et al, 1985; Johnson, 1988). One such compound, perindopril, has been used as an illustrative example in the preceding chapter.

The purpose of the following review is to highlight the evidence available for the existence and functional importance of a tissue based renin angiotensin system and the importance, if any, of differential interaction between ACE inhibitors with tissue and plasma ACE in a variety of sites.

3.1. DEFINITION OF THE TISSUE RENIN ANGIOTENSIN SYSTEM

Tissue based elements of the renin angiotensin system have been studied for the past ten years in an attempt to delineate the contribution of circulating and "tissue fixed" systems to biological effects in a range of key organs (Ganten et al, 1983). In general terms membrane bound ACE is known to be susceptible to hormone based induction which is clearly demonstrable in the maturation of monocyte cells towards macrophages and the changes noticed in granulomatous diseases such as sarcoidosis (Friedland et al, 1978). The enzyme maturation and induction found in these circumstances is glucocorticoid dependent and relatively specific (Hinman et al, 1979; Vuk-Pavlovic et al, 1989). Studies in experimental animals have shown that the tissue renin angiotensin

renin gene expression (Metzger et al, 1988). These and other general controlling factors are summarised in Table 3.1.

The evidence regarding the tissue specific distribution of ACE activity and a tissue RAS will be considered systematically for the major sites (i.e. vascular, pulmonary, cardiac, brain and renal) in the following sections. One tissue which has received considerable attention which will not be considered in detail is the ACE activity which has been identified in the testis. This isoenzyme of ACE is known to have a different peptide structure from either plasma or pulmonary enzyme and has been widely demonstrated as inaccessible to the effects of circulating ACE inhibitors in a variety of acute animal studies (El-Dorry et al, 1982; Velletri et al, 1985; Jackson et al, 1988; Johnston et al, 1989). The role of this enzyme, presumably during developmental reproductive biology is as yet unclear.

3.1.1. Vascular renin angiotensin system and ACE activity

The tissue based RAS of the vascular tree has been intensively studied as the most logical source mediating the haemodynamic effects of ACE inhibition. A major assumption in the majority of experimental work is that studies in large bore conducting vessels have relevance to more distal resistance vessel structure and response. All the components of the RAS are evident in isolated vascular tissue when studied using the techniques of molecular biology in animals (Dzau, 1986). The key elements of renin and angiotensinogen have been documented to the level of messenger RNA. The peptides can be generated locally or can be taken up from the circulating pool. Due care must be taken to exclude artefactual generation or degradation of angiotensins from simple in vitro pH changes or non specific protease activity (Swales & Hegerty, 1987; Campbell, 1985, 1987). The production of angiotensin II by synthesis and release into culture media has been demonstrated in vitro and in several experimental preparations of isolated

Table 3.1: Some general factors controlling the renin angiotensin system

- Na^+/RENIN STATUS
- GLUCOCORTICOIDS
- ANDROGENS
- ACE INHIBITOR THERAPY

and perfused hind limbs without circulating renin or angiotensinogen (Okamura et al, 1986; Mizuno et al, 1988). In these studies exogenous renin was shown to produce a temporal dissociation of an induced pressor response to angiotensin in nephrectomised animals whose circulating renin activity was low or absent. Measured arteriovenous differences in the clearance of exogenous renin implied tight binding of the enzyme to tissue sites with consequent activation of the tissue based renin angiotensin system and subsequent angiotensin II production. Similar experiments documented by Oliver and Sciacca (1984) demonstrated that the pressor response to angiotensinogen and angiotensin I in isolated perfused hindquarters could be ablated by concurrent captopril or peptide renin antagonist. In this situation the tissues generated renin activity over and above the quantity supplied in the infusate.

Although the accurate determination of angiotensin peptides in plasma and tissue are well known to be susceptible to methodological and analytical difficulties (de Silva et al, 1988) including the possibility of alternative routes for the hydrolysis of angiotensin I (Johnson & Drummer, 1988; Ideishi et al, 1990) careful studies on peptide kinetics have given valuable further evidence.

Campbell (1985), considering experimental data on the arteriovenous gradients of angiotensin peptides within subjects calculated a substantial production of angiotensin II in addition to the inactivation of arterial angiotensin II by tissues as demonstrated in classical isolated organ experiments (Ng & Vane, 1968). Using approximations of 40% angiotensin I conversion and 65% for the rates of angiotensin I and II inactivation he calculated that local production of angiotensin I was responsible for 83% of the total venous concentration of angiotensin I and similarly that local production of angiotensin II accounted for 68% of the total venous angiotensin II.

These approximations were recently borne out by elegant studies conducted in man to define the regional generation of angiotensin I in the renal, antecubital,

hepatomesenteric and femoral circulations using steady state tracer infusions of exogenous peptide and concomitant captopril therapy. Schalekamp and colleagues demonstrated that 40-90% of venous angiotensin I in these vascular beds was in fact generated by regional production from angiotensinogen (Schalekamp et al, 1989; Admiraal et al, 1990). Similar, though less extensive, studies have been documented by other groups (Reams et al, 1989).

ACE activity has been demonstrated immunohistochemically on vascular endothelium (Ryan et al, 1975; Caldwell et al, 1986) and furthermore the amino acid sequence and molecular structure of vascular ACE has been defined by cDNA cloning techniques (Alhenc-Gelas et al, 1989). The existence of a tissue based renin angiotensin system seems accepted from a number of different viewpoints. However, each element in the system of course must be demonstrated to be co-localised and to interact. As was suggested above for the case of angiotensin I to angiotensin II processing it has to be clear that only one specific enzyme is mediating any extra-vascular processing. This is questioned with regard to renin, since a significant fraction of renin-like activity appears not to be inhibited by renin specific antibodies in some studies (Rosenthal et al, 1984). Whereas co-localisation, specific action and functional interaction have been agreed for some tissues in experimental animals this full picture in the vasculature has been questioned by some authors (Corvol et al, 1989). A key element must be the degree of interaction and interchange of substrates with the circulating endocrine renin angiotensin system.

3.1.2. Pulmonary renin angiotensin system and ACE activity

The pulmonary circulation, on the basis of ex vivo pharmacology (Ng & Vane, 1968), has long been regarded as the most important if not exclusive site for the conversion of angiotensin I to angiotensin II by ACE activity. It has been regarded as the

major endocrine function of the lungs (Said, 1982). This view has undergone revision with the more precise definition of tissue renin angiotensin systems in many other sites. Within the lung, ACE activity has been localised on the endothelium of the pulmonary vasculature (Ryan et al, 1980; 1985) but is also present on other parenchymal sites and is widely recognised as a marker of lung injury (Kelley, 1988; Foresti et al, 1989) or of hypoxia (Joderlinic et al, 1988; Oliver et al, 1989).

The anatomy of the pulmonary circulation has allowed this site of tissue ACE activity to be studied using elegant single pass kinetic studies in animals which allow the definition of the enzyme in vivo (Chen et al, 1984; Catravas & White, 1984; Moalli et al, 1985; Linehan et al, 1990). Multiple indicator dilution techniques employing time paired samples of a relevant indicator and an ACE substrate have been applied to the pulmonary circulation (Linehan et al, 1985). The kinetic profile on single transpulmonary passage is mapped employing the synthetic substrate tritiated benzoyl-phenylalanyl-alanyl proline (^{3}H-BPAP) which is specific substrate for ACE. This sophisticated technique has not been applied to the human pulmonary circulation and has only been used to define the broad responsiveness to ACE inhibition rather than comparing different agents. However Chen et al (1984) showed a markedly attenuated lung metabolism of BPAP produced by 6 days of intravenous captopril bolus dosing in the face of rapid recovery of plasma ACE activity. The time course of pressor responses to angiotensin I after captopril followed the pattern of plasma ACE inhibition, i.e. rapid recovery after each dosing, and did not reflect continuing pulmonary tissue ACE inhibition between dosing. Of great importance was the fact that the mean hypotensive response followed neither the pattern of plasma ACE inhibition nor pressor response to exogenous angiotensin I but the pattern of persistent pulmonary ACE inhibition. This finding is qualitatively similar to the patterns observed in both ex vivo and in vitro studies of the temporal course of pulmonary ACE inhibition after a variety of ACE

inhibitors (Cohen & Kurz, 1982; Sakaguchi et al, 1988; 1989; Welsch et al, 1989).

As with other sites direct comparative studies on the reactivity of pulmonary ACE to different ACE inhibitors are complex in that equipotent doses should be administered preferably by intravenous injection or infusion. Unger et al (1985) demonstrated a slower recovery of pulmonary ACE after single dose ramipril than after enalapril in experimental animals. Relevant information on human tissue ACE, particularly at steady state dosing with ACE inhibitors, is not available. Further experimental studies show that induction of pulmonary ACE occurs on chronic ACE inhibitor therapy (Fyhrquist et al, 1982). Such enzyme induction is a general biochemical principle and may be relevant to the mediation of responses as considered later in the thesis.

In contrast to other circulations there are limited data on the transpulmonary conversion of angiotensin I in man. It may be that this is predominantly related to vascular endothelial ACE and its interaction with the circulating renin angiotensin system. However like other circulations it is complicated by the tissue generation and metabolism of peptides, previously described peptide assay problems and difficulties in differentiating between the tissue and plasma elements. A novel study by Neilly and colleagues (1987) demonstrated a wide range of transpulmonary angiotensin II formation rates in a rather heterogeneous population of cardiac and respiratory patients with cor pulmonale. The latter group of hypoxic patients showed no changes in transpulmonary angiotensin II formation during oxygen supplementation. This is perhaps somewhat surprising given the documented sensitivity of the enzyme to hypoxia and ion balance in vitro (Skoglof et al,1990).

3.1.3. Cardiac renin angiotensin system and ACE activity

As the central organ of the cardiovascular system the heart has been of particular

myotropic actions of angiotensin II (Koch-Weser, 1964) and its indirect neurogenic facilitation of the sympathetic tone (Xiang et al, 1985). A summary of the actions of angiotensin II on the heart is given in Table 3.2.

As with other tissues the key elements of the renin angiotensin system have been demonstrated using molecular biology and once more the co-localisation and functional significance of the findings is controversial (Mebazaa et al, 1989; Dzau, 1988). Studies of the localisation of angiotensin II, its specific receptors and generation of the peptide have shown that the right sided cardiac chambers and the right atrium in particular are key sites for angiotensin II production (Lindpainter et al, 1987; Rosenthal et al, 1987). Persistent detection and generation of angiotensin II can be seen in the heart following ablation of the circulating renin angiotensin system by bilateral nephrectomy (Mebazaa et al, 1989).

ACE activity is readily demonstrable in the heart with the similar increased activity in right sided chambers, especially the atrium, that is found with angiotensin peptide (Johnson et al, 1989). It is suggested that the majority of the enzyme is found in association with the vasculature and that only a small component is associated with myocardium. There is a high concentration on the surfaces of the cardiac valves. No ACE activity is found in association with the conducting system or in the region of the inter-ventricular septum (Saito et al, 1987; Yamada et al, 1989). There is some evidence to suggest that the molecular weight (Polsky-Cykin & Fanburg, 1979), carbohydrate content, primary (Iwata et al, 1982) and tertiary structure (Polsky-Cykin & Fanburg, 1979; Velletri et al, 1985) are different for cardiac with respect to soluble plasma ACE. Certain in vitro physicochemical properties of ACE isolated from the human heart similarly differ from that isolated from other tissues (Sakharov et al, 1987). There is no data to suggest that these laboratory findings are of functional significance.

ACE inhibitors have not been frequently used to define the specificity of the

Table 3.2 : Actions of Angiotensin II on the heart

- **Vascular constriction**	-	direct	
	-	indirect	- (autonomic tone) - (prostaglandin synthesis) - (kinin potentiation)
- **Inotropic effects**	-	direct	
	-	indirect	- (sympathetic facilitation)
- **Mitogenic effects**	-	potentiation of myocyte growth	
	-	fibrosis	
- **Myocardial conduction**	-	? secondary to	- structural changes - altered vasomotor tone/ ischaemia

interaction with cardiac tissue ACE but Fabris and colleagues (1989) have done a small experimental study in rat tissue. They were able to confirm concentration dependent binding in which the relative binding affinity of a range of ACE inhibitors was higher in right than left sided myocardial ACE. A similar rank order of potency was found for six ACE inhibitors of differing lipophilicity but the differences in ligand binding properties were not reflected in a small examination of the temporal degree of ACE inhibition to the compound quinapril. These authors therefore felt that the altered binding affinity between atrial and ventricular ACE was not of functional importance. They did not compare the temporal binding characteristics of the full range of the ACE inhibitors employed.

The functional importance of the renin angiotensin system to the heart is supported by clinical and experimental studies showing that ACE inhibitor therapy can reduce infarct size (Ertl et al, 1982), inhibit the progressive ventricular dilatation following experimental and clinical myocardial infarction (Pfeffer et al, 1985, 1988, 1992; SOLVD 1991,1992a) and have an important role to play in limiting ventricular fibrosis and remodelling ventricular hypertrophy from a variety of causes (Yoshimura et al, 1989; Swynghedauw, 1989; Michel et al, 1988). Most recently it has become clear that ACEI therapy can reduce the incidence of reinfarction in chronic heart failure patients whose initial cardiac damage originated in an index infarction (SOLVD 1992b) As in the lung there are a number of separate issues in relation to the role of the renin angiotensin system in the modulation of coronary vasomotor tone during normal perfusion or ischaemia which will not be considered in detail here (Ertl, 1988; Van Gilst et al, 1988; Gasic et al, 1990; Packer,1992).

Evidence to suggest interactions of tissue ACE with the conducting system is controversial and based more on indications of putative antiarrhythmic activity with some ACE inhibitors but not with others (Van Gilst et al, 1986; Westlin & Mullane, 1988;

Ferrari et al,1992). The relevance of the tissue based renin angiotensin system to this phenomenon is unclear but there is experimental evidence confirming alterations in ventricular hypertrophy with ACE inhibitor therapy are associated with beneficial changes in electrophysiological conduction (Thollon et al, 1989).

3.1.4. Brain renin angiotensin system and ACE activity

A brain renin angiotensin system has been identified in a number of species and has important functions in stimulating thirst to increase extracellular fluid volume (Ganong, 1984). Angiotensin converting enzyme has been specifically localised in the central nervous system (Strittmatter et al, 1985) and its relationship to angiotensin receptors defined (Chai et al, 1986; Allen et al, 1988). The inhibition of central ACE activity may be an important point in generating the chronic haemodynamic response to ACE inhibition as is suggested by the hypotensive response to intra-cerebroventricular ACE inhibitors in animals with experimental hypertension where there is no peripheral ACE inhibition (Phillips & Kimura, 1986; Sakaguchi et al, 1988). Brain ACE is active in vivo as is demonstrated by the inhibition of the pressor response to intra-cerebroventricular angiotensin I but not to angiotensin II in the presence of concurrent intraventricular ACE inhibitor treatment. Studies of the penetration of ACE inhibitors beyond the blood brain barrier have revealed a concentration dependent phenomenon. Although the circumventricular organs lying outwith the blood brain barrier are readily inhibited by circulating ACE inhibitors excessive "pharmacological" doses are required to penetrate deeper structures such as the basal ganglia in acute studies and indeed some agents do not penetrate in the doses chosen (Sakaguchi et al, 1988). Again the issue of relative potency and the doses chosen in such studies is of critical importance. The significance of these acute studies to chronic therapy in man is unclear.

Although these animal studies involved the circumventricular organs which do

not have a major role in human central vasomotor control the tissue angiotensin system is also evident in areas of the ventrolateral medulla in post mortem human specimens (Chai et al, 1990). These areas are more closely associated with blood pressure control (Allen et al, 1988). Animal studies with respect to this region of the brainstem indicate that the angiotensin system may modulate the control of vasomotor outflow (Chevillard & Saavedra, 1982; Andreatta et al, 1988).

3.1.5. Renal renin angiotensin system and ACE activity

Tissue based elements of the renin angiotensin system are of central importance in the kidneys due to the role of this organ in normal fluid and electrolyte balance and in the pathological genesis and perpetuation of hypertension (Klahr, 1989) and chronic cardiac failure (Stanton & Bremner, 1986). The origin of the circulating renin angiotensin system within the kidney is well established. However, a local paracrine and autocrine function for tissue generated angiotensin II is now supported by increasing evidence. As with the other organs there is certainly an element of renal ACE related to vascular endothelium. However the enzyme can also be identified on the tubular cells and intercellular membranes.

As with the other sites renin, angiotensinogen and ACE have been identified using the techniques of molecular biology in fetal renal tissue and it has been suggested that these tissue based elements have a role in controlling organ development (Mournier et al, 1987). As with the other tissues the local functional significance of these observations is still a matter of debate.

ACE is associated primarily with the proximal tubular cell in man but is also present in more distal structures in other species (Takada et al, 1982a). Experimental work in isolated nephrons by Marchetti et al (1987) suggests that there may be a gradation of activity from proximal to more distal segments of the tubule. However these

animal studies must be set in the context of interspecies differences which have been shown 1) in *in vitro* characteristics of kidney ACE (Takada et al, 1982b), 2) in the total content and distribution of the enzyme (Morin et al, 1989) and 3) with the known presence of other non specific tubular peptidases.

The biochemical structure of kidney ACE is again suggested to be altered with respect to carbohydrate content from the circulating soluble enzyme. However the authors who described these changes declared them to be irrelevant to functional activity and regarded the immunoreactivity of renal ACE as identical to that in the lung (Alhenc-Gelas et al, 1983).

Interestingly the temporal inhibition of renal ACE activity following ACE inhibitors tends to follow the pattern set by aortic vascular ACE which recovers more rapidly than that extracted from the lung (Sakaguchi et al, 1988; Jackson et al, 1988). As with other tissues renal ACE inhibition is markedly dissociated from the profile of plasma ACE inhibition (Unger et al, 1985). Intercomparison between agents is again complicated by variable potency and the use of potentially non equivalent doses. Even so, different patterns of inhibition in renal cortical ACE have been demonstrated between agents for example in the studies of Chevillard and colleagues using enalapril and trandolapril (Chevillard et al, 1989).

The generation of angiotensin II within the renal circulation has a potent local vasoconstrictor effect particularly affecting the postglomerular intravascular pressure which controls glomerular filtration and hydrostatic pressure. The effect of changes in circulating angiotensin II are therefore most evident in sodium deplete states and in the presence of renal artery stenosis (Navar & Rosivall, 1984). From studies in the isolated rat kidney it has been suggested that the relatively reduced quantity of ACE available may allow ACE to take on the role as the rate limiting step in angiotensin II formation (Misumi et al, 1989) which is not the case in other sites (Erdos, 1975). Studies of the

regional extraction of angiotensin I across the renal circulation give estimates in the region of 80%. Local generation of angiotensin I results in only a marginal arteriovenous gradient (Schalekamp et al, 1989; Admiraal et al, 1990).

The effects of ACE inhibitor therapy are predominantly focused on the haemodynamic effects, on renal function and certainly on the possibility of detrimental effects on kidneys affected by renal artery stenosis (Burnier et al, 1989). The beneficial effects of ACE inhibitor therapy in kidneys affected by diabetic hyperfiltration (Taguma et al, 1985; Marre et al, 1987; Romanelli et al, 1989) or hypertensive nephropathy (Ikeda et al, 1989) are equally felt to be based primarily on haemodynamic alterations (Raij et al, 1989). The interaction between overall blood flow, regional intrarenal flow and glomerulotubular pressures are complex and there is still considerable debate as to the relative importance of tissue based endothelial or tubular ACE in generating positive or negative responses to ACE inhibitors in various renal diseases. This is reflected in the continuing clinical debate over the net efficacy of ACE inhibitor treatment in progressive renal insufficiency (Keane et al, 1989).

3.2. ACE INHIBITORS AND TISSUE ACE INHIBITION

3.2.1. Analytical aspects

The definition of ACE activity in plasma and in tissue extracts is associated with well defined problems of methodology. The majority of analyses in clinical use are based on the generation of a chromophobe from a variety of peptide substrates for the quantitative description of peptide hydrolysis (Piquilloud et al, 1970; Cushman & Cheung, 1971; Ryan et al, 1977). Alternatively ACE activity has been expressed employing competitive binding inhibition of a radiolabelled tyrosyl derivative of enalaprilat (^{125}I-MK351A) which has the advantage of describing either ACE activity, by competitive displacement, or the concentration of any given ACE inhibitor (Fyhrquist

et al, 1984; Jackson et al, 1986; Johnson et al, 1987).

The results achieved with different chromophobes have been criticised by some workers who believe that inhibition of the renin angiotensin system either in plasma or tissue must be related directly to local or circulating ratios of angiotensin II to angiotensin I (Biollaz et al, 1982; Nussberger et al, 1988, 1989). This is not easily achieved. The cross reactivity of angiotensin peptides, and there are a variety of more or less bioactive breakdown products, hexapeptides, heptapeptides etc, causing an overestimation of key effector substance, AngII octapeptide. This can only be circumvented by incorporating preliminary separation of peptides with high pressure liquid chromatography and subsequent sensitive angiotensin II octapeptide radioimmunoassay. This is a particularly time consuming and difficult analysis which is expensive to perform. There is disagreement whether or not even the effects of different ACE inhibitors can be compared even using the same chromophobe assay to detect ACE activity (Burnier et al, 1989).

In vitro studies of serum ACE activity as defined using chromophobe techniques have demonstrated significant differences in the kinetic and inhibitory constants to captopril between species. In addition the level of circulating enzyme is well described as being variable between species (Ibarra-Rubio et al, 1989). Thus comparisons of plasma and tissue ACE deduced from animal experimentation using various methodologies should be treated with caution.

3.2.2. Acute vs chronic ACE inhibitor treatment

A large volume of published work studying serum and tissue ACE is based on experimental and clinical studies in which drug therapy is given either on a single dose basis or on short term administration lasting days or at the most a few weeks. There is little doubt that the acute and chronic responses of the renin angiotensin system to ACE inhibitor therapy differ considerably. There is an interaction between the analytical

technique used to define plasma ACE and the definition of the changes on chronic therapy. Studies in normal volunteers have shown reactive hyper-reninaemia which is associated with the re-appearance of bioactive angiotensin II octapeptide despite maximal ACE inhibition when defined by chromophobe techniques (Mooser et al, 1990). Such hormonal changes are of considerable importance in the definition of haemodynamic responses in that some authors suggest that both renin and by implication angiotensin II entirely explain the response to ACE inhibitors (Case et al, 1977). In effect responses to ACE inhibitors, for example in hypertension, can be defined on the basis of renal perfusion and local renin production in the kidney (Cody, 1984; Buhler, 1986; Laragh, 1989). These theories do not explain the re-emergence of circulating angiotensin II and a continuing and developing response to ACE inhibition with chronic treatment nor evidence of responses to ACE inhibitors in low renin states (Chatterjee & Opie, 1987).

3.2.3. Kininase II inhibition

In terms of alternative substrates for ACE the major interest in haemodynamic and stuctural effects mediated by ACE inhibiton lies in the local potentiation of bradykinin mediated by kininase II/ACE inhibition. Mammalian ACE can more efficiently degrade bradykinin ($K_m = 8 \times 10^{-7}$ M) than activate angiotensin I ($K_m = 3.3 \times 10^{-5}$ M) (Ryan, 1983) and it has been suggested that the secondary association of ACE with the renin angiotensin system seen from phylogenetic studies supports the importance of kinins as the major substrate for ACE (Lipke & Olson, 1988).

There is some evidence from experiments in anaesthetised animals that the acute hypotensive effects of captopril can be partially reversed by competitive antagonists of bradykinin in excess of the pressor effect caused by the infusion of the kinin antagonist alone (Seino et al, 1989). The antihypertensive effect of chronic ramipril therapy in SHR

rats can be partially reversed by the addition of a bardykinin antagonist (Bao et al,1992). A remarkable series of studies by Linz and colleagues has suggested that kinin antagonism can block the anti hypertrophic response seen following low dose ACE inhibition in rats who have left ventricular hypertrophy induced by surgical aortic constriction (Linz et al,1992). This series has also demonstrated a remarkable concentration dependency in that the anti hypertrophic effects were evident at doses of the ACE inhibitor which were devoid of haemodynamic effect (Linz et al,1989).

In addition localised studies on human forearm vascular tone by Benjamin and colleagues showed that without generalised ACE inhibition or haemodynamic changes, infusion of the ACE inhibitor enalaprilat has local effects whose onset and extent are mediated through angiotensin II but possibly also through bradykinin (Benjamin et al, 1989). This and similar studies showing potentiation of a range of bradykinin mediated effects such as intradermal wheals (Ferner et al, 1989) or increased forearm blood flow in response to intra-arterial bradykinin (Kiowski et al, 1982) have been used to suggest that this effect might explain the hypotensive effects of ACE inhibitors in low renin states (Swartz et al, 1979). The key element of course is the nature of the interaction: whether this is direct, as suggested by the workers above, or is in fact simply an indirect phenomenon. Nonetheless kinins are emerging as a strong candidate for future research as mediators of some ACE inhibitor effects (Kiowski et al,1992).

3.2.4. Tissue distribution of ACE inhibitors

The matter of physicochemical differences between ACE isolated from different organs and from different species has partly been addressed in preceding sections. Tissue and plasma ACE are likely to originate from one gene product but the possibility of a variety of isoenzymes responsible for variable properties is acknowledged (Strittmatter et al, 1985; Bernstein et al, 1988). Recently there has been much speculation

as to the functional significance of variations in ACE genotype in defining increased cardiovascular risk in a non-obese population of patients with myocardial infarction who had an otherwise low rating of "conventional" cardiovascular risk factors (Cambien et al;1992). Structural differences are highlighted particularly for the testicular enzyme (Iwata et al, 1982; Velletri, 1985) but the again the functional significance of this site and its differing structure remain unclear from ex vivo studies (Jackson et al, 1988).

The distribution of the drug between the plasma compartment and the tissue sites whether they are endothelial, epithelial or in deeper structures must be a concentration dependent equilibrium. This depends upon the quantitative availability of binding sites in the plasma and tissue and a range of factors related to the structure of the ACE inhibitor and the tissue enzyme such that interaction can occur. These factors are outlined in Table 3.3. The majority of comparative data on different ACE inhibitor profiles for tissue ACE inhibition have necessarily been gathered in animals using short term or acute doses of drugs. A large body of work has come from the group at the University of Melbourne (Johnston et al, 1988, 1989) using temporal ex vivo radioligand binding studies of tissue ACE. Whether simple tissue bioavailability or true differing tissue interactions with the enzyme are responsible for differential regional effects between ACE inhibitors is unclear. As the physicochemical properties of the ACE inhibitor also provide the more general pharmacokinetic properties of the drugs the interaction between plasma concentration time profiles and tissue binding has achieved considerable interest especially as there are marked differences in the conventional pharmacokinetic profiles between agents (Kubo & Cody, 1985; Belz et al, 1988).

3.2.5. Pharmacokinetics and pharmacodynamics of ACE inhibitors and tissue ACE

Circulating plasma ACE inhibition, regardless of the technique of determination,

Table 3.3: Factors influencing the tissue distribution of ACE inhibitors

-	PLASMA CONCENTRATION (ESTER/DIACID)
-	LIPOPHILICITY
-	RELATIVE PLASMA/TISSUE BINDING SITES
-	ESTER/DIACID INTERCONVERSION (PLASMA/TISSUE)
-	MEMBRANE BARRIERS (BLOOD/BRAIN/TESTIS)
-	ELIMINATION

is thought to correlate closely with simultaneous plasma drug concentrations without any significant hysteresis in this biochemical effect of the drug (Ajayi et al, 1987; Francis et al, 1987). However, theoretically this relationship should obey the laws of mass action and a non linear saturable binding phenomenon should be observed. In many instances the accumulation of drug is such that the very earliest phases of drug accumulation and ACE inhibition are not well described even in oral dosing studies (Francis et al, 1987). Similar non linear relationships have been described where in vivo ACE inhibition is defined using angiotensin peptide ratios (Biollaz et al, 1982) although the exact description of the observed data is a matter of debate (Kelman et al, 1983). Preliminary studies using low dose intravenous infusions of ACE inhibitors have suggested that a sigmoid drug accumulation profile in venous plasma is evident. This is best described by non linear saturable binding in which the model incorporates terms indicative of the proportions of drug bound to both plasma and tissue ACE (Lees et al, 1989). These studies have attempted to include statistical validation of the "goodness of fit" of the model but require further definition of the validity of the parameters generated. These parameters may give an alternative means of describing human tissue ACE inhibition *in vivo*.

The clinically important relationship centres upon that relating drug concentration to haemodynamic effect. Thus although ACE inhibition has been frequently compared to haemodynamic effects in a large number of normal and pathological states with a range of age groups and renin status, rarely if ever has drug concentration been quantitatively applied to individual subjects in the general terms of drug concentration effect modelling (Holford & Sheiner, 1981; Donnelly et al, 1989). In terms of acute hypotensive responses to ACE inhibitors or renin inhibition, temporal dissociation from circulating ACE activity or drug concentrations is well documented in a variety of animal models (Waeber et al, 1980; Boomsma et al, 1981; Blaine et al, 1984; Unger et al, 1986;

Kamei et al, 1989). The interpretation of this basic observation is at the heart of the complex and previously discussed points regarding definition of plasma ACE inhibition, peptide analysis and interaction with alternative vasoactive substrates are clearly pertinent. Although the existence and importance of tissue ACE are frequently cited as the explanation for this failure to correlate observations with response it should be noted that there are a number of single dose animal studies which suggest a close correlation between ACE inhibition and blood pressure fall (Sweet et al, 1981; Jackson et al, 1984). Studies with alternative means of blocking the renin angiotensin system such as specific renin inhibitors or angiotensin receptor antagonists (see chapter 8) are as yet too limited and do not define the temporal excursion of angiotensin II in sufficient detail to explain the discrepancy although again there is limited evidence of a direct relationship (Szelke et al, 1985).

In quantitative terms the discrepancy between drug concentration and haemodynamic effect is described over time a prosteresis (anticlockwise hysteresis) where the haemodynamic response lags behind drug concentration (Belz et al, 1989). This is common to a number of vasoactive agents (Donnelly et al, 1989). The suggestion that the delay is due to the equilibration of drug into key tissue sites and interaction with tissue ACE is tempting. Kirch and colleagues (1988) found that the shape of the blood pressure to ACE inhibition curve for cilazapril in hypertension was retained after 2 weeks therapy with a more rapid initial response to dosing and some reduction in the area of hysteresis. They suggested therefore that the eventual degree of haemodynamic effect was well described after the initial dose but that the duration of the effect was underestimated.

3.2.6. Activated renin angiotensin system in heart failure

In explaining the haemodynamic effects of inhibiting the renin angiotensin system it has been suggested that the fluid and sodium status and changes in the activation of the circulating renin angiotensin system combine to define completely the extent of the first dose response to an ACE inhibitor (Dzau, 1989). For example, in renovascular hypertension it is suggested that where there is no increase in extra-cellular volume nor sodium loss then there is in effect no acute blood pressure response to ACE inhibitor treatment. This is not a universal experience and marked hypotension is documented in this setting (Hodsman et al, 1983). The role of diuretic mediated natriuresis and volume depletion and secondary hyper-reninaemia are of significance. Equally in renovascular hypertension there is some evidence to suggest tissue based vascular ACE is the unquantified element in describing this haemodynamic response (Okamura et al, 1986).

Hyper-reninaemia was first suggested to play a role in the vasoconstriction of the chronic cardiac failure syndrome in the mid 1940s (Merrill et al, 1946). In addition to the circulating system there is clearly scope for quantitative alterations in the tissue elements of the renin angiotensin system in response to chronic changes such as occur in hypertension (Lever, 1989) and in cardiac failure (Anand et al, 1989). Animal studies support the activation of the tissue based renin angiotensin system at a number of levels in experimental heart failure (Fabris et al,1990; Hirsch et al,1991,1992). Both tissue and circulating systems are liable to be influenced by prior or concomitant drug therapy known to act directly or indirectly with the renin angiotensin system, diuretic therapy in particular (Bayliss et al, 1987).

The renin angiotensin system is centrally involved in the response to heart failure as one of the primary homeostatic systems controlling cardiovascular function. These are relevant to the pathology of heart failure in generating peripheral regional

vasoconstriction acting via the pressor peptide AngII. Activation is mediated by the fall in renal perfusion during heart failure activating baroreceptor mediated renin release. The sensitivity of various vascular beds to angiotensin II varies (Motwani & Struthers,1992) and this affects the response to activation of the system in heart failure. Fluid and electrolyte balance is also altered resulting in potassium loss, sodium and water retention intrinsic to the heart failure syndrome. This gives rise to the fluid overload at least partially responsible for the symptoms of heart failure (Schrier,1988). Management with diuretic drugs in patients with heart failure and a variety of other vasodilator agents also acts as a stimulus to the renin angiotensin systems (Doig et al,1992).

In chronic cardiac failure the first dose response to ACE inhibition has been associated with marked hypotension usually catalogued in small series or in isolated case reports (see Table 3.4). The exact incidence and severity of these events is unclear yet strategies of dose reduction, hospitalisation for initiation of therapy, and an initial choice of short acting ACE inhibitors appear to be common in clinical practice when this has been audited (McMurray et al, 1989). It is as yet unclear whether these episodes reflect a distribution of individual responsiveness, whether they are agent or dose related, or whether they simply reflect vagally mediated reflex hypotension which is common with a number of hypotensive drugs (Semple et al, 1988). The belief in an idiosyncratic first dose response which is not dose related has emerged to the extent that some groups employ small "test doses" prior to treatment although the rationale for these is unclear (Rademaker et al, 1986). As has been suggested for renovascular hypertension, hyper-reninaemia has been employed whether related to disease or diuretic therapy as the whole explanation for the magnitude of first dose response (Cody, 1984; Packer et al, 1985; Kubo et al, 1987; Dzau, 1989). However individual patterns of the response, where presented in detail, frequently show a sudden collapse in blood pressure (Cleland et al, 1985; Lantz et al, 1984) rather than any more rapid decline than expected and the

Table 3.4: Reports of hypotension following ACE inhibition

Report	n	NYHA Grade	Diuretic	Agent	Dose
Acampora et al (1989)	1	II	-	Enalaprilat	0.625mg(IV)
Ader et al (1980)	10(2)	III-IV	+	Captopril	25 mg
Cleland et al (1984)	10	III-IV	+	Captopril	6.25mg
Cleland et al (1985)	26	II-IV	+/-	Enalapril	5/ 10mg
Kramer et al (1982)	15	II-IV	+	Captopril	25 mg
La Barre et al (1982)	1	IV	?	Captopril	6.25mg
Lantz et al (1984)	16	III	?	Captopril	12.5/6.25mg
Mujais et al (1984)	10	III-IV	+	Captopril	25 mg
Packer et al (1983)	7	IV	+	Captopril	25 mg

has never been documented and indeed only very recently has a placebo controlled trial of the initial response to ACE inhibitor therapy in chronic cardiac failure been published (Herrlin et al, 1990). The importance of placebo control to any scientific study is as true for heart failure as any other area of therapeutics (Packer,1990).

Although ACE inhibitors are available as intravenous formulations (Kubo et al, 1985; Hornung & Hillis, 1987; Semple et al, 1987; De Marco et al, 1987; Walinsky et al, 1987) and have been suggested to be valuable in the management of severe acute heart failure (Rademaker et al, 1986; Flynn et al, 1988) they have not been studied in detail with respect to the concentration effect relationship of their first dose response.

Although this pattern of haemodynamic response to ACE inhibitors may provide further valuable insights into the balance between circulating and tissue ACE activity in generation of a response it should be noted that where studied this response apparently does not reflect a likelihood of overall clinical benefit from chronic therapy with ACE inhibitors in heart failure (Massie et al, 1984; Packer et al, 1985). Patterns of symptomatic response in individual patients appear to be variable and certainly emerge over 6-8 weeks of continuous therapy (Packer et al, 1983). Unfortunately many studies concentrate on acute short term central haemodynamic changes which have little relevance to long term efficacy (Lipkin & Poole Wilson, 1985; Packer, 1988). Moreover such invasive studies involve well documented placebo changes in pressures related to cardiac catherisation and as stated above placebo therapy is rarely documented (Massie et al, 1984; Packer et al, 1985; Siemienczuk et al, 1986; Gibbs et al, 1989).

Chronic therapy with ACE inhibitors has been demonstrated in large numbers of patients to reduce the morbidity and mortality of chronic cardiac failure. However many if not all of these studies incorporate concomitant diuretic and digitalis therapy (Cleland et al, 1984,1985; Consensus Study Group, 1987; Giles et al, 1989; Gheorghiade et al, 1989; Cohn et al,1991; SOLVD,1991,1992a,b). It is clear that beneficial effects produced

by such combination treatment may not be entirely related to haemodynamic changes. Alternatives include amelioration of the chronic electrolyte abnormalities of the syndrome (Cleland et al, 1987) or attenuation of primary or secondary neurohormonal changes induced by diuretic therapy (Bayliss et al, 1987). These may be a major cause of mortality, an important mechanism of action of ACE inhibitors and possibly a reason for differences in efficacy among ACE inhibitors (Packer et al, 1986a,1987a,1987b).

The significance of initial hypotensive effects for patients is unclear although cerebral and renal hypoperfusion have been documented with transient dysfunction (Rajogopalan et al,1984; Mujais et al, 1984). Similarly in many patients with cardiac failure secondary to ischaemic cardiomyopathy or coronary artery disease transient episodes of significant systemic hypotension might be expected to decompensate coronary perfusion and cause silent myocardial ischaemia. There is little definitive evidence in this important area although Gibbs et al (1989) found individuals with chronic stable angina pectoris who tolerated enalapril therapy poorly when assessed by electrocardiographic changes to exercise. Similarly, a retrospective analysis of previously published data have also suggested an aggravation of myocardial ischaemia, with subjective and objective indices, in heart failure patients who have active chest pain during controlled studies of ACE inhibition (Cleland et al,1992). The direct effects of ACE inhibitors on coronary perfusion are complex. Whereas some authors have suggested a "coronary steal" phenomenon diverting blood away from the ischaemic areas (Ertl, 1988) others conclude the opposite (Gasic et al, 1990). In balance the exact role of ACE inhibition in the ischaemic myocardium is as yet unclear. It is essential that the selected populations who recieve overall benefit should not be confused to the extent that ACE inhibition becomes a ubiquitous treatment for "heart disease"!. There is a worrying trend towards this assumption in recent published commentary about these drugs.

CHAPTER 4

CHRONIC HEART FAILURE AND ITS THERAPY

4.1. BACKGROUND AND INTRODUCTION

The characteristic symptoms of cardiac failure are lethargy, limitation of exercise capacity, breathlessness on progressively less exertion or at rest, and fluid retention and oedema (Poole-Wilson,1988). The exact relationship of the clinical signs and symptoms of heart failure to underlying central or peripheral perfusion, primary or secondary neurohormonal responses and tissue oxygenation and substrate or electrolyte metabolism is unclear (Massie,1988;Editorial,1989). What is abundantly obvious is the relentless morbidity and mortality of this sequence of events (Sutton, 1990;Kannel et al,1988;Schocken et al,1992). This is manifest as a cycle of episodes of fluid overload and pulmonary oedema requiring hospital admission with progressive reduction in mobility. Furthermore and possibly of differing origin is the high prevalence of sudden cardiac death in this patient group which is often attributed to the presence of life threatening arrhythmias (Cleland et al,1987).

Estimates of overall mortality for heart failure are complex as clinical diagnosis is fraught with problems despite the simplicity and common nature of the complaint (Wheldon et al,1993). Symptoms are relatively non-specific particularly where there is coincident pulmonary disease. Clinical signs (Remes et al,1991;Ghali et al,1991) and radiological features (Chakko et al,1991) are open to misinterpretation. Aetiology and subclassification of predisposing illness are rarely stratified eg sex, diabetes, hypertension, cardiomyopathies of varying types, non-stenotic valvular heart disease, myocardial ischaemia and/or post myocardial infarction. These may have variable outcomes and differing prognoses (Packer,1988). Despite these caveats and the potential

for wrong diagnoses and misclassification the mortality of severe heart failure in community terms is comparable, and in individual instances greater than, other terminal illnesses such as common neoplasms (Kannel,1989).

Somewhat distinct from this group but also relevant in terms of discussing the place of ACE inhibitor therapy and ventricular dysfunction are the population of patients with minimally symptomatic or asymptomatic cardiac dysfunction evident only during quantitative stress testing or non invasive monitoring of cardiac function for example using echocardiography (Yusef et al,1990).

Another relatively asymptomatic group of patients who have come under scrutiny as a target for ACE inhibitor treatment are those patients with recent myocardial infarction who have the "potential" for progressive ventricular dysfunction.

4.2. GENERAL THERAPEUTIC STRATEGIES

All patients with heart failure are managed within a strategy of graded exercise. This is somewhat of a reversal of previous general policies of bed rest and exercise limitation (Drexler,1992). The change is clearly based on well controlled clinical trials published in recent years (Coats et al,1990).

4.3. DRUG TREATMENTS

4.3.1. DIURETICS, FLUID BALANCE AND SODIUM STATUS

General management of the patient with heart failure is primarily based on control of fluid balance, in the first instance by combination of dietary sodium and fluid intake restriction. All patients with will require the use of diuretic drugs and in the majority this will imply the use of more potent loop acting agents in order to achieve adequate diuresis and natriuresis. A small proportion will be adequately controlled on less potent (in diuretic terms) thiazide agents. In more severe fluid imbalance

combination therapy may be required using aldosterone antagonists or ultimately both thiazide and loop acting drugs together which is a particularly potent natriuretic and diuretic regimen (Dargie,1989). It is clear from clinical observational studies that optimal fluid balance and diuretic administration can be achieved by simple clinical assessment and drug titration in accordance with reproducible and accurate measurement of body weight (Anand et al,1989b). There is general agreement that monotherapy with ACE inhibitor drugs is not an adequate treatment, regardless of the degree of myocardial dysfunction, in symptomatic patients (Cowley et al,1986;Richardson et al,1987;Anand et al,1990)

4.3.2 VASODILATORS

For the most part vasodilator therapy in heart failure is based on the simple relationship between central cardiac filling pressures and cardiac output. With cardiac failure of whatever cause the optimal pressure-output relationship at rest and/or during exercise is disrupted. In the process of trying to sustain optimal perfusion the neurohormonal modulators of cardiac function, peripheral blood flow and autonomic tone (such as catecholamines, angiotensin, natriuretic factors, vasopressin etc) generate a peripheral vasoconstriction inappropriate for optimal organ perfusion and function. The responses initially designed to maintain homeostasis ultimately perpetuate the syndrome generating sodium and fluid retention. A variety of vasodilator drugs have been tested in attempts to break this cycle. Some act primarily on the arteriolar system, some the venous system, some affect both to a variable degree and others have accessory properties such as positive inotropic activity (table 4.1.).

Examples of these agents will be considered in turn. In real terms not only individual activity is important. Most vasodilators will, in practice, be used with other drugs. Heart failure treatment is one of the few areas of clinical therapeutics where

Table 4.1 Vasodilator therapy for heart failure

A:	**Arterial vasodilators**
(i)	α_1 adrenoceptor antagonists e.g. prazosin, trimazosin, doxazosin
(ii)	Calcium channel blocking drugs e.g. dihydropyridines, nifedipine etc; diltiazem
(iii)	Dopaminergic agents, e.g. dopexamine, ibopamine, fenoldopam (SKF 82526)
B:	**Mixed acting vasodilators**
	Flosequinan (BTS 49465); hydralazine; pimobendan (UDCG 115 BS)
C:	**Venous vasodilators**
	Nitrates; Nicorandil
D:	**Inotropic drugs with vasodilator activity**
	Phosphodiesterase III inhibitors: e.g. Milrinone; Enoximone (MDL 17043); Piroximone (MDL 19205)

Vasodilator drugs have obvious haemodynamic endpoints and in principle are expected to alter central cardiac haemodynamics. This is the first activity which tends to be documented for new and existing agents to suggest efficacy in heart failure. Unfortunately central haemodynamic changes have little relevance to clinical symptomsin chronic heart failure patients and do not reliably predict whether these will be improved by treatment (Chattergee,1989; Franciosa et al,1981). Furthermore, haemodynamic studies, although relatively easy to perform in the cardiac catheterisation laboratory, are notoriously difficult to interpret. Placebo treatment is frequently omitted from studies. The placebo response in heart failure mimics a vasodilator drug (Packer,1990a). Supine haemodynamics are less relevant than the response to exercise although it is the former which are usually documented. Everyday activities such as feeding have well described "vasodilator" effects (Herrlin et al,1990). These are often specifically excluded from study and therefore the true response in practice remains unknown. The incidence of acute myocardial ischaemia/infarction and the decompensation of CCF follows a well described diurnal pattern with a peak onset of events during night-time (Muller et al,1989). Effective treatment should cover this nocturnal phase yet this is rarely studied with respect to haemodynamic drug responses (Giles,1991). Open, sequential or stepped dose pilot studies in small numbers of patients are of limited value but seem to be common relative to double-blind placebo controlled designs. This is a particular problem in heart failure (Lipkin et al,1985). Of prime importance, although some studies do support the relationship between certain central haemodynamic parameters and the length of survival, mortality studies with vasodilator treatments are rare with notable exceptions.

A. Arterial vasodilators

Predominantly arteriolar dilatation has been studied with alpha$_1$ antagonists (prazosin, trimazosin) calcium channel blocking drugs (dihydropyridine drugs, eg, nimodipine, nifedipine etc or diltiazem) or prostacyclin analogues. Studies largely document the effects of acute treatment on reducing cardiac filling pressures specifically right atrial pressure, pulmonary capillary wedge pressure and reductions in calculated systemic vascular resistance and arterial pressure. These studies are relevant to the management of acute heart failure, for example, during acute myocardial infarction. In chronic cardiac failure persistent haemodynamic effects have not always been evident. This is particularly relevant to alpha blockade (Bayliss et al,1985; Kirlin et al,1985). Prostacyclin derivatives, with no oral bioavailability, have not been tested on repeated administration.

The use of calcium channel blocking drugs in chronic heart failure has been described as a matter of concern (Editorial,1991; Packer,1990b). Although persistent changes in haemodynamic parameters can be documented in some studies, the failure of these drugs to increase exercise tolerance in small randomised, double-blind studies (Tan et al,1987) and evidence of clinical deterioration with increased hospital admission during treatment (Dunselman et al,1990; Jezek et al,1990) has led to a reappraisal of their value. Diltiazem shares these deleterious effects (Goldstein et al,1991). There is little to distinguish any calcium antagonist suggested to have less negative inotropic properties. Despite these results conflicting reports based on the haemodynamic efficacy of calcium antagonists in heart failure continue to occur (Reicher-Reiss & Barasch,1991; Thomas et al,1989).

Drugs acting to stimulate arterial vasodilatation through vascular dopamine receptors have been studied in heart failure. These agents have complicated dose and concentration dependent pharmacology and some such as ibopamine also act as alpha$_1$

and beta$_2$ adrenoceptor agonists (Marchetti,1990). The most frequently studied agent in this class is ibopamine. Evidence of clinical efficacy with this group of agents is limited to demonstrations of vasodilatation on central haemodynamic studies. However, there are studies which show a symptomatic improvement with ibopamine (Barbino et al,1991) based on clinical assessment of heart failure. Diuretic effects of this group of drugs have been highlighted as an additional beneficial property. The diuresis is at best modest (Baumann et al,1990; Wehling et al,1990) and quantitatively unlikely to be relevant to the management of heart failure. As with some other drugs even in acute studies partial haemodynamic tolerance has been shown to occur during fenoldopam infusion within 24 hours (Munger et al,1990).

B. "Balanced" vasodilators

Mixed arterial and venous dilatation is produced by a variety of drugs dependent on dosage. The most recent agent studied with this predominant mode of action is flosequinan. This has the characteristic haemodynamic profile (Reigger et al,1990; Markus & Cowley,1991) and has been suggested to improve exercise parameters (Silke et al,1992, Elborn et al,1990). It is felt that the venous dilatation is relevant to the mode of action of this drug and a degree of positive inotropic activity is also suspected (Corin et al,1991). The place of this drug in treatment is unclear but early studies based on improved exercise capacity are promising.

C. Venous "offloading"

Oral nitrate therapy has long been a part of heart failure treatment. For the most part this is felt to act by venous dilatation and reduced right heart pressure. However, it has become apparent that high doses of nitrates are required in the treatment of heart failure and selective venodilatation is unlikely to be relevant (Cohn,1985). Tolerance to

haemodynamic effects is readily produced unless a nitrate free interval is present (Sharpe et al,1987; Elkayam et al,1991; Jordan et al,1985) although there is some interesting if flawed evidence that combinations of nitrovasodilators and ACE inhibitors might ameliorate this problem (Katz et al,1991;Mehra et al,1992). Alternative routes of administration such as transdermal preparations (Jordan et al,1985) or different structures such as nicorandil (Galie et al,1990) probably have little to offer over oral nitrate. A recent study employing seven day therapy with a range of ACE inhibitors in normal volunteers using forearm venous plethysmography to assess the response to a low dose transdermal nitrate patch has suggested that nitrate tolerance might be avoided with this combination (Katz et al,1991). With the established role of ACE inhibitors in treatment this finding merits further study in heart failure. Fluid imbalance or sub-optimal diuretic therapy may also be a factor in nitrate tolerance in heart failure (Varriale et al,1991).

D. Vasodilator drugs with inotropic properties

A final group of agents with a component of vasodilator activity in addition to more direct positive inotropic properties has been extensively studied in heart failure. Based largely on favourable changes in the acute haemodynamic profile, a series of orally active, selective, inhibitors of phosphodiesterase III; eg enoximone, milrinone and piroximone, have been developed. Despite the favourable changes recorded in cardiac pressures, even in the early stages of clinical study, concern was expressed about a lack of symptomatic benefit and increased prevalence of arrhythmias in treated patients (Rubin et al,1985). Well designed studies have failed to document changes in exercise capacity and have confirmed an increase in mortality on longer term treatment with these drugs (Uretsky et al,1990; Packer et al,1991). Although this class of treatment appears to have little future in population terms for the management of chronic heart failure they may still prove useful in acute heart failure and exceptionally in individual patients in the

longer term (Chattergee,1989).

E. Vasodilators and mortality in heart failure

Due to the undoubted need for combinations of drugs in the treatment of heart failure it is interesting to note that the best evidence for symptomatic efficacy and reduced mortality through vasodilator therapy comes from a combination regimen. The two multicentre Veterans Administration Cooperative studies examined large numbers of patients receiving hydralazine (300 mg/day) and isosorbide dinitrate (160mg/day) in a double blind randomised study compared to prazosin or placebo in the first study (Cohn et al,1986) or to the ACE inhibitor, enalapril, in the second study (Cohn et al,1991). Although of borderline statistical significance (not significant statistically speaking but regarded as clinically significant), the first study showed a reduction in mortality (%) with hydralazine/ISDN (25.6) compared to placebo (34.3) or prazosin (34.1) at 2 years. Although the second study revealed significantly greater benefits in terms of reduced mortality for the patients receiving the ACE inhibitor enalapril over the vasodilator combination, it was felt that the pattern of mortality differed between the treatments. The incidence of sudden death was particularly affected by enalapril and the combination of vasodilator treatment with ACEI treatment could provide a greater mortality reduction. The success of the hydralazine/isosorbide dinitrate therapy in both Veterans Administration studies is clear. It appears to be dose dependent for both agents and the role each of each component in the overall response remains unclear (Remme,1989).

F. Development areas of vasodilator therapy

The scope for further improvements in the response and use of vasodilator treatments in heart failure is considerable. Although many invasive studies of acute pressure changes in small numbers of patients do not seem to reliably identify those

treatments which will have an impact on symptoms and mortality, they do indicate drugs which should prove efficacious at least in acute heart failure. Furthermore on an individual patient basis the pattern of aetiology of heart failure, long neglected as a prognostic or therapeutic indicator, may be employed with haemodynamic response in attempts to select sub-populations who will benefit from treatments at least on a symptomatic basis. What needs to be used in combination with this are simple methods for controlled assessment of response which are readily applied to individual subjects (Guyatt et al,1986). As the Veterans Administration Heart Failure investigators suggest the future undoubtedly revolves around developing better combinations of vasodilators, exploring the efficacy of high dose nitrates and hydralazine and the power of combinations involving other vasodilators, diuretics, digoxin, ACE inhibition and antiarrhythmic drugs.

4.3.3 ACE INHIBITION IN HEART FAILURE

The theoretical basis for the use of angiotensin converting enzyme inhibitors (ACEI) in the management of congestive cardiac failure is blockade of the neuroendocrine activation which accompanies heart failure and in response to the management of fluid overload with diuretic drugs (Packer,1988). The primary effector peptide of the renin angiotensin system (RAS), Angiotensin II (AngII), has detrimental effects on cardiac structure (Tan et al,1991; Pfeffer et al,1991), perfusion (Ertl,1988) and overall function (Mochizuki et al,1992). There are additional deleterious effects on renal function, overall electrolyte balance and peripheral skeletal muscle perfusion. Ultimately the neurohormonal reflexes, primarily those involving the renin angiotensin system but with important contributions from the sympathetic nervous system (Francis,1989) and other humoral factors such as natriuretic peptides (Uretsky et al,1990) ,initially designed to maintain peripheral organ perfusion, become deleterious (Packer,1992).

Blockade of the renin angiotensin system by ACE inhibitors has been shown unequivocally to have beneficial effects on the key end points in heart failure. These are overall patient mortality, whether related to decompensation of heart failure to a terminal state of fluid imbalance and organ failure or sudden cardiac death (CONSENSUS Trial Study Group,1987;SOLVD investigators,1991;1992). In addition frequency of hospital admission, an excellent indicator of impact on morbidity and general quality of life, is also significantly reduced in real terms by ACE inhibitor therapy (Cohn et al,1991). Although exercise capacity and the related parameters of oxygenation, symptoms of dyspnoea, or heart failure classification are very difficult parameters to stratify and interpret in controlled therapeutic trials (Poole-Wilson,1989) in general terms there is a considerable body of data to attest to the efficacy of ACE inhibitor therapy on such "symptom based" parameters in patients with heart failure (Cleland et al,1984;Creager et al,1985). With this data available drug treatment of individual patients should include an ACE inhibitor as a cornerstone of treatment along with diuretic therapy (Braunwald,1991;Editorial,1992).

4.4 PHARMACOLOGY OF ACE INHIBITION IN HEART FAILURE: THE EXAMPLE OF PERINDOPRIL

4.4.1. Experimental studies - general principles

In experimental animals the syndrome of heart failure is simulated by a variety of techniques. Either volume or fluid overload of the heart can be created by a variety of surgical techniques eg, aortic or pulmonary banding or ligation, or valve rupture. Failure of the ventricles can be induced by a variety of pacing techniques (Dibner-Dunlap & Thames,1990). Direct injury to cardiac muscle can be created eg by cryo-injury (Lefer et al,1986),ligation of coronary arteries (Howes et al,1991) or microsphere occlusion of

coronary arteries (Gorodetskaya et al,1990). A toxic cardiomyopathy can be induced by a variety of agents, the most commonly used being the cytotoxic antibiotic, doxorubicin (Arnolda et al,1985;Wanless et al,1987). A range of genetically based cardiomyopathies are available (Hirakata et al,1990; Haleen et al,1991). Although each of these models has its own limitations (Smith & Nuttall,1985) in general low output models with a chronic course which generate the appropriate neurohormonal responses to those seen in man are preferred. Pharmacological treatments can be employed in an attempt to mimic the autonomic dysfunction common in heart failure (Hof et al,1992). In the main the animals studies do not receive any additional therapy such as diuretic drugs which are intrinsic to the management of human heart failure. Such models have been used to characterise the pharmacology of perindopril and perindoprilat.

4.4.2. Hormonal effects

Experimental studies have shown that the renin angiotensin system is an essential component to the response to heart failure resulting in a peripheral vasoconstriction affecting perfusion of the kidneys and skeletal muscle beds. The former responds by initiating fluid and sodium retention (Anand et al,1989) and the latter may be involved in the generation of further reductions in exercise capacity typical of heart failure (Myers & Froelicher,1991). Perindopril has been shown to effectively provide blockade of the renin angiotensin system resulting in ACE inhibition, reactive elevation in renin, reduction in infarction related rises in natriuretic factor but little effect on plasma aldosterone (Michel et al,1988).

Experimental myocardial infarction and heart failure are associated with activation of the sympathetic nervous system at a variety of levels primarily due to increased secretion and occurring in association with receptor down regulation (Abraham et al,1990;Bristow et al,1989). Activity of the sympathetic nervous system as portrayed

in elevated serum catecholamines has been defined as a sensitive indicator of poor prognosis in heart failure (Cohn et al,1984). In experimental animals Howes and co-workers demonstrated that perindopril treatment whether introduced early or late after experimental infarction produced complete attenuation of infarction related augmentation in sympathetic activity as indicated by the ratio of dihydroxyphenylethylene glycol to noradrenaline (Howes et al,1991).

4.4.3. Vascular structure

The effect of perindopril in reversing the vasculopathy associated with experimental hypertension has been documented in detail (Levy et al,1991). Altered vascular structure and reactivity are features of the response to heart failure and deficits in blood flow during exercise may at least play a partial role in the generation of fatigue in heart failure patients (Drexler et al,1988). It is clear that altered muscle structure and metabolism are common in heart failure and these effects may interact with flow abnormalities or even dominate the peripheral generation of symptoms (Arnold et al,1990;Buller et al,1991; Massie et al,1987)

4.4.4. Cardiac hypertrophy/infarction and remodelling

During all its clinical phases from the asymptomatic stage through to terminal pump failure the myocardium during heart failure undergoes a complex process of adaptation. This involves concurrent thinning, regional dilatation and expansion and compensatory hypertrophy of the ventricular walls, altered myocardial structure and composition (Swynghedauw,1989; Pfeffer et al,1991). The renin angiotensin system is at least a major element, if not the major element controlling these responses to myocardial damage and/or heart failure (Katz,1990;Weber et al,1992).

As has been recorded for other ACE inhibitors perindopril effectively blocks or

reverses the process of ventricular dilatation and increased heart size following experimental myocardial infarction (Howes et al,1991). Sensitive studies by Michel and colleagues demonstrated a trend towards normalisation of the ventricular myosin isoenzyme profile and collagen content following experimental infarction in rats treated with perindopril (Michel et al,1988).

Electrophysiological abnormalities are common in all patients with heart failure and sudden cardiac death, often attributed to the onset of a lethal arrhythmia, is a common mode of death for patients with this disease (Cleland et al,1987). Experimental models have been employed to examine this aspect of heart failure pathology only infrequently (Doherty & Cobbe,1990). Thollon and colleagues (1989) examined the effect of perindopril therapy on the electrophysiological consequences of myocardial infarction. They observed increased action potential lengthening in association with cardiac remodelling after infarction. Perindopril therapy prevented the development of cardiomegaly and attenuated the increase in action potential duration significantly. Direct effects of perindoprilat on the inward calcium current of isolated porcine ventricular myocytes has also been demonstrated which acts to inhibit the action of noradrenaline (Enous et al,1992). These findings suggest potential anti-arrhythmic effects of perindopril treatment relevant to heart failure. A small acute study in a peri-infarction model in pigs failed to demonstrate significant abnormalities despite a significant reduction in mortality in the perindopril treated group (Tobe et al,1992)

4.4.5. Tissue renin angiotensin systems

Although human heart failure is widely regarded as being accompanied by an activation of the renin angiotensin system much confusion is caused by the concurrent use of diuretic therapy. In untreated heart failure there may only be small elevation in circulating renin in keeping with the increased intravascular fluid volume (Anand et

al,1989). However in experimental models of low output heart failure activation is demonstrable at the level of the tissue based system (Hirsch et al,1991,1992; Schkunert et al,1990;Fabris et al,1990). In addition it is clear that other non converting enzyme dependent pathways for the local generation of AngII are also activated in heart failure (Urata et al,1990).

4.4.6. Mortality

Clearly one of the most significant levels of efficacy in heart failure treatment must be the effect on mortality. Experimental studies in rats treated with captopril after myocardial infarction clearly show reductions in long term mortality with ACE inhibitor treatment (Pfeffer et al,1985). Short term studies with perindopril in similar models of left ventricular infarction in rats show no appreciable difference between sham operated placebo treated controls and perindopril (Michel et al,1988). This is largely due to the duration of study than any real suggestion of anything other than a class related activity. In longer term studies with a similar long acting ACE inhibitor, quinapril, hamsters with a genetic cardiomyopathy showed a decreased mortality compared to placebo controls (Haleen et al,1991). Similar results have been documented with enalapril (Sweet et al,1987). A relatively small study examining the effect of intravenous perindoprilat (0.06mg/kg) on experimental myocardial infarction in pigs showed a significant reduction in the death rate 7 of 12 controls compared with 2 of 12 treated animals over the 2 weeks following coronary occlusion (Tobe et al,1992).

In the context of experimental studies the reasons given for the observed reductions in mortality tend to focus on myocardial changes. Altered myocardial structure is often cited as a major factor. Specifically the reduction in myocardial fibrosis following infarction demonstrated clearly by Michel and colleagues (1988) using perindopril is felt to be a central element in the concept of "cardioprotection" manifest as

improved cardiac haemodynamics and coronary perfusion, attenuated progression of myocardial failure and inhibition of acute events such as the tendency towards arrhythmia and sudden cardiac death (Gavras & Gavras,1991). While theoretically appealing the concept originates in animal observations and the treatments employed do not always mimic the clinical situation (McMurray et al,1991). This remains an exciting area for further definition of the mechanism of action of ACE inhibitor treatment. Experimental studies tend to examine the contribution of one of the above mechanisms in isolation and tend not to give an integrated answer to the mechanism of action in man.

4.5. CLINICAL STUDIES

4.5.1 PHARMACOKINETICS

A. GENERAL

It is important to remember that the duration of action of ACE inhibitors has important functional implications. The incidence of a variety of cardiovascular emergencies follows a well described periodicity. The majority of events, and this includes the onset of acute cardiac decompensation, peak in the late evening or early hours of the morning (Cugini et al,1990;Muller et al,1989). This has important implications for drug therapy and it is a notable omission that many heart failure treatments are not studied for efficacy at the peak hours of onset of the disease, namely in the middle of the night (Giles,1990). This has recently been a focus of attention in the description of the efficacy of anti-hypertensive drugs .

The pharmacokinetics of perindopril in heart failure are affected by some simple principles applicable to most drugs used in this condition (Table 4.2).

Table 4.2: General effects of heart failure on the pharmacokinetics of cardiovascular drugs

ABSORPTION	- reduced blood flow to gut and muscle
FIRST PASS METABOLISM	- reduced hepatic blood flow
	- reduced hepatic metabolism
VOLUME OF DISTRIBUTION	- interstitial fluid accumulation (little impact)
ELIMINATION	- interaction with alterations in volume of distribution, associated renal/hepatic dysfunction

moderate to severe heart failure, continued on their normal diuretic regimen but off vasodilators for 48 hours, the time to peak perindopril concentration (2 hours) was only slightly prolonged compared with that expected in healthy age matched controls receiving a single 4mg oral dose. This was thought to be due to a delay in drug absorption. The peak plasma concentration of perindopril was higher (112.6±12.7ng/ml) and apparent half life (3.54±0.46hr) prolonged. Perindoprilat appearance was delayed (8.22±0.85hr) as was ACE inhibition in plasma. Calculated elimination constants are similar to healthy controls but their definition hinges largely on the sensitivity of the assay system employed. The peak plasma ACE inhibition remained unaltered.

B. AGE

People who have heart failure are in the main elderly. The condition has a high prevalence in those above the age of 65 years. It is responsible for nearly 10% of all acute hospital admissions in this age group (McMurray & McDevitt,1990). Elderly patients may be particularly susceptible to the haemodynamic effects of these drugs (Reid,1987). Considering age alone there is clear evidence of reductions in the clearance of perindopril. Lees and colleagues showed that the mean AUC for a single oral dose of 8mg perindopril was increased from 119.7 ng/ml/hr to 295.3 ng/ml/hr comparing young and elderly normal volunteers. This may be related to a reduction in renal clearance of perindoprilat but the relationship is not direct. This is similar to the findings for other prodrug ACE inhibitors (Hockings et al,1986).

Gilgenkrantz and Flammang (1987) studied 33 heart failure patients (35-77 years) over three months perindopril therapy in the dose range from 1-4mg once daily. Model independent pharmacokinetic parameters increased in a dose related manner both for perindopril and perindoprilat (see table 4.3).

Table 4.3: Pharmacokinetics of perindpril in heart failure patients. data on file from study PKH 07.02, Gilgenkrantz and Flammang,1991; Servier Europe Courbevoie, France.

Population	t_{max} (h)	C_{max} ($ng.ml^{-1}$)	AUC ($ng.ml^{-1}.h$)	$t_{\frac{1}{2},2}$ (h)
Heart failure patients (n = 10)	1.85 ± 0.39 (0.5 - 4.0)	112.55 ± 12.67 (28.7 - 172.1)	577.01 ± 111.46 93.3 - 1261.9)	3.54 ± 0.46 (1.3 - 6.0)
Healthy subjects	1.0** (0.5 / 3.0) (n = 66)	73.27* 19.74 / 126.79 (n = 66)	168.90* (38.77 / 298.94) (n = 66)	0.89* (0.31 / 1.47) (n = 54)

* = **mean (95% confidence limits)**

** = **median (extreme experimental values)**

three month treatment interval. The increment in perindoprilat AUC of approximately 1.5 is typical of that seen with other ACE inhibitors and heart failure does not significantly alter this phenomenon in comparison to normal individuals or patients with hypertension.

Reduction in the total body clearance of perindopril and perindoprilat occurs in elderly patients with heart failure as seen by the higher plasma concentrations again in comparison to reference data from normal volunteers or patients with hypertension.

C. RENAL IMPAIRMENT

Renal responses are central to the development of heart failure. Through a variety of mechanisms renal impairment is a common feature of many patients (Ritz & Fliser,1991;Naschitz et al,1990). As the major route of elimination of perindopril is by metabolism, largely hepatic, and that of perindoprilat is excretion by the kidneys as expected renal impairment affects the pharmacokinetics of perindoprilat rather than perindopril. In stable chronic renal impairment there is a direct positive correlation between cumulative clearance of perindoprilat and creatinine (Verpooten et al,1991;Sennesael et al,1992; see figure 4.1).

This results in elevation of maximal plasma perindoprilat concentrations and AUC in proportion to the degree of renal impairment. Terminal elimination half life, which reflects in part distribution of the drug to tissue compartments (Lees et al,1989), is unaltered by renal failure. Proportionally a reduction in perindopril dosage is required where renal function is impaired.

D. HEPATIC IMPAIRMENT

Liver dysfunction is a feature of heart failure. In the majority of instances this is a reflection of hepatic congestion which can be in part corrected by appropriate diuretic

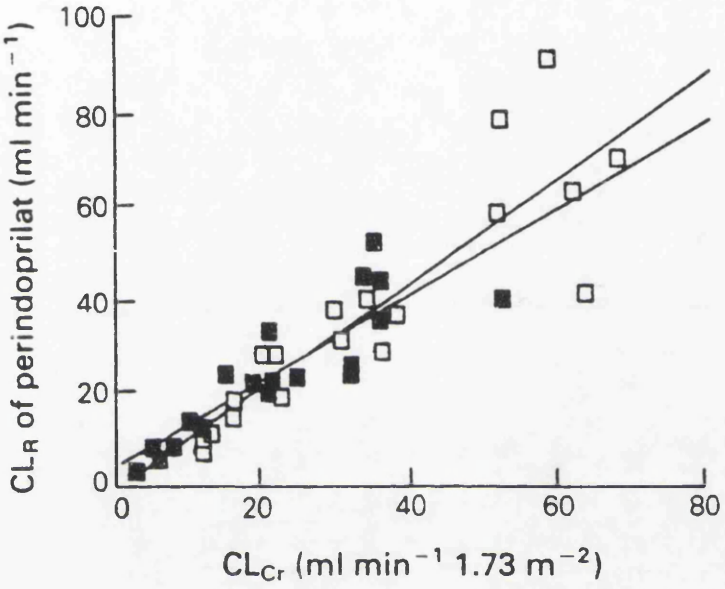


Figure 4.2 The relationship between cumulative renal clearance of perindoprilat ($CL_R$and creatinine clearance (CL_{Cr}) (data from Sennesael et al,1992)

unclear. The de-esterification of prodrug ACE inhibitors is a diffuse process occurring in a variety of sites. However the majority of this step is conducted in the liver (Larmour et al,1985). Studies by Tsai (Tsai et al,1989) and Thiollet (Thiollet et al, 1992) have examined clearance of perindopril and its metabolites in patients with established cirrhosis. Both these investigators suggested increases in the AUC for perindopril, roughly doubled (eg 602 ± 294 ng/ml/hr vs 266 ± 70 ng/ml/hr), but similar values for perindoprilat (eg 134 ± 139 ng/ml/hr vs 120 ± 29 ng/ml/hr) to those found in normal volunteers (Devissaguet et al,1990).

On the assumption that perindoprilat is the entity which generates the metabolic effects of treatment it was concluded that no dosage alteration was necessary. Similar observations have been made using other prodrug ACE inhibitors (Ohnishi et al,1989). Comparable studies are not available in heart failure but liver dysfunction per se does not appear to pose specific risks of drug accumulation requiring alterations in daily dose.

E. FOOD

Food has an important effect on drug bioavailability. In the context of heart failure responses and ACE inhibitor drugs this is doubly important as a meal will in addition have independent effects on blood pressure. The haemodynamic effects resemble the effect of vasodilator drug treatment and effectively enhance or overestimate the effects of ACE inhibitor treatment (Herrlin et al,1990).

Generally the bioavailability of captopril and its haemodynamic effect are reduced and delayed respectively by concommitant food (Singhvi et al,1982;Mantyla et al,1984). This does not appear to be relevant to longer acting drugs such as enalapril (Swanson et al,1984). In twelve healthy volunteers Lecocq and colleagues (1990) found reductions in the bioavailability of perindoprilat and its fractional urinary excretion associated with reductions in the metabolism of perindopril to perindoprilat. Perindopril

disposition was unaltered in this single dose study in which 4mg perindopril was studied. Plasma ACE activity was affected by the altered perindoprilat profile (Table 4.4).

The reduced interconversion of perindopril to perindoprilat was not due to altered total absorption as total urinary excretion remained unaltered.

4.5.2 PHARMACODYNAMICS

A. Hormonal effects

The effects of heart failure on the renin angiotensin system alone are difficult to address. In most instances patient studies are complicated by concurrent treatments which directly or indirectly affect the system. The most common example is diuretic therapy (Bayliss et al,1986), but any vasodilator treatment will also activate the renin angiotensin system (Dcig et al,1992). Rare studies in untreated severe heart failure indicate elevated renin activity but on a variable and unpredictable basis (Anand et al,1990). With the technical limitations of most common renin activity measurements this probably underestimates the extent of the elevation of renin in these circumstances (Plouin et al,1990).

Induction of the renin angiotensin system in human heart failure has been suggested to occur on chronic treatment with ACE inhibitors in man (O'Neill et al,1992). This may relate to the disease process per se in addition to being an effect of the drug treatment itself. In general the blockade of the renin angiotensin system is felt to be central to the efficacy of the drugs despite their non specific profile. The direct relationship to beneficial effects on symptoms or in reducing mortality is not yet clear. In the same respect the optimal dosage employed within the range of drugs available is also unclear both in terms of symptomatic relief and reducing mortality.

In an open study of 10 patients with moderate(5) and severe(5) heart failure

Table 4.4.: Pharmacokinetic parameters of perindoprilat following oral perindopril (4mg) in the fasted and fed states (data from Lecocq et al,1990).

	Fasted	*Fed*	*p Value*
C_{max} (ng · ml^{-1})	4.7 ± 1.6	3.6 ± 1.5	NS
t_{max} (hr)	3.6 ± 2.2	3.9 ± 2.5	NS
AUC(0-t) (ng · hr · ml^{-1})	52 ± 22	29 ± 17	$p < 0.05$
$t_{1/2}$	10.9(8.3-31.1)*	12.0-14.2**	—
F_e (% dose)	19 ± 7	13 ± 4	$p < 0.01$
CL_R (ml · min^{-1})	171 ± 70	177 ± 70	NS
Relative amount of biotransformation (AUC_{fed}/AUC_{fasted})	0.65 ± 0.42		

*Median (range); n = 6** range; n = 2.
Data are mean values ± SD.
C_{max}, Peak serum concentration; t_{max}, time to reach maximum concentration; AUC(0-t), area under the serum concentration-versus-time curve; $t_{1/2}$, half-life; F_e, fractional urinary excretion; CL_R, renal clearance; NS, not significant.

significantly greater and more prolonged than that observed in healthy normal volunteers Thuillez and colleagues (1990) showed that a single dose of perindopril 4mg, after 24hr diuretic withdrawal in sodium restricted state, produced approximately 70% inhibition of plasma ACE (Figure 4.2). There was an associated rise in plasma renin and fall in aldosterone (Lees & Reid 1987a,b). In summary Perindopril produces rapid onset of dose dependent inhibition of plasma ACE of a protracted nature with appropriate changes in renin and aldosterone (Table 4.5).

B. Cardiac Haemodynamics

Central cardiac haemodynamic studies are conducted in order to define the initial status of agents used in the treatment of heart failure. They serve as indicators of compounds which are likely to prove valuable in the management of acute heart failure through beneficial alterations in the Frank-Starling relationship defining cardiac output in relation to initial LVEDP and cardiac work. They may be valuable in the selection of therapy for individual patients with severe heart failure who have a poor prognosis despite multiple therapies (Chatterjee, 1989;Uretsky & Hua,1991).

Such studies are not efficient predictors of individual symptomatic responses to long term therapy in chronic heart failure. Some significance has been attributed to the pulmonary vascular response as a therapeutic predictor of response to long term ACE inhibitor therapy in individual patients (Packer et al,1985). This is surprising given the known resistance of the pulmonary circulation to the haemodynamic effects of AngII (de Bono et al,1966). Patients may have acute responses compatible with haemodynamic improvements which are either not sustained on chronic therapy or are not translated to functional benefits in terms of exercise studies.

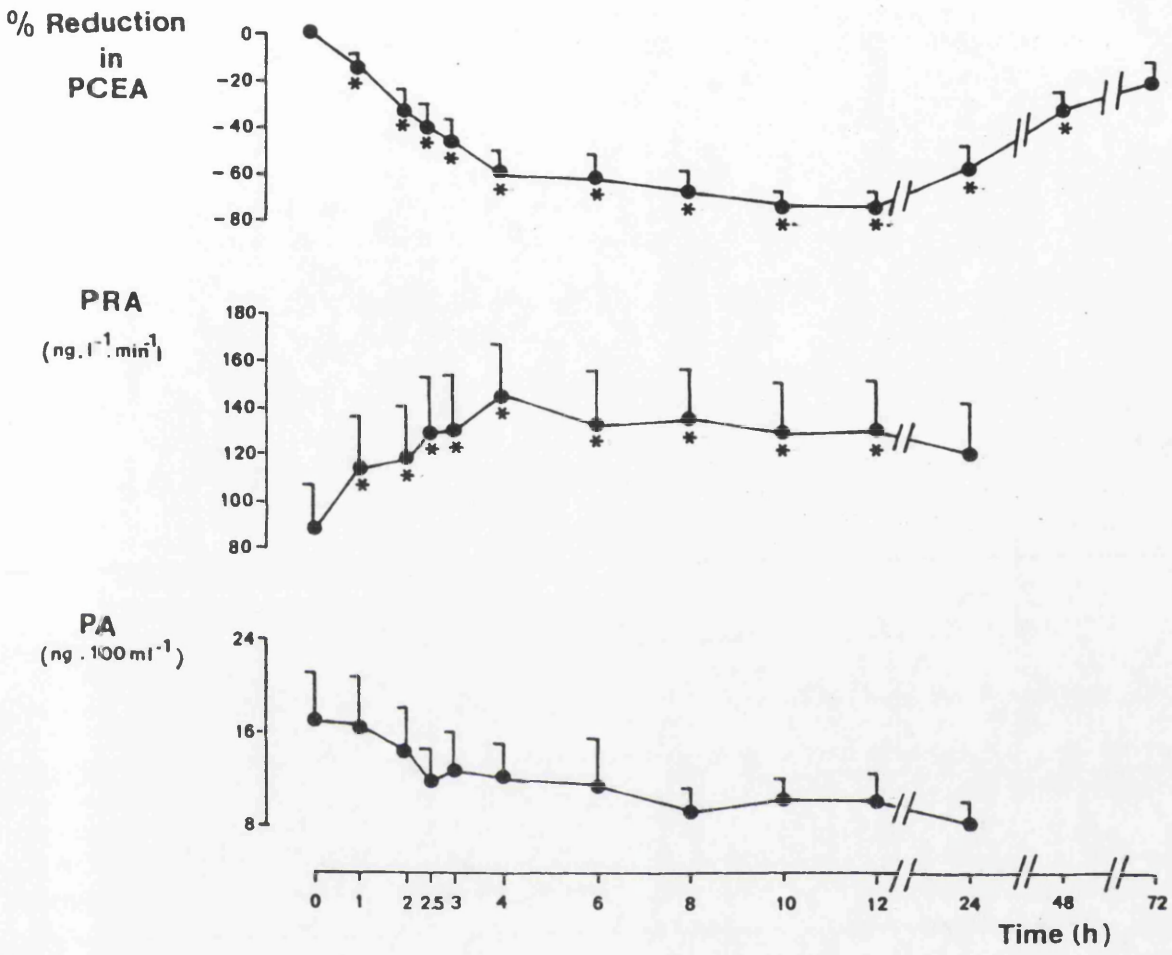


Figure 4.2: Effects of oral perindopril (4mg) on plasma converting enzyme activity (PCEA), renin activity (PRA) and aldosterone (PA) in heart failure patients (data from Thuillez et al,1990)

Table 4.5: Inhibition of ACE following acute and chronic dosing with perindopril in a range of unpublished reports in heart failure patients (data on file from expert report, Dargie,1990)

ACE inhibition	CP 04.04 acute		CP 04.01 acute		CP04.02 acute 2 mg	CP04.03 acute 4 mg	CP04.05/2 chronic 4 mg	PKH06.02 chronic 4 mg
	1 mg *(n = 11)*	2 mg *(n = 9)*	2 mg *(n = 6)*	4 mg *(n = 6)*	*(n = 12)*	*(n = 10)*	*(n = 11)*	*(n = 12)*
Peak inhibition	44 % at 4 h	71 % at 12 h	53 % at 6 hr	79 % at 6 h	63 % at 12 h	72 % at 12 h	86 % at 4 h	89 % at 5 h
Inhibition at 24 h	34 %	59 %	50 %	59 %	45 %	56 %	57 %	67 %

heart cardiac catheterisation with pressure monitoring and cardiac output determinations by thermodilution techniques. None of these studies has a placebo control. Interpretation is therefore difficult in the light of a well characterised placebo effect of instrumentation (Packer,1990), spontaneous improvements in cardiac function (Francis et al,1990), and diurnal variation in the haemodynamic aspects of the disease (Caruana et al,1988) and the response to treatment. However changes can be described with respect to baseline values.

Thuillez and colleagues (1990) demonstrated a significant and long lasting fall in systemic vascular resistance (-18%), right atrial pressure (-60%), and mean capillary wedge pressure (-28%) following perindopril 4mg in 10 patients with moderate to severe heart failure. There was an associated rise in cardiac index (+12%). Anguenot and colleagues (1987) saw delayed falls in systemic vascular resistance and mean pulmonary artery pressure at 24hrs compared to 12hrs in 14 patients with severe refractory heart failure. These are in keeping with the expected profile of vasodilatation expected to occur with acute ACE inhibition.

C. Regional blood flow

Thuillez and colleagues (1990) in their invasive study also observed the regional vascular responses to perindopril in heart failure patients. The regional sensitivity of the circulation to AngII is well described (Motwani and Struthers,1992). ACE inhibition because it affects systems other than AngII generation would be expected to produce a pattern slightly different from simple AngII withdrawal and would be modified by the disease process itself.

Thuillez found that in addition to the central haemodynamic changes described above there was a marked increase in brachial (+130%) and renal (+34%) blood flow. The falls in forearm and renal vascular resistance appeared to be related to the basal

circulating plasma noradrenaline concentrations rather than renin. As expected the vasodilatation was inhomogeneous in different vascular beds. In keeping with the insensitivity of the pulmonary circulation pulmonary arterial resistance was virtually unaltered.

These alterations in regional blood flow may be important in determining the beneficial effects of treatment on exercise symptoms originating in the poorly perfused skeletal muscle vascular beds (Cohen-Solal,1989). There appears to be a gradual increase in skeletal muscle flow with long term ACE inhibition (Drexler et al,1988;Drexler,1992). Although there is not a marked initial increase in flow the key element may be intra muscular redistribution of flow mediated by the blockade of the localised renin angiotensin system.

D. Blood pressure and first dose response

Where there is felt to be activation of the renin angiotensin system and a circulation dependent on AngII pressor tone, acute ACE inhibition has occasionally been associated with marked and symptomatic falls in blood pressure. The nature of this phenomenon is unclear but most individuals assume a direct relationship with the circulating or localised contribution of AngII to vascular tone. Concern has arisen as to the functional significance of such falls in blood pressure in heart failure patients where there is frequently concurrent renovascular, cerebrovascular or coronary vascular disease. Attention has been focused by anecdotal case reports (Cleland et al,1985;Lantz et al,1988;La Barre et al,1982) and small series of patients where detrimental effects particularly on renal function have been documented in renovascular hypertension and heart failure (Hodsman et al,1983;Mujais et al,1984). The incidence of the problem and its overall clinical importance in the light of the potential benefits of ACE inhibitor therapy (Editorial,1992) are unclear and controlled studies are lacking. The phenomenon

is seen with many cardiovascular agents (Semple et al,1988) or placebo and may represent effects on arterial, venous tone and autonomic function (Capewell & Capewell,1991).

The lack of initial haemodynamic response does not, as expected (Massie et al,1984) have implications for beneficial increases in exercise tolerance (see section 4.2.5). Longer term therapy with 2 or 4mg perindopril once daily was similarly not associated with significant falls in blood pressure (Bounhoure et al,1989). For this drug at least symptomatic improvement can occur without overt reductions in mean arterial pressure. This is, at present, a unique feature among the ACE inhibitors available for the treatment of heart failure.

E. Exercise Capacity

This is one of the key areas of documenting the beneficial effects of drug treatment in heart failure. This appears to have little to do with the acute effects of drugs on blood pressure or cardiac haemodynamics (Packer et al,1985;Massie et al,1984). Like haemodynamic studies placebo control, training effects and structured assessment are essential (Lipkin & Poole-Wilson,1985).

Kirlin and colleagues (Kirlin et al,1988) have suggested that the hormonal effects of ACE inhibitor drugs are affected by exercise. They found that a dose dependency was evident only following exercise which was not evident at rest. In their study the focus was concentrated around the short duration of effects on hormones found with the short acting drug captopril and the long acting drug, lisinopril. The authors conclude that full blockade of the system was important and that this may not always occur during exercise on chronic dosing despite similar resting levels of inhibition. Using hormonal responses (reactive rise in renin or falls in aldosterone) this was not achieved adequately. Although lisinopril is not a prodrug the profile of inhibition seen with perindopril most closely

resembles this pattern.

Bounhoure and colleagues studied the effects of perindopril treatment (2 or 4mg daily) or matched placebo in a double blind parallel group design on clinical symptoms and exercise parameters (Bounhoure et al,1989). Patients received three months therapy. Little change was noted in blood pressure or heart rate. Placebo treatment was associated with initial reductions in symptom scores, NYHA classification and exercise time at 1 month. This is a common feature of most controlled studies, but as expected this was not sustained at the three month assessment. In contrast perindopril treatment produced significantly greater and sustained improvement in all these parameters at both 1 and three months of active treatment (Table 4.6). In addition there was also a small but statistically significant fall in cardiac size as reflected in cardiothoracic ratio. These results are in keeping with the emergence of efficacy in the 6-8weeks following the initiation of ACE inhibitor treatment (Massie et al,1984,Packer et al,1983)

It is clear that the dose of perindopril appropriate for the management of heart failure (2-4mg daily) is below that normally employed in the management of hypertension with this agent (Lees et al,1989).

4.6 SAFETY AND TOLERABILITY

Perindopril has been studied during long term treatment in relatively small numbers of heart failure patients in order to observe its record of safety and tolerability. In controlled studies there have been no major reports of significant biochemical or haematological adverse events. In particular there were no significant changes in biochemical indices of renal function although as expected a small but statistically significant rise in serum potassium can be documented.

Treatment withdrawal from controlled studies as expected shows a significant incidence of death and progression of heart failure (Table 4.7). Matched placebo data are

Table 4.6: Exercise response to oral perindopril table 5 study C 04.01/2

	Before treatment	After 3 months treatment
Excercise test duration (sec)		
Bicycle - placebo *(n = 36)*	563.6 *(25.9)*	594.4 *(33.8)*
- perindopril *(n = 36)*	493.8 *(23.6)*	619.4 *(28.2)*
Treadmill - placebo *(n = 16)*	530.3 *(49.4)*	544.7 *(70.4)*
- perindopril *(n = 18)*	597.6 *(50.0)*	778.4 *(60.1)*
NYHA class		
- placebo *(n = 56)*	II = 38 III = 18	I = 9 II = 32 III = 12 IV = 3
- perindopril *(n = 56)*	II = 30 III = 26	I = 12 II = 37 III = 7 IV = 0
Heart failure score		
- placebo *(n = 55)*	4.5 *(0.3)*	3.7 *(0.4)*
- perindopril *(n = 56)*	5.5 *(0.4)*	2.4 *(0.3)*

Table 4.7: Summary of treatment withdrawals in heart failure patients during a range of clinical trials of oral perindopril therapy (data on file from Dargie,1990).

	Study C 04.01/2		Study C 04.02 (n = 320)	Study CP 04.05/2 (n = 15)	Study PKH 06.02 (n = 12)
	S 9490 (n = 61)	placebo (n = 64)			
Total no. of withdrawals	5	7	75	5	0
Reasons					
Death	0	1	10	4	0
Adverse event	2	4	38	1	0
Worsening of heart failure	1	0	9	0	0
Poor compliance	0	1	1	0	0
Loss to follow-up	2	1	7	0	0
Other	0	0	10	0	0

not available for comparative purposes.

Generalised adverse events reported in the safety studies cover the usual range of non-specific symptoms (Table 4.8). There is little to distinguish the pattern of symptoms from those generally attributable to the class effects of ACE inhibitor treatment.

Table 4.8: Adverse symptoms reported (total number and percentage) during clinical studies with oral perindopril in heart failure patients (data on file, Dargie,1990)

Symptom	C 04.01/2 3 months				C 04.02 up to 30 months		PKH 06.02 1 month	
	S 9490 *(n = 61)*		placebo *(n = 64)*		S 9490 *(n = 320)*		S 9490 *(n = 12)*	
	n	%	n	%	n	%	n	%
Cardiorespiratory								
- anginal pain					8	2.5		
- orthostatic discomfort					6	1.9		
- dizziness	2	3.3	3	4.7	13	4.1	2	17
- palpitations	1	1.6	0	0.0	5	1.6		
- heavy legs	1	1.6	0	0.0	1	0.3		
- oedema of lower limbs	1	1.6	1	1.6	2	0.6		
- cough	2	3.3	0	0.0	32	10.0	1	8.0
- atypical chest pain	1	1.6	0	0.0	2	0.6	1	8.0
- dyspnoea					2	0.6	2	17
CNS and behaviour	--							
- mood and/or sleep disturbances	1	1.6	1	1.6	4.1	1.3		
- tremor	0	0.0	1	1.6				
- concentration and coordination disturbances	0	0.0	1	1.6				
ENT and opthalmology								
- tinnitus	1	1.6	1	1.6	1	0.3		
- runny nose	0	0.0	1	1.6	1	0.3		
- lacrimation	1	1.6	0	0.0				
Digestive system								
- taste disturbance	0	0.0	1	1.6				
- dry mouth	1	1.6	0	0.0	1	0.3		
- diarrhoea	3	4.9	1	1.6	7	2.2	1	8.0
- nausea	1	1.6	0	0.0	6	1.9		
- vomiting	0	0.0	1	1.6	1	0.3		
- epigastric pain	5	8.2	2	3.1	7	2.2		
- abdominal pain	1	1.6	0	0	3	0.9		
Locomotor system								
- joint pains	1	1.6	3	4.7	3	0.9		
- muscular cramps	3	4.9	0	0	5	1.6	1	8.0
General symptoms								
- tiredness	4	6.6	2	3.1				
- headache	2	3.3	2	3.1	8	2.5	1	8.0
- sweating	2	3.3	1	1.6	4	1.3		
- hot flushes	0	0.0	1	1.6	3	0.9		
- cutaneous signs	1	1.6	1	1.6	10	3.1		
- anorexia	1	1.6	0	0.0	3	0.9		
- asthenia					13	4.1	1	8.0
- angioneurotic oedema					1	0.3		

STUDIES ON ANGIOTENSIN CONVERTING ENZYME INHIBITION

CHAPTER 5: GENERAL RECURRENT METHODS

5.1. Patient and Volunteer Studies

5.1.1. Blood Pressure and heart rate measurement

During all clinical studies, whether in patients or normal volunteers, the technique of blood pressure and heart rate measurement was uniform. Data was recorded using an established (Johnson & Kerr,1985) semi-automatic device (Sentron, Bard, Sunderland UK), maintained and calibrated at regular intervals by the hospital Clinical Physics Department. This machine operates on an ocsillometric technique for indirect blood pressure and direct heart rate measurement. All subjects had supine blood pressure measurements recorded in triplicate after at least 30 minutes supine rest.

During the heart failure studies (Chapter 6) blood pressure was recorded continuously at 2 minute intervals to establish the baseline pressure, for safety reasons and to establish a trend analysis. In addition triplicate blood pressure measurements were meaned and constituted the data set used to compare drug effects.

Where relevant erect blood pressures were determined in triplicate at 1,2 and 5mins and meaned to constitute the data point.

5.1.2. Protocol and Patient Consent

All studies involving patients or human volunteers in clinical projects were the subject of detailed pre study protocols laid out in a standard format for the relevant local Research and Ethical committee. In all cases each protocol was reviewed and granted clearance prior to the conduct of the study with only minor modifications.

All human subjects whether patient or normal volunteers were screened in terms of their clinical history and physical examination as suitable for inclusion in the particular study by myself. An information sheet as to the aims and details of the procedures involved in any given study was provided and written and informed consent to participated was subsequently obtained.

5.2. Analytical Methods

5.2.1. ACE activity

The determination of angiotensin converting enzyme (ACE) activity was based on the method of Chiknas (1979). This method is based on the measurement of the formation of hippuric acid from the synthetic tripeptide substrate Hippuryl-Histidine-Leucine (Hip-His-Leu). By quantifying the amount of hippuric acid formed by the action of ACE on Hip-His-Leu an indirect index of ACE activity can be calculated. One unit of ACE activity (1 EU/l) is that which produces 1 mole hippuric acid per minute at 37^{o}C under controlled reaction conditions *in vitro*.

SOLUTIONS

100 mM potassium phosphate buffer pH 8.3 (substrate buffer)

5.705 g of K_2HPO_4 and 4.375 g NaCl were dissolved in 250 ml distilled water. 1.361 g of KH_2PO_4 was dissolved in 100 ml distilled water. The monopotassium salt was then added to the dipotassium salt until the pH reached 8.3.

5 mM Hip-His-Leu (substrate)

21.48 mg Hip-His-Leu was dissolved in 10 ml assay buffer. This was freshly made for each assay since there is a gradual degradation of the substrate to hippuric acid, the reaction product.

Internal standard

20.4 mg phthalic acid was dissolved in 20 ml methanol and made up to 100 ml with distilled water. The solution was then diluted 1:2:5 with distilled water to give the working solution (0.41 mmol/l).

Hippuric acid standards

179.2 mg of hippuric acid was added to 100 ml of distilled water, 2 ml of which was taken and made up to 20 ml with drug free plasma (5 mmol/l). A standard calibration line of hippuric acid over the range 0.05 to 1.0 mmol/l was prepared.

Mobile phase

5.44 g KH_2PO_4 (HPLC grade) was dissolved in 1.5 l distilled water and the pH adjusted to 4.0 with orthophosphoric acid. The volume was made up to 2.0 l with distilled water, 140 ml discarded and replaced with 140 ml methanol. The mobile phase was filtered through a 0.8 micron aqueous filter and finally degassed by bubbling helium through for a minimum of 10 minutes.

ANALYTICAL PROCEDURE

Procedure for standards

Into 4 ml polypropylene tubes in duplicate were placed;

a) 200 ul substrate buffer.

b) 50 ul 50%HCL (v/v). Vortex briefly.

c) 20 ul standard plasma.

d) 50 ul internal standard solution. Vortex briefly.

e) 50 mg (approximately) NaCl. Vortex briefly.

f) 1 ml ethyl acetate. Vortex for 15 seconds.

g) Centrifuge at 2000 rpm for 5 minutes.

h) Remove 500 ul of the organic layer to a clean 4 ml. polypropylene tube and concentrate under air/N_2 at 37°C.

i) When dry add 100 ul of mobile phase and vortex briefly.

j) Inject 20 ul onto HPLC system.

Procedure for unknown samples

Into 4 ml polypropylene tubes in duplicate were placed:

a) 200 ul 5 mmol Hip-His-Leu in substrate buffer.

b) 20 ul unknown plasma sample. The reaction was started by vortexing briefly.

c) Samples were incubated in a water bath at 37°C for 30 minutes.

d) Reaction stopped by the addition of 50 ul 50% HCl and vortexing.

e) Procedure as from step d) in procedure for Standards.

Quality control data

The minimum limit of quantitation was 0.2 EU/l. Using a quality control sample supplied by Sigma Chemicals the accuracy of the method was 104% whilst 10 replicate analyses of this 18 EU/l standard revealed intra- and inter-assay variability to be 2.0 and 3.3% respectively.

5.2.2. Drug concentration measurements

Drug concentrations of ester and diacid ACE inhibitors were analysed using the

standardised inhibition assay described by Tocco and colleagues (1982) and later modified by Francis (Francis et al,1987). It is based on the above assay for angiotensin converting enzyme using Hip-His-Leu as substrate and generated hippurate detected by HPLC to quantify the presence of inhibitor. The source of high activity plasma ACE is rabbit plasma. Drug concentrations are determined against known standard curves. Ester prodrug ACE inhibitor concentrations can be determined by alkaline degradation of an aliquot in addition to the determination of free diacid. Simple subtraction of diacid from total substrate inhibition allows the definition of both ester and diacid from a plasma sample where both are present.

This assay is applicable to all prodrug ACE inhibitors and was used extensively for perindopril, perindoprilat; and enalapril, enalaprilat in the studies reported here. The minimum limit of quantitation was 0.5 mg/ml. Using quality control samples revealed intra- and inter-assay variability to be in the region of 3.5 and 6% respectively for both prodrugs and diacid metabolites.

5.2.3. Plasma renin activity

Plasma renin activity was determined by quantification of the rate of angiotensin I formation using exogenous sheep angiotensinogen added to plasma samples. Angiotensin I was measured by a sensitive and specific RIA with a detection limit (in terms of renin activity) of 0.1 ngAI/ml/hr and an inter assay coefficient of variation of 7% (Derkx et al,1979)

5.2.4. Plasma aldosterone

Plasma aldosterone was kindly measured in the steroid laboratory of Dr Robert Fraser of the MRC Blood Pressure Unit at the Western Infirmary. An established RIA was employed based on a commercial kit (Aldosterone MAIA, Biodata, SPA, Italy).

Intra and inter assay coefficients of variation were 2.5 and 4.6% respectively.

5.2.5. Plasma angiotensin II

Plasma angiotensin II was kindly measured in the laboratory of Dr JJ Morton of the MRC Blood Pressure Unit at the Western Infirmary using the current technique of his own published radioimmunoassay system (Morton & Webb,1985). Coefficients of variation are 5% and 7.8% respectively for intra and inter assay comparisons. The limit of detection of the assay is 0.5 pg/ml.

5.2.6. Plasma catecholamines

Plasma concentrations of noradrenaline and adrenaline were measured using a technique based on HPLC analysis of alumina extracted plasma. Samples collected and frozen (-70^{o}C) after collection were processed following washing in acid washed alumina. They were eluted from alumina after washing with perchloric acid. Samples were separated on a reversed phase Spherisorb column after preliminary separation. Quantification was by concurrent internal standards and detection was based on an electrochemical signal system using a silver electrode (Howes et al,1985). The limits of detection for noradrenaline and adrenaline were 0.1nmol/L respectively. Inter and intra-assay coefficients of variation were 8% and 3% respectively at the time of analysis.

5.3. Statistical Methods

Blood pressure and heart rate recordings were taken in triplicate at each recording time (section 5.1). The mean of these three recordings, rounded to the nearest whole digit, was used for the statistical analysis. Where relevant mean arterial pressure was calculated from systolic and diastolic pressure using the formula MAP = DBP + (SDP - DBP)/3. All blood pressure data are expressed in mmHg and all heart rate

data in beats per minute. Absolute or baseline corrected values of heart rate, mean arterial pressure and systolic or diastolic pressures were subjected to repeated measures analyses of variance using the statistical package, RUMMAGE on an ICL 3988 mainframe computer. The model included two fixed factors (time, with variable levels and treatment with variable levels) and one random factor (patients, with 8-12 levels dependent on the individual study). Treatment time interactions were estimated using Bonferroni correction for repeated comparisons, based on the premise that the only comparisons of interest were those between treatments at any given time point, i.e. individual times within each treatment were not compared.

Where the pre-treatment values for heart rate, mean arterial pressure or rate pressure product were significantly different between treatment groups, the data were baseline corrected by subtracting the pre-treatment value from all subsequent values before being subjected to statistical analysis. Statistical significance is assumed if $p<0.05$ was achieved.

Angiotensin converting enzyme inhibition was calculated from the formula:

% inhibition = 100 x (1-ACE/pre-treatment ACE).

The graphical representation of all data is based on mean values for each treatment group at a given time point with the error bars representing one standard deviation of the mean. All graphs show times following dosing on a linear scale with the 24-hour or later recordings shown separately on the far right.

Demographic data and baseline sodium creatinine and renin values etc were compared amongst the treatment groups using a Freidman one way analysis of variance. In chapter 6 the New York Heart Association Classification of Heart Failure Severity was compared amongst the treatment groups using an analysis of variance by ranks (Kruskal-Wallis) The effect of treatment on serum creatinine and other biochemical

indices was assessed by comparing for the four treatment groups the difference between post-treatment and pre-treatment values, using one way analysis of variance.

Specific techniques applied only in certain sections are described at the appropriate point.

CHAPTER 6

ANGIOTENSIN CONVERTING ENZYME INHIBITION IN HEART FAILURE

6.1 STUDIES WITH ORAL PRODRUG ESTER ACE INHIBITORS

This section set out to compare three oral agents in an observational study using standard clinical practice for introducing ACE inhibitors in heart failure. A non-invasive, double blind, randomised, placebo-controlled, parallel group study was conducted in elderly patients with stable chronic heart failure. The two standard ACE inhibitor in U.K. practice, captopril (6.25 mg) and enalapril (2.5 mg) were to be compared with each other and with the more recent long acting prodrug ACE inhibitor, perindopril (2 mg). These drugs were compared with placebo therapy in their recommended low starting doses for heart failure on a background of diuretic withdrawal and hospital supervision. The haemodynamic and neurohormonal responses of these drugs or placebo and the drug accumulation profiles were the main features to be studied.

6.1.1. PATIENTS AND METHODS

An observational study was conducted in unselected patients (n=48) (59 - 86 years; 31:17, M:F) admitted to hospital for initiation of ACE inhibitor treatment as adjunctive therapy for the management of heart failure. Patients were symptomatic on diuretic therapy (> 80 mg frusemide or equivalent daily) and none had significant fluid imbalance. All had stable renal function and normal serum sodium status (Na $\geq$ 135 mmol) prior to treatment. The diagnosis was confirmed by symptomatic enquiry, clinical history, physical, radiological and electrocardiographic examination prior to treatment. The diagnosis had initially been established by a member of the consultant staff. The majority had impaired left ventricular function documented by echocardiographic

examination or left ventriculography. All had NYHA symptoms Grades II - IV, the majority were grade III, symptomatic on minimal exertion. The clinical details of the four patient groups are given in Table 6.1.

Diuretic therapy was withdrawn under supervision for at least 24 hours prior to ACE inhibitor treatment and in the majority this was for 48 hours. All concomitant vasoactive drugs such as calcium antagonists or nitrate preparations were withheld on the day of treatment and until after monitoring was completed at 24 hours. Digoxin, where prescribed, was continued. On the morning of treatment (≈ 0730 hours) a heparinised peripheral venous cannula was inserted for blood sampling and the patients rested undisturbed, semi-supine in bed while baseline blood pressure was recorded semi-automatically at 2 minute intervals for at least 30 minutes (Sentron, Bard, Sunderland, U.K.). Oral treatments (captopril 6.25 mg, enalapril 2.5 mg, perindopril 2 mg, lactose placebo) were administered double blind in their standard formulations within a white hard gelatin capsule prepared in accord with a randomisation schedule held by the Department of Pharmacy. All patients received their normal meals throughout the study. They were kept supine from the start of the study till 10 hours after dosing. Patients were allowed up after this but were supine for at least 45 minutes prior to sampling and blood pressure measurement at 24 hours. Following supervised oral administration blood pressure was recorded supine at 2 minute intervals with supplemental triplicate determinations at set observation points when blood samples were also drawn for the determination of drug concentration, ACE activity and plasma renin activity.

At 24 hours following dosing, blood pressure, drug concentrations, hormones, routine biochemistry and haematology were determined after a period of supine rest. The nature of therapy was then obtained from the pharmacist to allow appropriate further treatment with an oral ACE inhibitor and re-instatement of diuretics and vasodilators. Patients who had received placebo continued on diuretic withdrawal and received oral

Table 6.1: Demographic data of study patients

	PLACEBO (n=12)	CAPTOPRIL (n=12)	ENALAPRIL (n=12)	PERINDOPRIL (n=12)
Male/Female (n)	11:1	8:4	6:6	6:6
Age (Mean±1SD)	68.2±5.7	67.8±5.6	65.9±7.3	69.2 ± 7.9
NYHA Class				
II	3	6	4	3
III	9	5	8	9
IV	-	1	-	-
Aetiology of Heart Failure				
Ischaemic heart disease	6	7	6	4
Alcohol related cardiomyopathy (CM)	1	-	-	-
Dilated CM	1	-	3	3
Valvular disease	-	1	-	-
Combinations	4	4	3	5
Atrial fibrillation	2	2	-	3

captopril 6.25 mg as open therapy under normal nursing observation in hospital. These patients were **not** included in any subsequent active treatment group.

6.1.2 RESULTS

A. General

Each of the parallel groups contained patients of a similar age distribution, NYHA class, pretreatment serum sodium, creatinine and plasma renin activity (Table 6.2). No patient enrolled in the study experienced significant symptoms during the study day or in the preceding period of diuretic withdrawal prior to treatment. All left hospital after initiation of therapy adjustment of diuretic dosage and other drugs without ill effect. No treatment group showed a significant alteration in serum sodium, potassium, urea or creatinine 24 hours after study. In all cases there was the expected small fall in haemoglobin associated with venesection which was well tolerated. The individual changes in pre- and post-treatment laboratory indices are given in Table 6.3.

The pretreatment baseline blood pressure and heart rate values were not equal between the groups. Baseline mean arterial pressure (MAP) was significantly higher ($p < 0.05$) in the group who received enalapril (106.4±11.6 mmHg) than those who received captopril (98.4±10.8), perindopril (98.2±11.0) or placebo (100.3±12.8). In addition the baseline supine heart rate was significantly lower ($p < 0.05$) in the group who were treated with captopril (75.9±9.8 bts/min) than those who received enalapril (83.3±15.9), perindopril (77.1±10.4) or placebo (81.1±12.8). For these reasons the temporal pattern of response is described as the change from individual baseline pressures and heart rate rather than absolute values. Baseline values are meaned from the 2minute recordings recorded during the pretreatment period of 30-60 minutes rest.

Table 6.2:Summary of comparisons of pre and post treatment laboratory data, age and NYHA class between treatment groups

	PLACEBO	CAPTOPRIL	ENALAPRIL	PERINDOPRIL	ANOVA P=
Age	-68.2±5.7	67.8±5.6	65.9±7.3	69.2±7.9	0.677
Na^+	138.9±2.5	141.2±3.4	140.3±2.7	140.8±2.4	0.220
ΔNa^+	-0.2±2.8	+0.1±2.3	-0.5±2.1	+0.3±1.2	0.794
K^+	4.1±0.6	4.2±0.3	4.2±0.6	3.9±0.6	0.407
ΔK^+	+0.02±0.6	-0.15±0.4	+0.05±0.6	+0.08±0.4	0.684
Urea	8.5±5.9	6.3±1.7	7.2±2.5	8.6±3.2	0.380
ΔUrea	-1.4±4.0	-0.1±1.1	-0.4±1.3	-0.4±1.8	0.570
Creat	107±15	98±22	102±29	122±36	0.150
ΔCreat	-0.6±19	-4.0±12	-8.2±16	-6.6±15	0.651
Hb	14.4±1.3	13.5±1.4	14.0±1.2	13.8±1.1	0.408
ΔHb	-1.2±0.6	-1.2±0.5	-1.8±0.5	-1.2±0.5	0.016
NYHA Class (Average rank)	26.7	21.3	24.0	26.0	0.681 (Kruskal-Wallis)

B.Haemodynamic effects

Heart rate

Only minor changes in heart rate were seen with any treatment, the greatest being a fall of 10.8 beats per minute 8 hours after dosing with enalapril. No reflex tachycardia was encountered with any treatment. The mean heart rate difference from baseline was -2.62 beats per minute for the placebo group, -2.96 beats per minute for the captopril group, -6.77 beats per minute for the enalapril group and -1.06 beats per minute for the perindopril group ($P = 0.034$). The mean heart rate was,therefore, lower for the enalapril group than all other groups and was higher in the perindopril group than in the captopril group. From the analysis of variance, the probability of a treatment time interaction was $P < 0.001$. Heart rate differences at individual time points were mainly apparent for the enalapril group. The heart rate in the enalapril group was lower than placebo from 1 hour to 8 hours after dosing. It was lower than the captopril group from 20 minutes until 24 hours after dosing and lower than the perindopril group from 20 minutes until 24 hours after dosing (Figure 6.1).

Mean arterial pressure

Mean arterial pressure fell by 10 mmHg from baseline, 4.5 hours after placebo. One and a half hours after captopril, mean arterial pressure had fallen by 17.7 mmHg from baseline. The fall in MAP after enalapril did not occur until 5 hours post-dosing and reached -23.2 mmHg. The lowest blood pressure after perindopril was seen 7.5 hours after dosing at -8.9 mmHg, which was higher than the placebo value at that time, and with a similar difference from placebo at 24 hours post-dosing. Analysis of Variance on the baseline corrected mean arterial pressure data revealed a significant mean effect of treatment ($P = 0.022$) and significant treatment time interaction ($P < 0.001$). The mean values for the 28 recordings in each treatment group were as follows:-

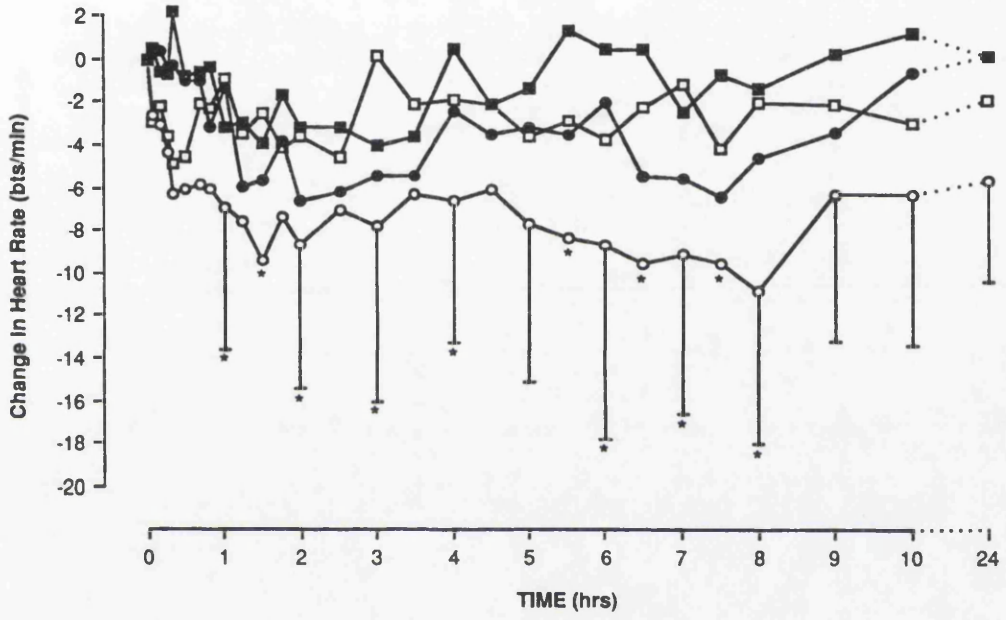


Figure 6.1: The baseline corrected mean effect (±1SD) of oral placebo (□), captopril 6.25mg (●), enalapril 2.5mg (○),or perindopril 2mg (■) on supine heart rate in patients with chronic cardiac failure.

Captopril - 8.82 mmHg

Enalapril -11.68 mmHg

Perindopril - 4.08 mmHg

Thus, the mean figures for captopril and enalapril were lower than for placebo or perindopril and the mean figure for enalapril was lower than that for captopril.

The comparisons at individual time points revealed the following:- The mean arterial pressure following captopril was lower than that after placebo from 40 minutes until 3 hours after dosing. Mean arterial pressure after enalapril was lower from 2.5 hours until 10 hours after dosing. The mean arterial pressure after captopril was lower than after perindopril from 15 minutes until 4 hours after dosing and was lower after captopril than enalapril from 50 minutes until 105 minutes after dosing. This reversed from 4 hours after dosing, however, with mean arterial pressure then being lower after enalapril than after captopril and this difference persisted until 10 hours after dosing. The mean arterial pressure after enalapril was lower than that after perindopril at 50 minutes and from 3 hours until 10 hours after dosing (Figure 6.2).

C. Drug concentration data and plasma hormonal responses

Sample collection procedures in a double blind study did not allow for the special preparations required in order to undertake collections for determination of captopril drug levels or measurement of ACE activity in these specimens.

Drug Concentrations

The pharmacokinetic profiles for enalapril and perindopril groups are illustrated in Figure 6.3. Concentrations of both parent ester and active diacid metabolite were higher for enalapril than perindopril. The mean (± 1 SD) time to peak ester concentrations (t_{max}) were 1.95 (±0.7) hrs for enalapril and 1.74 (±0.7) hrs for perindopril. The mean time to peak for

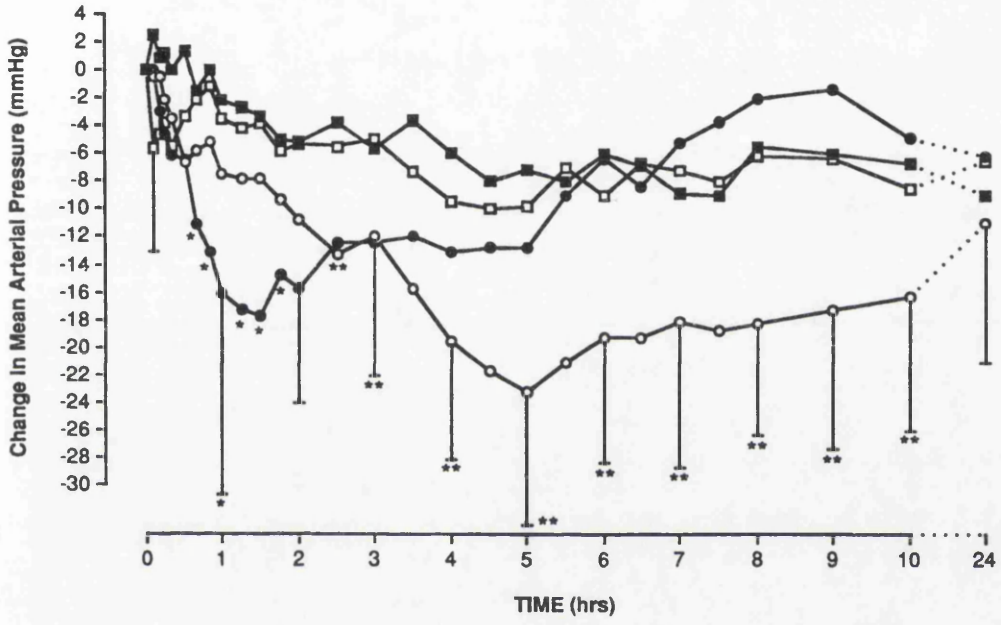


Figure 6.2: The baseline corrected mean effect (±1SD) of oral placebo (□), captopril 6.25 mg (●), enalapril 2.5 mg (○), or perindopril 2 mg (■) on supine mean arterial pressure in patients with chronic cardiac failure.

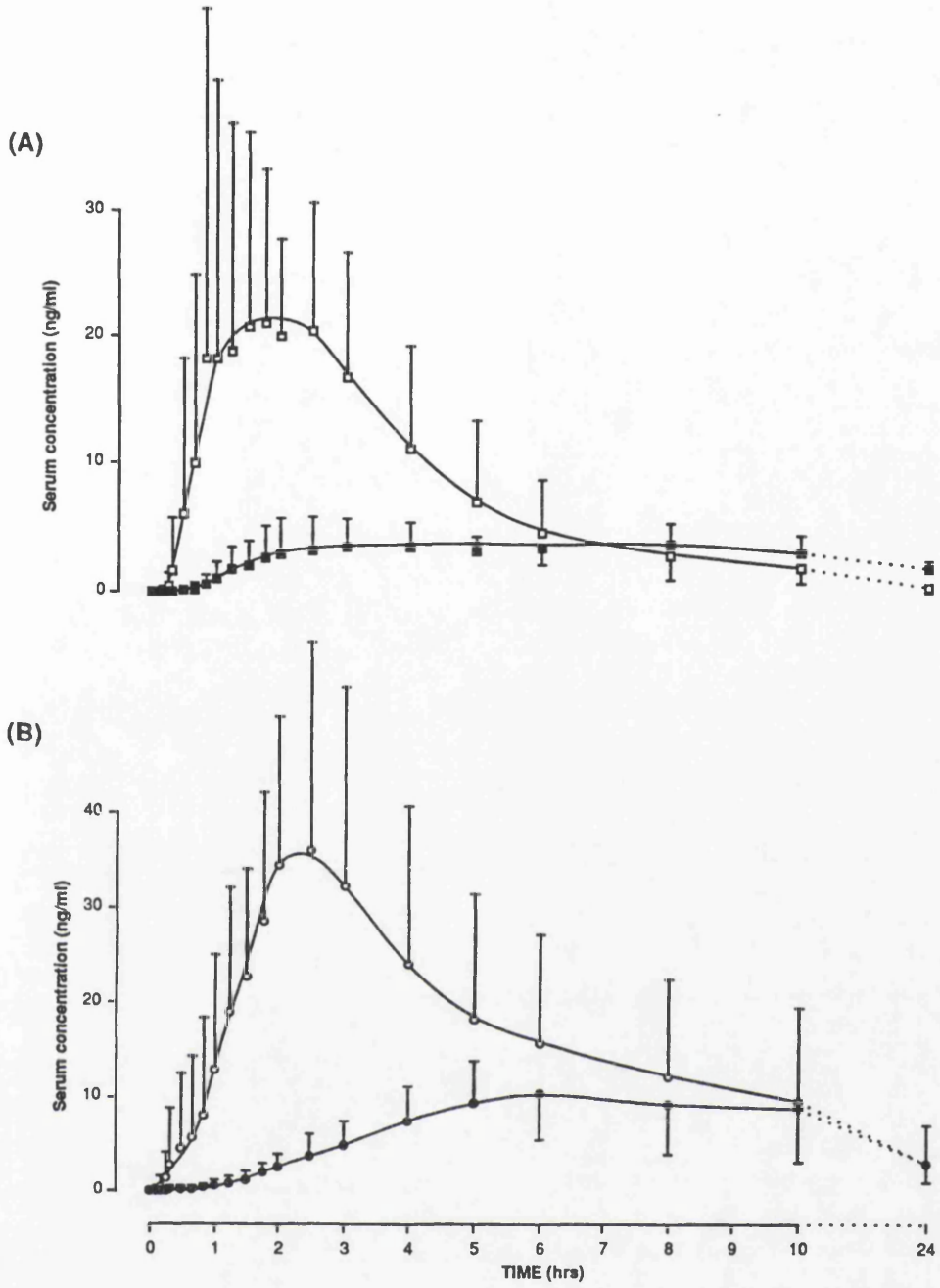


Figure 6.3: Mean serum concentrations (±1SD) of perindopril (□) and perindoprilat (■) (a) or enalapril (○) and enalaprilat (●) (b) following oral therapy in patients with chronic cardiac failure.

was the difference in t_{max} for either ester ($p = 0.684$) or diacid ($p = 0.229$) significantly different between the treatment groups (Kruskal-Wallis ANOVA). Generally speaking both treatments were associated with prolonged plateau concentrations of the active diacid.

ACE Inhibition

The analysis of variance for angiotensin converting enzyme inhibition confirmed a significant main effect of treatment between the enalapril and perindopril groups ($P = 0.002$) and significant treatment time interaction for these groups ($P < 0.001$).

Plasma ACE activity was significantly reduced in both perindopril and enalapril groups with respect to placebo (Figure 6.4). Maximal ACE inhibition was similar for both active treatment groups (enalapril 63%; perindopril 68%) at 8 hours post-dosing. The onset of inhibition was earlier with perindopril. Inhibition was significantly greater between 50 mins and 3 hours after perindopril compared to enalapril. ACE inhibition was also significantly greater at 24 hours post-dosing after perindopril.

From the ANOVA the mean ACE inhibition over the 28 recording times for perindopril was 30% and for enalapril was 23%. There were differences between the two treatments at individual recording times from 50 minutes until 3 hours and also at 24 hours. In each case, perindopril showed the greater inhibition of ACE.

Plasma Renin activity

Pretreatment plasma renin activity (geometric mean, 95% confidence limits) after 24-48 hr diuretic withdrawal was similar in all groups ($p = 0.183$, one way ANOVA) ; captopril group (1.1; 0.2, 3.8 AI/ml/hr) compared with placebo (3.2; 0.5, 11.7), enalapril (1.7; 0.5, 44.7) and perindopril (2.5; 0.2, 11.9). There was no significant difference ($p = 0.222$ Kruskal Wallis ANOVA) in the prestudy dose of diuretic drugs between those patients who received captopril

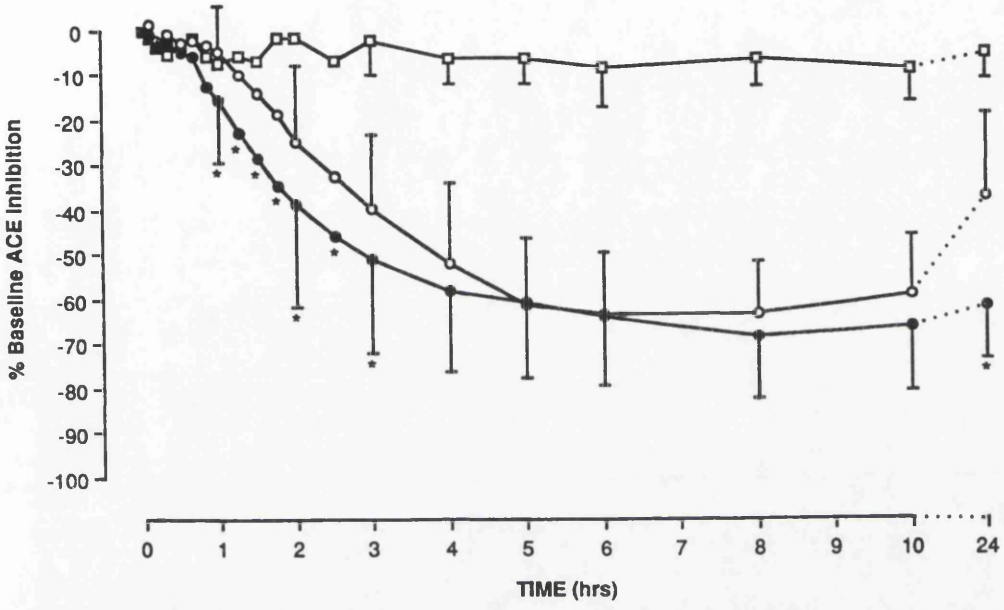


Figure 6.4: Plasma ACE inhibition (% baseline activity EU/ml, mean±1SD) following placebo (□), enalapril (○) or perindopril (●).

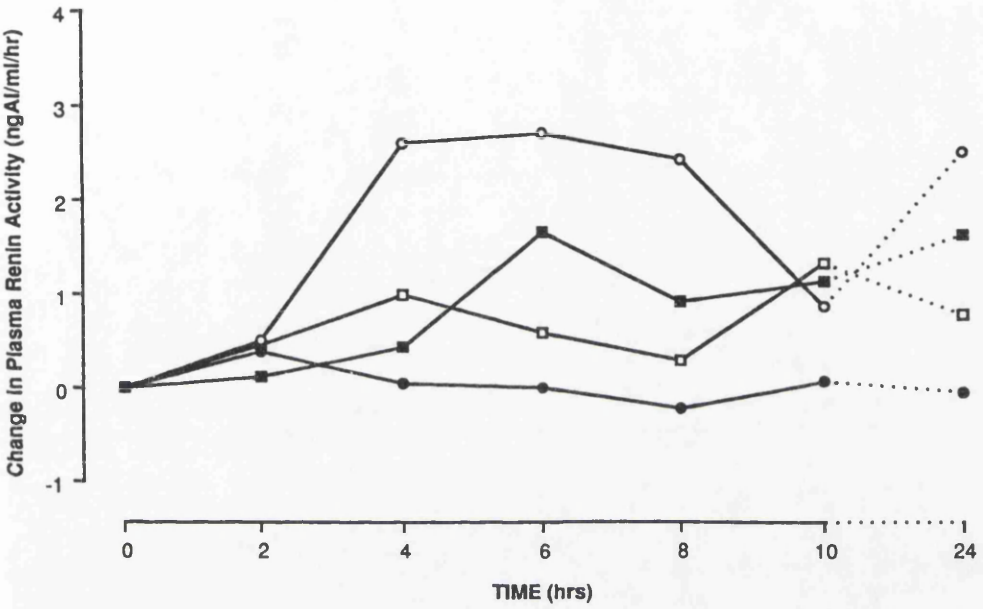


Figure 6.5: Plasma renin activity following oral placebo (□), captopril (●), enalapril (○) or perindopril (■): geometric mean values are illustrated.

All three active treatments produced elevations in PRA. The early rise associated with captopril was not marked which related to the initial sampling point at 2hrs after dosing. Later rises were associated with enalapril and perindopril (Figure 6.5).

6.2 STUDIES WITH INTRAVENOUS DIACID ACE INHIBITORS

In this section a comparison was made of the diacid metabolites enalaprilat and perindoprilat administered by intravenous infusion in a non-invasive, double blind, randomised, placebo-controlled, parallel group study in elderly patients with stable heart failure. Perindoprilat (1 mg) and enalaprilat (1.5 mg) were given in total doses which broadly approximated, on the basis of prior bioavailability, to the oral doses of perindopril (2 mg) and enalapril (2.5 mg) used in our parallel oral studies (see section 6.1). Both active drugs and placebo (saline) were prepared in normal saline (30 ml) and infused at a constant rate of 5 ml/hr over 6 hours. The haemodynamic and neurohormonal responses of these drugs or placebo and the drug accumulation profiles were the main features to be studied.

6.2.1 PATIENTS AND METHODS

This study was conducted in patients (n=36) (60-87 years; 24:12, M:F) admitted to hospital for initiation of ACE inhibitor as adjunctive therapy for the management of heart failure. The placebo group was common to section 6.1. Patients were selected in an identical fashion to the section on oral ACE inhibitor treatments. As before all symptomatic on diuretic therapy (> 80 mg frusemide or equivalent daily) and none had significant fluid imbalance. The clinical details of the three patient groups are given in Table 6.3; 6.4; & 6.5.

All diuretic therapy was again withdrawn under supervision for at least 24 hours prior to ACE inhibitor treatment and in the majority this was for 48 hours. All concomitant vasoactive

Table 6.3: PATIENT DEMOGRAPHY - PLACEBO THERAPY

STUDY NO.	SUBJT INITL	AGE	SEX	AETIOLOGY	NYHA CLASS	CARDIAC RHYTHM	DURATION OF INFUSION
3	JM	69	M	H/ACM	III	AF	6 HOURS
4	EW	60	M	IHD	III	S	6 HOURS
5	DR	76	M	ACM	III	S	6 HOURS
11	GH	67	M	H/IHD	II-III	S	6 HOURS
17	JB	74	M	IHD/MR	III	AF	6 HOURS
22	AJ	74	M	IHD	III	S	6 HOURS
37	TM	74	M	IHD	III	S	6 HOURS
41	JG	64	M	IHD	III	S	6 HOURS
55	DR	61	M	H/IHD	III	S	6 HOURS
62	WB	67	M	IHD	II	S	6 HOURS
66	JW	71	F	DCM	III	S	6 HOURS
27	AM	61	M	IHD	II	S	6 HOURS

IHD - Ischaemic heart disease
H - Hypertensive heart disease
ACM - Alcohol related cardiomyopathy
DCM - Dilated cardiomyopathy
MR - Mitral regurgitation
S - Sinus rhythm

AF - Atrial fibrillation

Table 6.4:PATIENT DEMOGRAPHY - ENALAPRILAT 1.5 mg

STUDY NO.	SUBJT INITL	AGE	SEX	AETIOLOGY	NYHA CLASS	CARDIAC RHYTHM	DURATION INFUSION	DOSE (mg)
13	EMcM	72	F	DCM	III	AF	6 HOURS	1.5
15	MMcD	71	F	IHD/H	III	AF	6 HOURS	1.5
19	AB	74	F	IHD	III	S	6 HOURS	1.5
23	JD	60	M	DCM	III	S	6 HOURS	1.5
28	PW	73	M	ACM	III	S	2 HOURS	0.5
32	MF	76	F	IHD	III	S	2 HOURS	0.5
39	CT	60	M	ACM/MR	II	AF	6 HOURS	1.5
40	MM	77	F	MR	III	AF	6 HOURS	1.5
46	JM	77	F	CP	III	S	3 HOURS	0.75
59	JG	70	M	IHD/H	III	S	4 HOURS	1.0
63	JB	66	M	IHD	II	S	6 HOURS	1.5
70	AK	63	M	DCM/MR	III	S	5 HOURS	1.5
						MEAN	4.8	1.2
						± SD	1.6	0.4

IHD - Ischaemic heart disease
H - Hypertensive heart disease
ACM - Alcohol related cardiomyopathy
DCM - Dilated cardiomyopathy
MR - Mitral regurgitation
S - Sinus rhythm
AF - Atrial fibrillation

CP - Cor Pulmonale

Table 6.5:PATIENT DEMOGRAPHY - PERINDOPRILAT 1 mg

STUDY NO.	SUBJT INITL	AGE	SEX	AETIOLOGY	NYHA CLASS	CARDIAC RHYTHM	DURATION INFUSION	DOSE (mg)
16	RW	64	M	DCM	III	S	6 HOURS	1
20	AW	84	F	IHD	II	AF	2.5 HOURS	0.42
26	MM	87	F	IHD	III	AF	6 HOURS	1
30	IB	62	F	IHD	III	AF	6 HOURS	1
34	JK	65	M	IHD	II	S	6 HOURS	1
35	HT	68	M	IHD	II	S	5 HOURS	0.83
43	JK	64	M	IHD/H	II	S	6 HOURS	1
45	JL	71	F	CP	III	S	5 HOURS	0.83
47	JM	63	M	IHD/CP	III	S	5 HOURS	0.83
52	LMcC	60	F	DCM	III	S	4 HOURS	0.67
65	TD	71	M	DCM/IHD	II	S	6 HOURS	1
72	JG	73	M	DCM/IHD/MR	III	S/FVPC	6 HOURS	1
						MEAN	5.3	0.88
						± SD	1.1	0.18

IHD - Ischaemic heart disease
H - Hypertensive heart disease
ACM - Alcohol related cardiomyopathy
DCM - Dilated cardiomyopathy
MR - Mitral regurgitation
S - Sinus rhythm
AF - Atrial fibrillation
CP - Cor Pulmonale
FVPC - Frequent premature ventricular complexes

drugs were withheld on the day of treatment and until after monitoring was completed at 24 hours.

On the morning of treatment (≈ 0730 hours) two heparinised peripheral venous cannulae were inserted for blood sampling and infusion purposes in contralateral antecubital fossae. The patient then rested undisturbed, semi-supine in bed while baseline blood pressure was recorded semi-automatically at 2 minute intervals for at least 30-60 minutes (Sentron, Bard, Sunderland, U.K.).Infusions (saline placebo, enalaprilat 1.5 mg; perindoprilat 1 mg) were administered double blind in accord a randomisation schedule held by the Department of Pharmacy. All patients received their normal meals throughout the study. They were kept supine from the start of the study till 10 hours after the start of infusion.

The protocol allowed early termination of the infusion under double blind conditions while the observations of blood pressure and sampling strategy continued, on the basis of a blood pressure fall to 70% of the MAP established during the baseline determinations prior to the start of infusion (2 minute recordings over 30-60minutes). This step was included in the protocol in order to limit potentially damaging falls in blood pressure. In addition it allowed us to test whether low dose constant rate infusion of a diacid ACE inhibitor was a readily controllable means of drug administration.

Patients were allowed up after the initial 10 hour observations but were supine for at least 45 minutes prior to sampling and blood pressure measurement at 24 hours. As before following initiation of the infusion blood pressure was recorded supine at 2 minute intervals with supplemental triplicate determinations at set observation points when blood samples were also drawn for the determination of drug concentration, ACE activity and plasma renin activity.

At 24 hours following dosing, blood pressure, drug concentrations, hormones, routine biochemistry and haematology were determined after a period of supine rest.

Again treatment coding was then obtained from the pharmacist to allow appropriate further management with an oral ACE inhibitor etc. Patients who had received placebo continued on diuretic withdrawal and received oral captopril 6.25 mg as open therapy and were not subsequently included in any other study group.

6.2.2 RESULTS

A. General

The three randomised parallel groups had comparable demographic profiles in terms of patient age, clinical severity of heart failure (by NYHA grading), pre-treatment diuretic dosage and plasma renin activity. The pre-study laboratory tests of serum electrolytes were similarly equally balanced (Table 6.6). Laboratory indices were not significantly influenced by acute ACE inhibitor with the exception of haemoglobin concentration (Table 6.6). The small fall documented in all three groups was similar ($p = 0.930$) and is attributable to the blood sampling protocol employed in this and previous studies.

All patients tolerated therapy well and no symptoms were reported. Two patients receiving active therapy (1, perindoprilat; 1, enalaprilat) showed transient elevation of serum urea and creatinine values (at 24 hrs) following therapy indicative of a decline in renal function. This pattern required discontinuation of maintenance oral enalapril (5 mg b.d.s.). Only moderate biochemical changes occurred and these rapidly reversed on discontinuation of oral ACE inhibitor therapy (Figures 6.6 and 6.7). Both patients remained asymptomatic throughout.

B. Haemodynamic effects

The group mean absolute blood pressure and heart rate values prior to therapy are illustrated in Table 6.7. These were not significantly different from each other ($p >$

Table 6.6: Comparison of demographic and laboratory parameters for the three treatment groups

	PLACEBO	ENALAPRILAT	PERINDOPRILAT	P = (ANOVA)
Age (yrs)	68±6	70±6	69±6	0.822
PRA (geom mean)	3.9	2.3	2.2	0.681
Frusemide (mg/day)	110±61	100±41	83±30	0.493
Na^+ ($mmol.l^{-1}$)	138.9±2.4	140.6±2.3	140.3±3.9	0.582
ΔNa^+ ($mmol.l^{-1}$)	-0.3±2.6	-0.9±2.8	+0.0±2.7	0.718
K^+ ($mmol.l^{-1}$)	4.1±0.6	4.0±0.4	4.3±0.4	0.363
ΔK^+ ($mmol.l^{-1}$)	0.0±0.6	0.0±0.4	-0.2±0.2	0.604
Urea ($mmol.l^{-1}$)	8.5±5.6	8.2±2.8	8.0±3.3	0.958
ΔUrea ($mmol.l^{-1}$)	-1.4±3.8	-0.3±2.1	-1.2±2.0	0.661
Creatinine ($\mu mol.l^{-1}$)	106.7±14	108.3±27	101.3±24	0.750
ΔCreatinine ($\mu mol.l^{-1}$)	-0.6±18	8.2±57.9	1.3±13.9	0.836
Hb (g/dl)	14.4±1.2	13.7±2	13.7±1.9	0.576
ΔHb (g/dl)	-1.3±0.5	-1.2±0.7	-1.3±0.8	0.004
NYHA Class (Average Rank)	19	20.5	16	0.390 (Kruskal-Wallis)

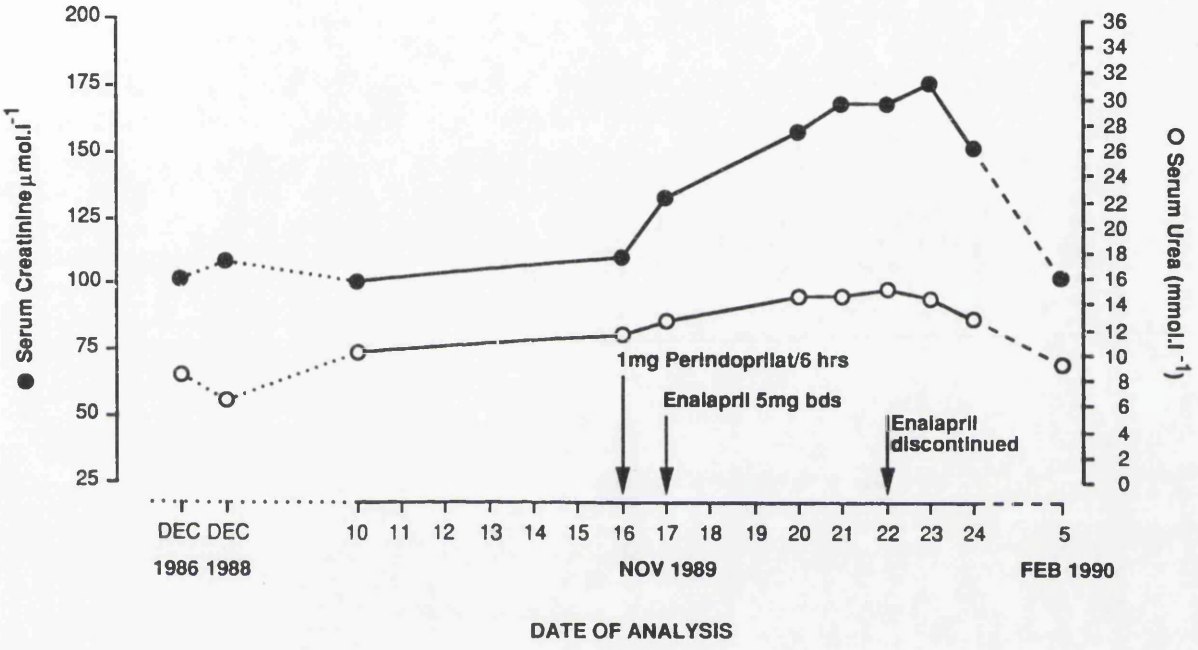


Figure 6.6: Individual pattern of renal biochemistry in subject JL (study no 45) receiving perindoprilat infusion (1mg over 6hrs) and subsequent oral enalapril (5mg bds).

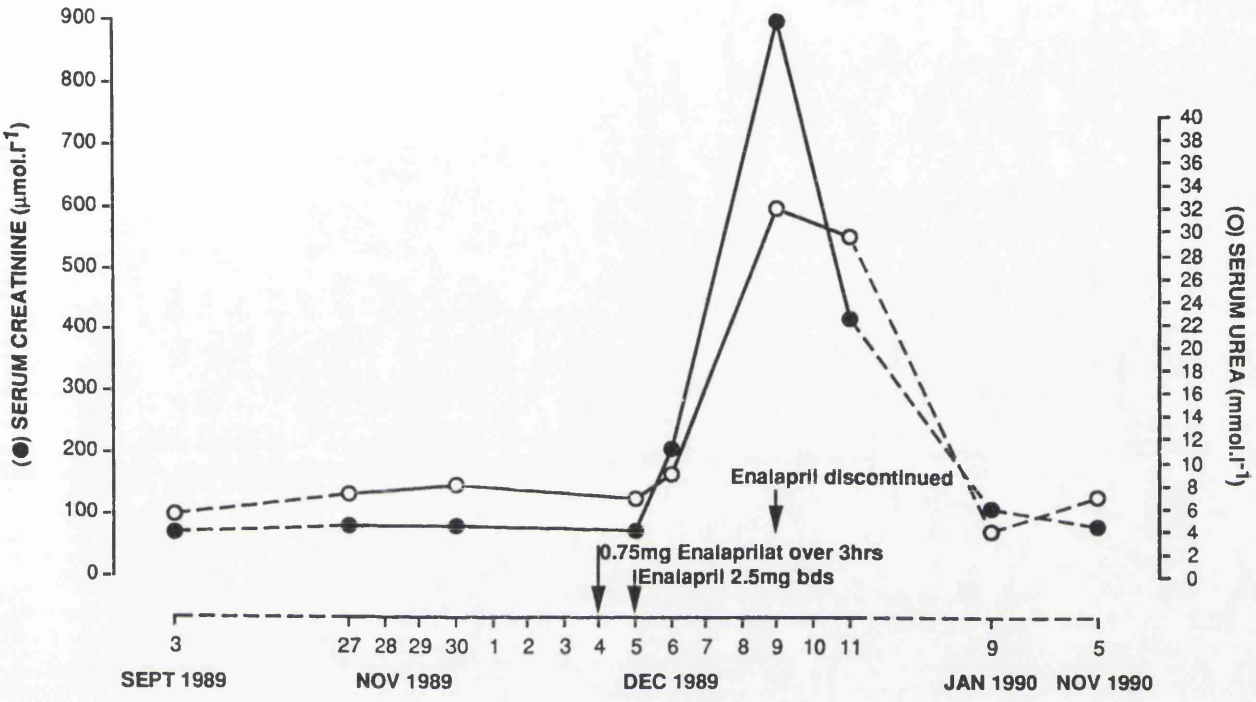


Figure 6.7: Individual pattern of renal biochemistry in patient JMa (study no 46) receiving enalaprilat infusion (0.75mg over 3hrs) and subsequent oral enalapril (2.5mg bds).

Table 6.7: Absolute starting values (group mean ± 1SD) in each of the three randomised parallel groups (n=12).

GROUP	HEART RATE (bpm)	SYSTOLIC (mmHg)	DIASTOLIC (mmHg)	MEAN ARTERIAL PRESSURE (mmHg)
Placebo	81.0±13	139.6±18	80.6±13	100.3±13
Enalaprilat	74.3±8	124.5±30	76.9±11	99.1±14
Perindoprilat	78.5±14	128.4±14	72.1±11	89.1±10

ANOVA, p = NS; Placebo vs Enalaprilat vs Perindoprilat all parameters.

0.05). However, as both active treatment groups tended to have lower starting blood pressures than the placebo and in view of the initial pattern of blood pressure changes results are illustrated as changes from the values established as baseline as in the previous section.

Heart rate

In absolute terms heart rate was significantly less in the enalaprilat group than in **Table 6.6:** Summary of statistical comparisons of demographic and laboratory data between groupsthe placebo group from 40 min - 8 hr (excepting 50 min and 4 hrs) and at 24 hrs. perindoprilat treated group had a lower absolute heart rate than placebo between 6.5 -> 8 hrs and at 3 and 4.5 hrs. There were no significant differences between enalaprilat and perindoprilat. The overall magnitude of the changes was small and comparable to our previous observations with low dose oral enalapril. There were no significant differences between either active group or placebo when the results were corrected to baseline pretreatment values (Figure 6.8).

Blood pressure

No placebo infusions were terminated. In each of the active treatment groups five infusions were terminated prior to the intended completion of dosing at 6 hrs: in the enalaprilat group at 2, 2, 3, 4 and 5 hrs; and in the perindoprilat group at 2.5, 4, 5, 5 and 5 hrs. The mean total dose of enalaprilat was therefore 1.2±0.4 mg and for perindoprilat was 0.88±0.18 mg. The mean responses beyond 2 hrs therefore do not reflect continuing infusion in all cases.

Baseline corrected blood pressure values also revealed an early transient rise in BP in both active treatment groups. This was significantly greater than placebo in the perindoprilat group at the 10 and 20 min time points (Figure 6.9).

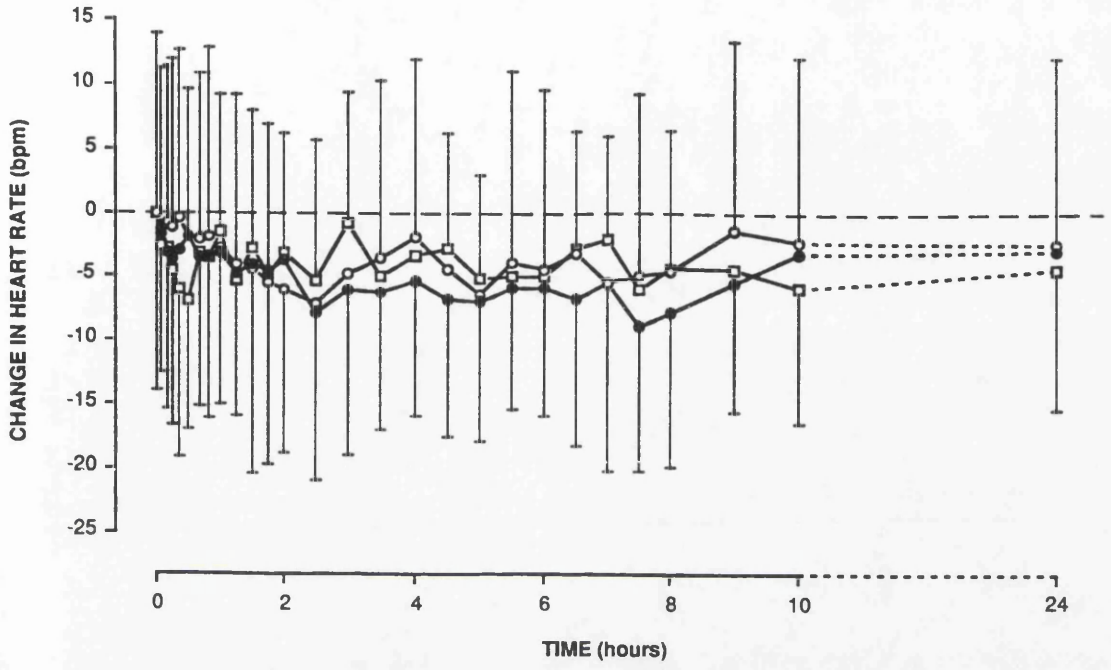


Figure 6.8: Mean baseline corrected change in heart rate (±1SD) following intravenous placebo (□), enalaprilat 1.5mg (○) or perindoprilat 1mg (●) in patients with chronic cardiac failure.

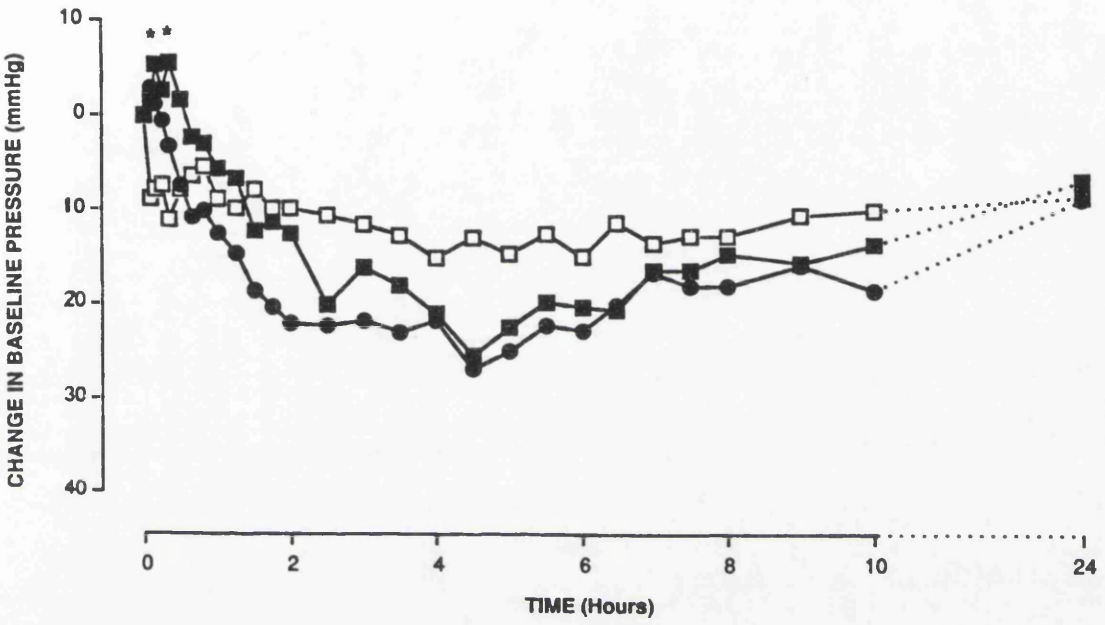


Figure 6.9: Mean baseline corrected change in supine systolic blood pressure (±1 SD) following intravenous placebo (□), enalaprilat 1.5mg (●) or perindoprilat 1mg (■) in patients with chronic cardiac failure.

Table 6.8: Individual maximal change from baseline of mean arterial pressure with time recorded and duration of infusion in each of the three treatment groups.

Case	PLACEBO MAP (mmHg)	Time (hr)	Infusion (hr)	ENALAPRILAT MAP (mmHg)	Time (hr)	Infusion (hr)	PERINDOPRILAT MAP (mmHg)	Time (hr)	Infusion (hr)
1	-4	0.33	6	-15	0.67	6	-9	4	6
2	-16	4.5	6	-23	3.5	6	-29	2.5	2.5
3	-8	6	6	-15	5.5	6	-30	5	5
4	-27	5.5	6	-19	4.5	6	-29	4.5	6
5	-17	4	6	-21	2	2	-47	4.5	2.5
6	-8	0.33	6	-19	2.5	2	-18	2.5	6
7	-10	2	6	-33	6	6	-11	4.5	6
8	-16	4	6	-38	2.5	6	-25	4	6
9	-12	4.5	6	-30	3.5	3	-29	4.5	5
10	-32	5	6	-45	5	4	-23	4.5	4
11	-10	7.5	6	-25	4.5	6	-15	4.5	6
12	-24	0.08	6	-40	4.5	5	-27	6	6

During subsequent infusion blood pressure fell in both active treatment groups (Figure 6.9). This was statistically significant for the enalaprilat group between 1.75 - 6.5 hrs (excepting 4 hrs) and for perindoprilat (4hrs) for SBP compared to placebo (Figure 6.9). A similar pattern was observed for DBP in the enalaprilat group (2.5, 3, 3.5, 4.5, 5.5, 7 hr) and the perindoprilat group (5.5 hr) compared to placebo.

The mean maximum recorded fall in SBP from baseline was -27±8 mmHg for placebo, -38.8±11 for enalaprilat and -34.9±12 for perindoprilat ($p = 0.046$).

The relationship between individual maximal recorded fall in MAP, the corresponding time, and the duration of infusion for all three treatment groups is illustrated in Table 6.8. The maximal mean fall in MAP was -10.4 mmHg at 6 hours during placebo infusion, -22.3 mmHg at 5 hours during enalaprilat infusion and -18.6 mmHg at 4.5 hours during perindoprilat infusion. Early termination of infusion successfully controlled the blood pressure fall. The minimum MAP always followed cessation of treatment by at most 15-30 minutes.

C. Drug concentration data and plasma hormonal responses

Drug concentrations

Drug concentrations of enalaprilat and perindoprilat are illustrated in figure 6.10. They include the data from the infusions discontinued prior to 6 hours. The mean time to peak for enalaprilat concentrations was 4.8±1.6 hr and for perindoprilat was 5.3±1.1 hr and as expected the drug concentrations were in each case maximal at the end of the individual infusions. Both treatments were associated with a sigmoid shaped drug concentration profile during infusion which has previously been reported (Lees et al, 1989). A sigmoid accumulation is apparent during constant rate infusion. This phase, before 2 hours is unaffected by discontinuation of drug infusions (always >2 hours).

Table 6.8: Individual maximal change from baseline of mean arterial pressure with time recorded and duration of infusion in each of the three treatment groups.

Case	PLACEBO MAP (mmHg)	Time (hr)	Infusion (hr)	ENALAPRILAT MAP (mmHg)	Time (hr)	Infusion (hr)	PERINDOPRILAT MAP (mmHg)	Time (hr)	Infusion (hr)
1	-4	0.33	6	-15	0.67	6	-9	4	6
2	-16	4.5	6	-23	3.5	6	-29	2.5	2.5
3	-8	6	6	-15	5.5	6	-30	5	5
4	-27	5.5	6	-19	4.5	6	-29	4.5	6
5	-17	4	6	-21	2	2	-47	4.5	2.5
6	-8	0.33	6	-19	2.5	2	-18	2.5	6
7	-10	2	6	-33	6	6	-11	4.5	6
8	-16	4	6	-38	2.5	6	-25	4	6
9`	-12	4.5	6	-30	3.5	3	-29	4.5	5
10	-32	5	6	-45	5	4	-23	4.5	4
11	-10	7.5	6	-25	4.5	6	-15	4.5	6
12	-24	0.08	6	-40	4.5	5	-27	6	6

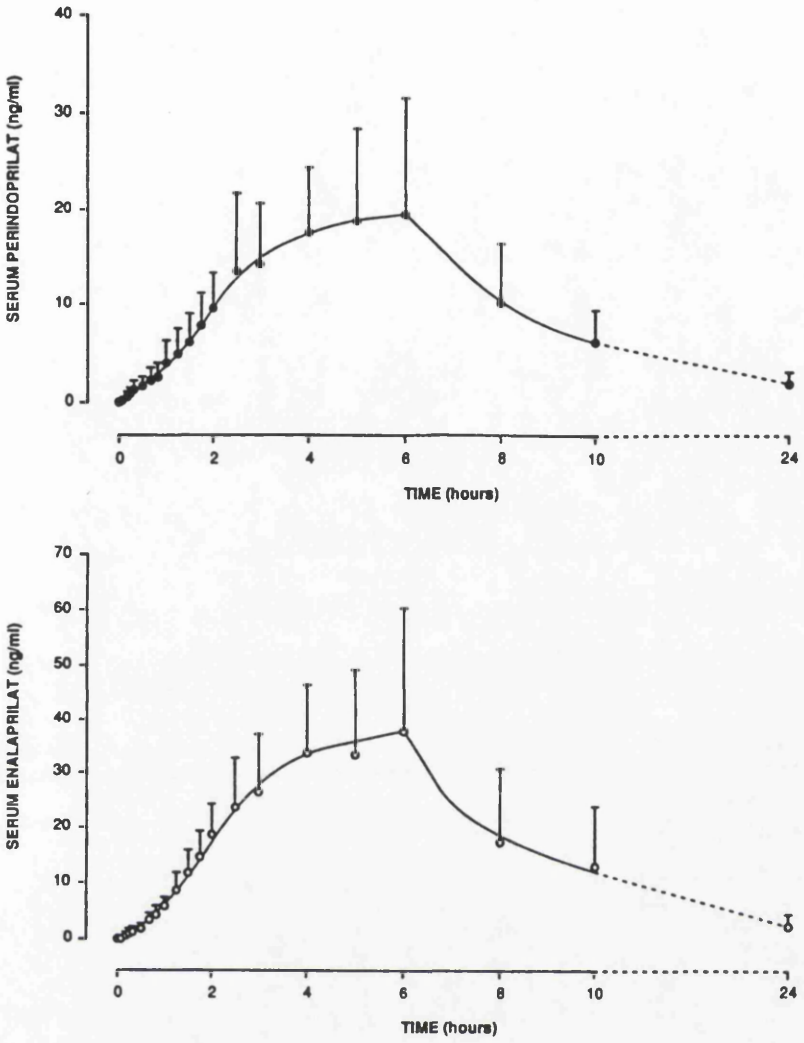


Figure 6.10: Serum drug concentrations (±1SD) following intravenous enalaprilat 1.5mg (○) or perindoprilat 1mg (●) in patients with chronic cardiac failure.

Plasma ACE inhibition

The profile of plasma ACE inhibition for both active treatments was similar showing a rapid onset and sustained plateau of inhibition (Figure 6.11). The mean maximal % inhibition of plasma ACE activity was significantly higher ($p = 0.004$) for the perindoprilat group (91.7±3%) compared to the enalaprilat group (86.5±4%). Mean maximal ACE inhibition across the whole group was achieved at 4.5±1.3 hrs in the perindoprilat group and 5.0±1.2 hrs in the enalaprilat group.

Plasma Renin Activity

Plasma renin activity was significantly elevated by both active treatments compared with placebo (Figure 6.12) and remained elevated after discontinuation of infusion.

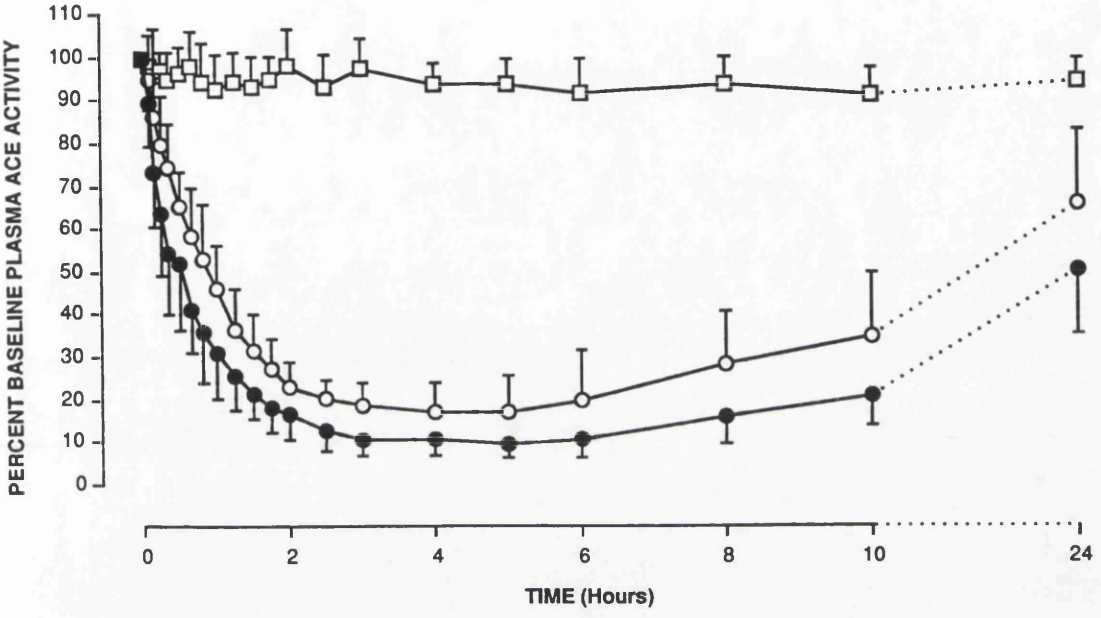


Figure 6.11: Plasma ACE inhibition (±1 SD) following intravenous placebo (□), enalaprilat 1.5mg (○) or perindoprilat 1mg (●) in patients with chronic cardiac failure.

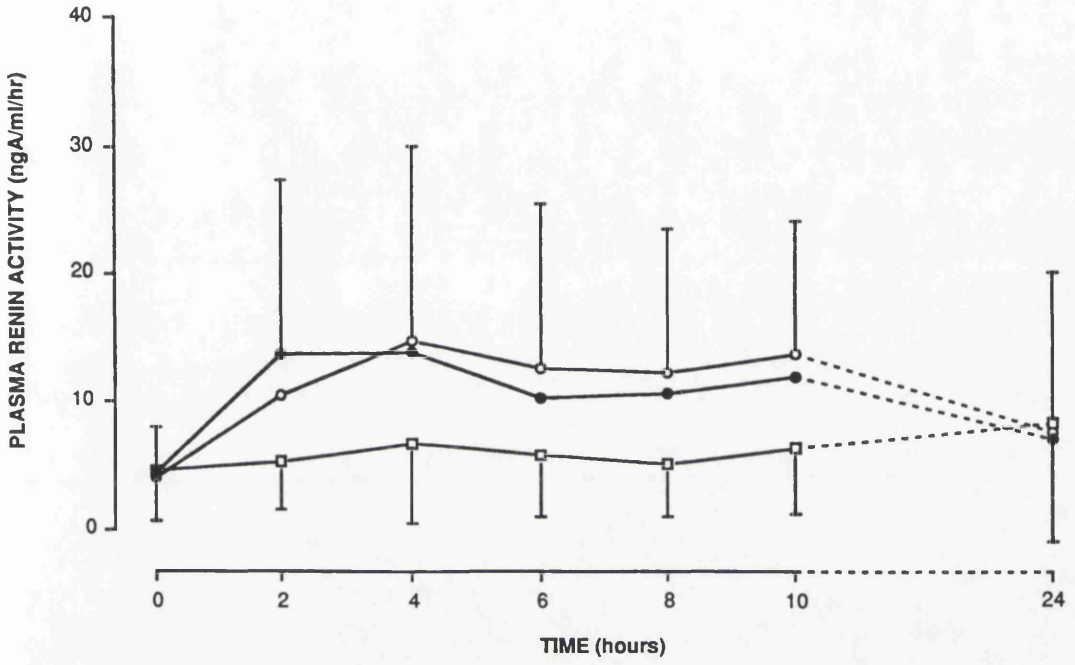


Figure 6.12: Plasma renin activity (±1 SD) following intravenous placebo (□), enalaprilat 1.5mg (○) or perindoprilat 1mg (●) in patients with chronic cardiac failure.

6.3.1 ORAL ACE INHIBITION IN CHRONIC HEART FAILURE

This study revealed significant differences in the responses to the recommended low doses of ACE inhibitors in elderly patients with stable heart failure in the commonly employed clinical setting of temporary diuretic withdrawal. The use of a placebo controlled randomised parallel group design in this study has permitted comparisons of drug effects. It is important to evaluate haemodynamic responses in these patients in the light of a placebo treated group to control for diurnal changes and feeding patterns (Packer et al,1985a). A parallel group design was chosen to avoid a carryover effect due to the protracted elimination phase associated with some ACE inhibitors (Belz et al,1988) and to avoid delay in the initiation of active therapy.

The first dose hypotensive response to ACE inhibitors in heart failure has received much attention in case reports and small series (see chapters 3 & 4). The mechanism and significance of the response in clinical practice remains obscure. The relationship of blood pressure fall to previous or concurrent diuretic therapy, the dose or type of ACE inhibitor employed and the importance of a spectrum of individual patient responses is poorly defined. A comparison was made of the degree and pattern of blood pressure response in the group of patients who could be considered to be at greatest risk of developing a hypotensive response. These are elderly, diuretic treated subjects with moderate to severe heart failure.

Despite employing the standard low doses of both captopril and enalapril both these drugs showed falls in blood pressure consistent with previous reports. If, as some workers suggest, the first dose blood pressure response is related to the prevailing activation of the renin angiotensin system, as predicted by circulating renin activity (Packer et al,1985b), it might be suggested that these observations would **underestimate** the general degree of response in the captopril treated group. Nevertheless we

documented predictable and significant supine blood pressure falls regardless of diuretic withdrawal and relatively (but not significantly) lower renin activity in this group. There is limited experience with lower oral doses of captopril e.g. 1 mg (Warren et al,1986) but formulation is compromised by chemical instability (Colucci et al,1989). Patterns of test dosing with captopril have been suggested but the individual relevance of this strategy to subsequent responses to higher doses of the same or different agents has not been documented. However there is some data to suggest that in fact the dose reduction of captopril at least between 6.25mg and 25mg makes little difference to the overall blood pressure response (McClay et al,1992). The design of this study was far from optimal but this study at least questions the validity of empirical dose reduction without observational and comparative data. The selection of captopril on the empirical basis that if symptomatic hypotension and hypoperfusion were to occur its duration would at least be limited in comparison to longer active drugs dose at least appears logical (Reid,1987). The observations with respect to the comparison between low dose captopril and enalapril confirm this pattern. In this section treatment with neither captopril nor enalapril caused symptoms or adverse changes in renal biochemistry.

In contrast, low dose perindopril was not associated with any change in blood pressure or heart rate. This was despite comparable plasma ACE inhibition which was if anything more protracted after perindopril than after enalapril. This may therefore reflect an important and clinically relevant agent specific difference in response between ACE inhibitors. The comparison was performed in patients who remained supine in a controlled hospital setting. The ambulatory blood pressure response would be expected to follow a similar pattern.

At the doses selected a similar differential response is observed with respect to heart rate. Altered autonomic tone and baroreceptor reflexes in heart failure patients are well known (Porter et al,1990) as are the anti-adrenergic or parasympathomimetic

properties of captopril (Campbell et al,1985), enalapril (Boni et al,1990) and perindopril (Ajayi et al,1986). This study was the first occasion in which a small but significant slowing of basal heart rate in the supine posture after low dose enalapril has been demonstrated with an appropriate placebo control group. This effect on heart rate was seen only briefly with captopril and not seen at all with perindopril therapy.

One of the mechanisms proposed to be responsible for the fall in blood pressure in a proportion of heart failure patients treated with ACE inhibitors is activation of the Bezhold-Jarisch reflex with vagally mediated hypotension and bradycardia (Mark,1983). In the current study one patient had a marked fall in blood pressure and associated bradycardia yet remained asymptomatic immediately following dosing with placebo (Figure 6.13). He subsequently tolerated open captopril therapy outwith the study on the following day without complications. His placebo response was **not** excluded from the analysis. This reflex mechanism may be relevant in a few reported cases of hypotension following ACE inhibitors in heart failure patients. The Bezhold-Jarisch reflex clearly has little relevance to specific ACE inhibitors, agent selection or general management policies designed to avoid hypotension in heart failure as it is unpredictable and related neither to dose nor to individual drugs (Mark,1983).

This acute study documented similar plasma ACE inhibition with both enalapril and perindopril yet divergent blood pressure responses. One explanation may involve differences in penetration and interaction with the tissue renin angiotensin systems. With both enalapril and perindopril the net interaction with tissue and plasma ACE will be a product of the parent ester, a weak but lipid soluble ACE inhibitor and the more potent but polar diacid metabolite. The potency ratios and polarity of ester and diacid vary substantially between different compounds despite similar basic structures (see 2.3.1.) and there is limited in vitro evidence that the interaction is significant at least with

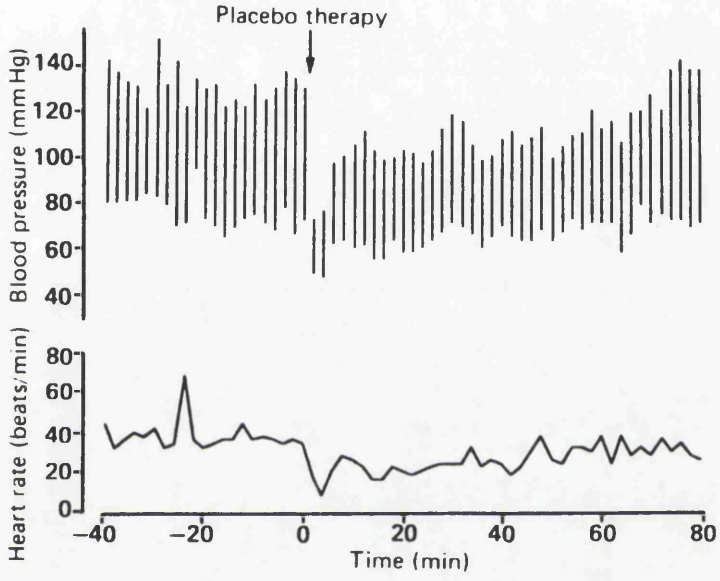


Figure 6.13: Individual blood pressure and heart rate profile of patient (study no.27) on double blind placebo therapy.

of each of these components would also be expected to vary and contribute further to potential differences in the pattern of tissue ACE inhibition. In the same fashion the tissue conversion of inactive ester to the active diacid as a local phenomenonmay also play a role in defining differential activity. As chronic therapy involves the attainment of an equilibrium the most likely setting in which potential differences would be apparent would be the first dose response.

The reactive rise in renin or aforementioned problems in documenting ACE inhibition with substrate based assays are unlikely to be relevant to the acute responses described in heart failure patients in this study. In addition they would be inadequate to explain the qualitative difference in blood pressure response between enalapril and perindopril.

The acute haemodynamic responses to ACE inhibitor drugs in patients with chronic heart failure are not markers of symptomatic benefit and conversely smaller blood pressure falls can be associated with benefit (Massie et al,1984). In general terms improvements in exercise duration and symptoms appear to require 6-8 weeks of therapy and alternative mechanisms may be important in reducing mortality (Packer et al,1987). As perindopril has been shown to provide symptomatic improvement in chronic cardiac failure (Bounhoure et al,1989), similar to other ACE inhibitors, the lack of an initial blood pressure fall does not appear to be an indication of a lack of long-term efficacy in heart failure at least with respect to exercise capacity. Reductions in mortality have not been shown with this drug and this may not be pertinent at these low doses. In addition, in acute heart failure oral perindopril is unlikely to share the haemodynamic benefits which have been demonstrated with other ACE inhibitor (Flynn et al,1988).

These results have general relevance since the patient population was not highly selected and since it included primarily the 'high risk' groups of the elderly, diuretic pretreated, severe heart failure patients. Any manoeuvre which reduces the risk of

hypotensive responses in these patients may have clinical and possibly resource implications although it has been stated on several occasions that the blood pressure responses per se are of poorly defined importance. The patient population studied , dose and agent employed and the adequate definition of haemodynamic and biochemical responses are important and the accurate descriptions provided in this study gave a hitherto unknown differentiation between similar agents.

6.3.2. INTRAVENOUS ACE INHIBITION IN CHRONIC HEART FAILURE

This section was designed to extend and complement the oral dosing study. The two intravenous diacid ACE inhibitor used produced broadly similar patterns of response when administered in low doses by constant rate infusion. The present intravenous protocol involving double blind discontinuation of therapy dependent on BP response worked well in practice and allowed effective control of the haemodynamic responses in these patients. It is a significant improvement upon bolus dose strategies employed in other studies with much higher doses of enalaprilat (Kubo et al, 1985; de Marco et al, 1987; Hornung and Hillis, 1987; Walinsky et al, 1987; Taylor et al, 1989) or other ACE inhibitors (Rademaker et al, 1986; Dickstein et al, 1987; Volpini et al, 1989; Ahmad et al, 1990). Blood pressure responses were intentionally limited by this manoeuvre and the low doses employed demonstrated in all cases a more or less smooth controlled blood pressure fall. No instance of sudden hypotension and bradycardia (or tachycardia) was noted in any patient receiving active therapy. Individual dose titration and optimisation of the blood pressure response may be valuable early after myocardial infarction (Nabel et al, 1991).

The study demonstrated a hitherto unreported early, transient pressor response to very small quantities of ACE inhibitor under the conditions of study. With the doses selected this was more clearly observed with perindoprilat. The same trend was apparent

with enalaprilat but this did not prove statistically significant. The origin of this response is unknown. It occurred after very low doses of drug (25-50 μg perindoprilat) during onset of plasma ACE inhibition. It preceded the onset of a controlled blood pressure fall during continued constant rate infusion. Clearly the response may be spurious and may only reflect the difference between parallel groups of patients. Nonetheless it was clearly evident during the data collection phase. It may reflect a transient partitioning effect on plasma and tissue based ACE and a further example of effects seen at doses of ACE inhibitor too low to affect blood pressure (eg Linz et al, 1992; Motwani & Struthers,1992).

Drug may be inhibiting plasma ACE prior to concentration dependent diffusion into tissue sites. During the lag phase prior to tissue uptake and thus prior to blockade of the RAS in tissue sites excess plasma generated Ang I may transiently mediate a BP rise through conversion by tissue ACE. As diacid concentrations rise this may overcome the molecules' lack of lipid solubility to gain access to tissue ACE sites and so inhibit Ang II mediated vasoconstriction.

This response has not been reported in previous studies. This could largely be because they have not employed the low dose protocol used in the present observations or have involved inadequate controls, infrequent blood pressure monitoring or bolus drug administration. As previous studies have not recorded BP until 30 mins post dosing clearly they would have missed this transient response. Similarly it is unclear if this response is agent related; or a feature only of patients with heart failure. Both drugs tended to show the pattern yet it was significant only after perindoprilat. Tissue ACE levels are known to be increased in heart failure from experimental observations (Hirsch et al,1991,1992; Fabris et al, 1990). The total angiotensin II generating activity may not be entirely related to angiotensin converting enzyme or susceptible to angiotensin converting enzyme inhibitor drugs (Urata et al, 1990). Increased tissue based enzyme

activity, if present in human chronic cardiac failure, may be pertinent to the transient elevation of blood pressure which we have observed.

The clinical use of angiotensin converting enzyme inhibitors has gradually evolved. Lower doses are now favoured yet surprisingly few studies have adequately examined haemodynamic or biochemical effects after these lower doses. The total dose administered and the rate of drug administration probably determine the observed dynamic response. Slow intravenous initiation of the angiotensin converting enzyme inhibitors is potentially useful in patients who may be at risk of excessive falls in blood pressure. Specifically this might be safer in patients with acute heart failure treated with diuretics around the period of myocardial infarction.

6.3.3. INTEGRATED FINDINGS OF HEART FAILURE STUDIES

These studies demonstrate under controlled observation a qualitatively different pattern of BP response between ACE inhibitors of similar chemical structure and metabolism. In the oral study the ester prodrug enalapril, given in low doses (2.5 mg) produced significant and long lasting falls in blood pressure and heart rate whereas perindopril (2 mg) caused no significant change from placebo. These responses were recorded despite comparable plasma ACE inhibition. In a similar randomly assigned group of patients with broadly comparable plasma ACE inhibition, the diacid metabolites of both drugs given alone reduce blood pressure.

The perindoprilat concentrations achieved after intravenous administration were higher than those previously achieved after oral perindopril. The contrasting effects of perindopril and perindoprilat may simply be confounded by differences in effective dose. Alternatively, the hitherto disregarded prodrug ester (perindopril) which appears first in the systemic circulation prior to the generation of significant quantities of the active diacid metabolite (perindoprilat) may influence the blood pressure response to perindopril.

After oral treatment with perindopril it may be that the lipophilic ester, with its weak angiotensin converting enzyme inhibiting properties, may undergo rapid tissue distribution and interaction with the tissue based angiotensin generating system. From in vitro work it appears that perindopril, the parent compound, may reduce inhibition of angiotensin converting enzyme by perindoprilat, the metabolite (Harrigan et al, 1989). Any interaction between the parent compound and metabolite would be seen primarily after the initial dose, it would be expected to be dose and concentration dependent, and it could produce effects which would vary among tissues dependent on the distribution of each element to different sites.

In general terms the clinical pharmacology of ACE inhibitors in a variety of settings has been plagued by a lack of information about the lower end of the dose response curve. It was a specific aim of this series of studies to address this issue and it has allowed differential and hitherto unreported responses to be demonstrated.

CHAPTER 7

STUDIES OF ANGIOTENSIN CONVERTING ENZYME INHIBITION IN NORMAL AND PATIENT VOLUNTEERS

7.1 TRANSPULMONARY PHARMACOKINETICS OF PERINDOPRILAT IN MAN.

The pulmonary circulation has traditionally been associated with the generation of angiotensin II from angiotensin I by the action of ACE. For many years it was felt that circulating AII was the product of pulmonary ACE activity and that this was in turn a major endocrine function of the lungs (Ng and Vane, 1968; Said, 1982).

In theory uptake of drug to tissue sites should alter the plasma concentration time profile of ACE inhibitors. Non linear pharmacokinetic models are known to describe ACE inhibitor disposition better than conventional techniques (Francis et al, 1987; Lees et al, 1989). Parameters describing such a profile for an ACE inhibitor could indirectly give an index of tissue ACE activity and inhibition.

This study was designed to examine the transpulmonary extraction of the intravenous diacid ACE inhibitor perindoprilat (see Chapter 2). As the pulmonary circulation is theoretically a rich source of ACE activity this central site might be expected to provide a good definition of tissue uptake and altered pharmacokinetic profile of an ACE inhibitor.

7.1.1. PATIENTS AND METHODS

A. Patients

The study was conducted in an open design in ten male patients undergoing diagnostic cardiac catheterisation for the investigation of chest pain. The demographic details of the study population are given in Table 7.1. All patients had stable clinical

Table 7.1: Demographic characteristics and concomitant therapy of the study population.

STUDY (DATE)	AGE YEARS	WEIGHT Kg	CARDIAC HISTORY	MEDICATION	VENTRICULOGRAPHY	ANGIOGRAPHY	OUTCOME
1 (6/3/89)	43	60	IHD Post infarct 1983	Atenolol 50mg ISMN 20mg bds	Inferior hypokinesia	RCA stenosis	Angioplasty
2 (14/3/89)	58	67	IHD Post infarct 1986	Atenolol 50mg ISMN 20mg bds Diltiazem 60mg tds Aspirin 150mg	Anterior hypokinesia	Distal LAD disease	Medical therapy
3 (23/3/89)	53	85	IHD Post infarct 1978	Atenolol 100mg Diltiazem 60mg tds Aspirin 300mg	Normal	Diffuse CAD Occluded RCA Proximal & distal LCA stenoses	Coronary artery bypass grafting
4 (18/4/89)	42	66	IHD Post infarct 1986	Diltiazem 60mg tds ISMN 20mg bds	Anterior hypokinesia	Proximal LAD disease	Medical therapy after -ve thallium scintigraphy
5 (25/4/89)	63	86	IHD Post infarct 1982	Atenolol 50mg Diltiazem 60mg tds	Inferior hypokinesia	Occluded RCA LAD & 1st diagonal stenoses	Coronary artery bypass grafting
6 (2/5/89)			IHD ? infarct 1985	Atenolol 100mg Nifedipine Retard 10mg bds Betahistine 8mg tds	Anterior hypokinesia	Severe proximal LAD & obtuse marginal stenoses	Angioplasty
7 5/5/89)	39	105	Atypical chest pain	GTN prn	Normal	Normal	Further investigation
8 (30/5/89)	48	85	Atypical chest pain	"Franol" prn	Normal	Normal	Further investigation
9 (4/7/89)	42	85	Atypical chest pain	Cimeditine 400mg nocte	Normal	Normal	? AV node dysfunction Further EP investigation
10 (11/7/89)	70	75	IHD Post infarct 1966	ISMN 20mg bds Diltiazem 60mg tds GTN prn Ranitidine 150mg bds	Normal	Occluded LAD	Angioplasty filling from (R) 70-90% RCA lesion diffuse disease

Patients were selected such that they had no clinical, biochemical nor radiological evidence of cardiac failure and had not received diuretic therapy in the 2 months prior to study.

B. Procedure

All patients underwent routine diagnostic left ventriculography and coronary arteriography by the Judkins technique using non ionic contrast media (Iopamidol; Niopam 370, Merck U.K.) between 1030 and 1230 hours. After completion of this procedure a Cournand catheter was placed in the central venous circulation also by a femoral approach. Right heart pressures through to the pulmonary capillary wedge pressure, and the on-line pressure from the descending aorta, were recorded at baseline and the right heart catheter was then withdrawn to the main pulmonary artery for continuous pressure measurement and blood sampling. Aortic pressures were recorded throughout. A peripheral vein on the dorsum of the hand was cannulated for drug infusion. The surface electrocardiogram was monitored throughout the study. Immediately prior to infusion the infusate was prepared in sterile saline to contain 1 mg perindoprilat, 0.5 mg/kg indocyanine green and 10 ml purified plasma protein solution in a total volume of 20 ml which was infused at a constant rate of 100 µl/min over 20 minutes. A Braun perfusor pump (Perfusor Secura E, Braun, Melsungen, FRG) was used with the infusate primed and running for 2-5 minutes prior to connection to the patient at the start of the infusion. The co-infusion of indocyanine green was employed as a marker for the intravascular space and transpulmonary transit analogous to previous animal experiments employing multiple indicator dilution (Linehan et al, 1985). The concentration of 0.5 mg/kg is known to be eliminated in an approximately linear fashion (Meijer et al, 1988). During the infusion (20 mins) and for the first 40 mins thereafter (i.e. total 1 hr) simultaneous right and left heart blood samples (5 ml) were drawn from

the appropriate catheters after removal of the dead space volume. Sampling was conducted at frequent intervals during the initial accumulation phase of the infusion (30 second intervals for 5 minutes) and during the early elimination phase after completion of the infusion (1 minute intervals for 5 minutes). The infusion was stopped exactly at 20 minutes and the intravenous line disconnected but not flushed. Transpulmonary sampling was continued for a period of 1 hour after which the central catheters and peripheral infusion cannula were removed. Later phases of drug elimination were monitored by samples withdrawn from a separate peripheral venous cannula up to 20 hours post commencement of the infusion. All patients remained supine for the full duration of the study and were monitored overnight in the cardiac investigation unit according to local practice.

C. Additional Biochemical methods

Indocyanine green was quantified after dilution of the plasma with 0.1% bovine serum albumin using standard photometric assay at 805 nm (Bjornsson et al, 1982; Svensson et al, 1982). The infusate formulation was shown to be stable in vitro for both perindoprilat and ICG at room temperature over 2 hours. There was no interference between the angiography dye and any of exogenous drug, ACE, plasma renin activity or ICG added to control plasma *in vitro*.

D. Pharmacokinetic analysis

To aid the interpretation of the accumulation profiles where multiple samples had been collected at close time intervals, and where assay variability therefore often exceeded the increment in drug concentration for pairs of samples, the raw data were subjected to mathematical smoothing before further statistical analysis. This was undertaken simply by substituting each concentration result with the mean of the

reference concentration and the preceding and subsequent ones (Three point running mean technique, see Velleman,1980). The concentrations reflecting the start and end of the infusion were not adjusted.

The circulatory delay was calculated from comparison of the early, linear portions of the ICG accumulation data from the right heart samples with those from the left heart samples by applying linear regression of time upon ICG concentrations: the difference in Y intercept was taken to represent circulatory delay. A similar approach was used to assess apparent circulatory delay for the ACE inhibitor. Results are expressed as the mean of the individual circulatory delays for the 9 subjects with complete ICG data.

Pharmacokinetic modelling was conducted on raw concentration data. A hierarchy of standard multi-exponential pharmacokinetic models (1- to 3-compartment open, zero order input) and three modified 1-compartment open models (incorporating non linear tissue binding parameters, non linear plasma binding parameters or non linear tissue and plasma binding parameters) were fitted to the data (see Section 7.2 for further details). This was conducted by least squares non linear regression analysis using the biomedical statistics package BMD and the derivative-free option PAR (Ralston et al, 1979) on an ICL 3980 series mainframe computer (University of Glasgow Central Computing Service). The derivation and application of these models had previously been described (Lees et al, 1989). The concentration time profiles employed were a combination of the right heart sampling concentrations during the transpulmonary catheterisation phase and the subsequent peripheral venous samples from 1 - 20 hours. Where appropriate, model comparisons were based on the use of the general linear test (F-ratio test) (Neter and Wasserman, 1974) and the Schwarz criterion (Schwarz, 1989).

A. Haemodynamic effects

There were no appreciable change in central or systemic cardiac pressures either during or up to one hour from the onset of the infusion (Figure 7.1).

B. Drug concentration and ICG profiles

The mean profile of plasma drug accumulation during transpulmonary catheterisation is illustrated in Figure 7.2. The ACE inhibitor did not approach steady state during the infusion. The small early gradient in perindoprilat concentrations between right and left heart samples is equivalent to a mean circulatory delay of 25 seconds, and is exactly mirrored by the control substance ICG (Figure 7.3) from which a mean circulatory delay of 25 seconds was calculated. Thereafter, ICG shows rapid accumulation towards steady state and then rapidly declines on cessation of the infusion, reflecting the known short half-life of this compound. The full pattern of drug elimination employing right heart samples followed by peripheral venous samples is shown in Figure 7.4. There was no evidence during the early post-infusion period of any relative increase in left heart concentrations compared with those from the right heart, to reflect dissociation of drug from pulmonary binding sites.

C. Plasma ACE activity

ACE activity in circulating plasma from both pre-pulmonary and post-pulmonary sampling shows virtually complete inhibition of ACE within 90 seconds of starting infusion (Figure 7.5). There was little evidence for either a transpulmonary gradient in ACE activity or reduction in post-pulmonary ACE after discontinuation of infusion. Marked and persistent ACE inhibition was noted during the later period of monitoring with substantial inhibition of ACE activity remaining at 20 hours after the start of the infusion.

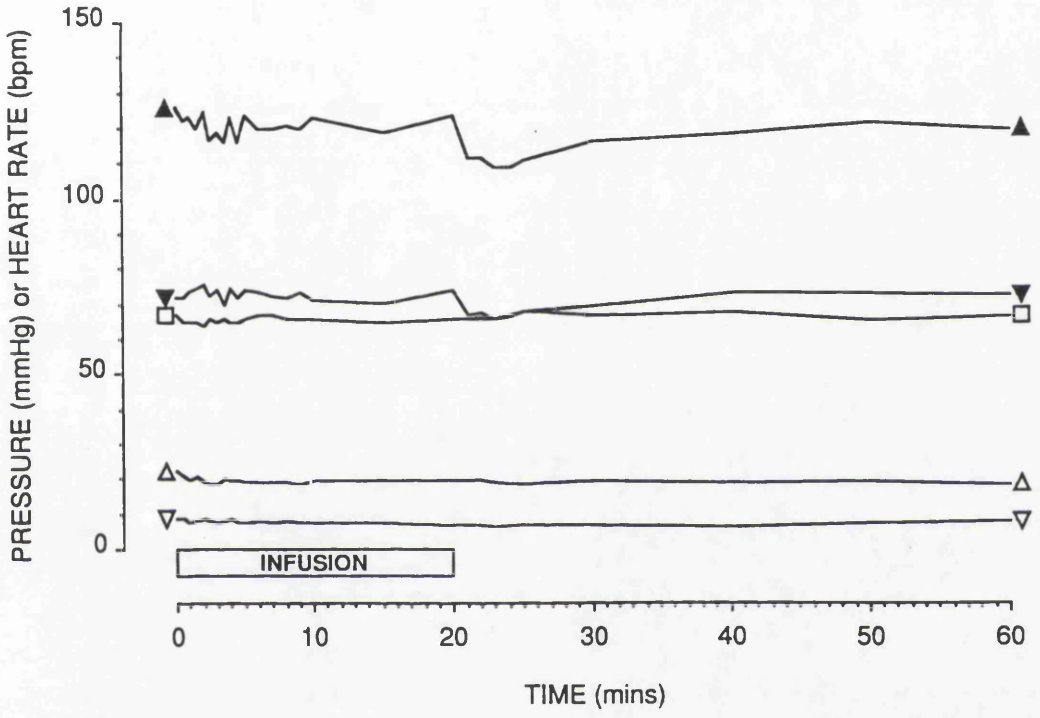


Figure 7.1: Mean blood pressure profiles (n=10) in aorta (systolic, ▲ ; diastolic,▼) and main pulmonary artery (systolic, △ ; diastolic,▽) with mean heart rate (□) during transpulmonary catheterisation (1 hour) and infusion of perindoprilat (1 mg, 20 mins).

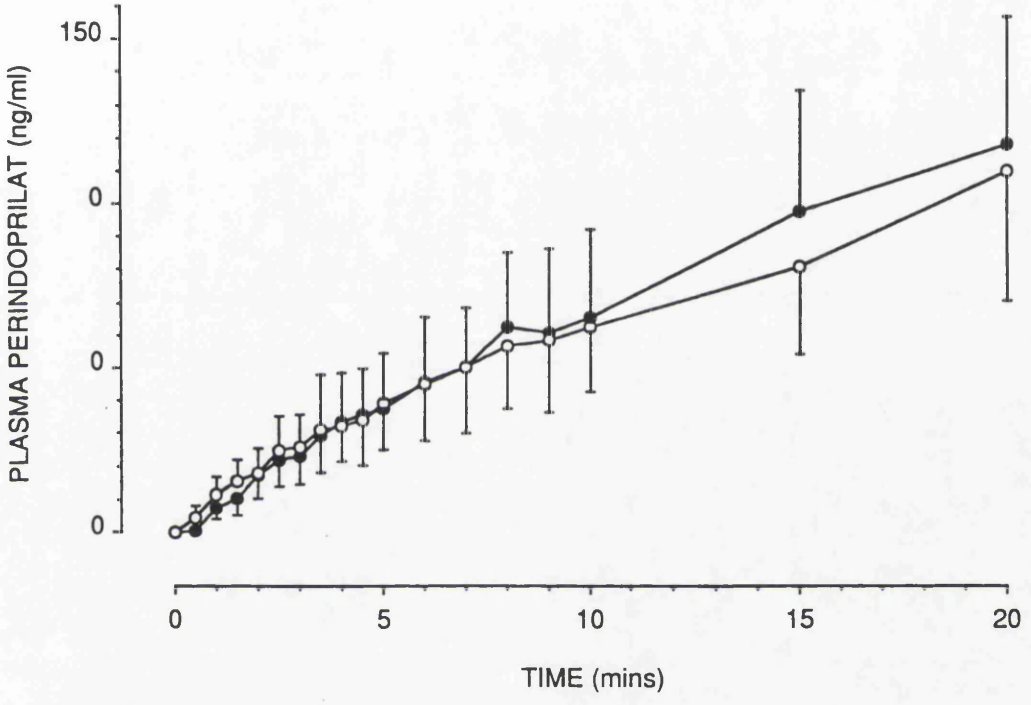


Figure 7.2 Accumulation concentration time profile (mean±1 SD, n=10) of perindoprilat in simultaneous plasma samples from right (○) and left (●) heart catheters during constant rate infusion (1 mg, 20 mins).

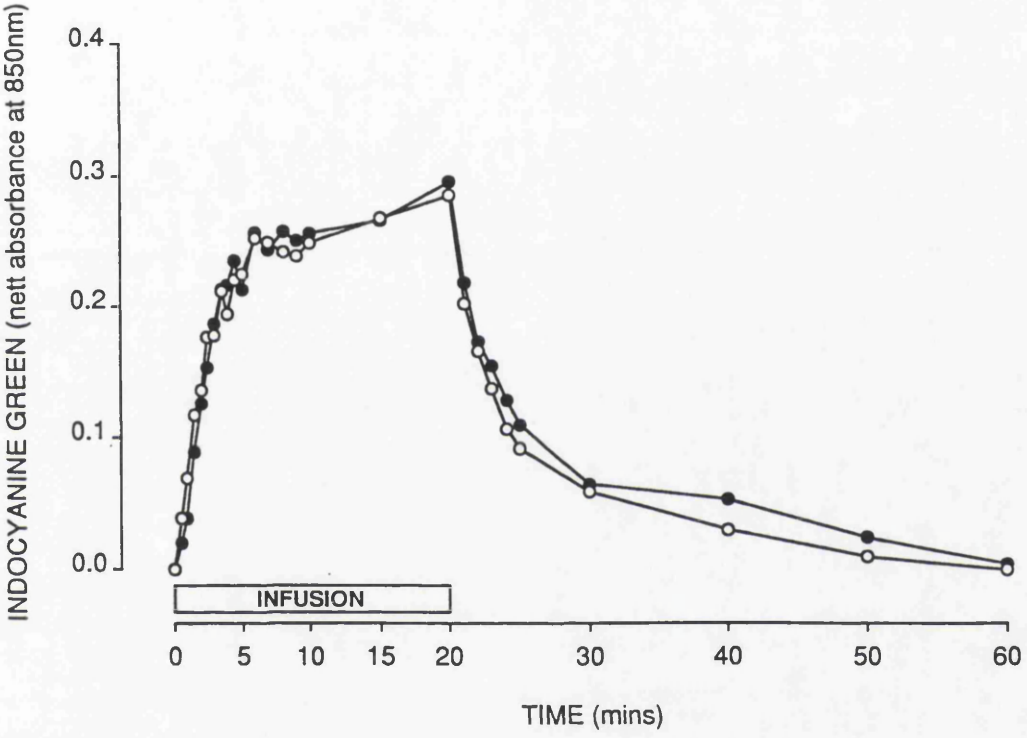


Figure 7.3: Mean concentration time profile (n=10) of indocyanine green in right (○) and left (●) heart catheters expressed as absorbance during infusion (0.5 mg/kg, 20 mins) and elimination.

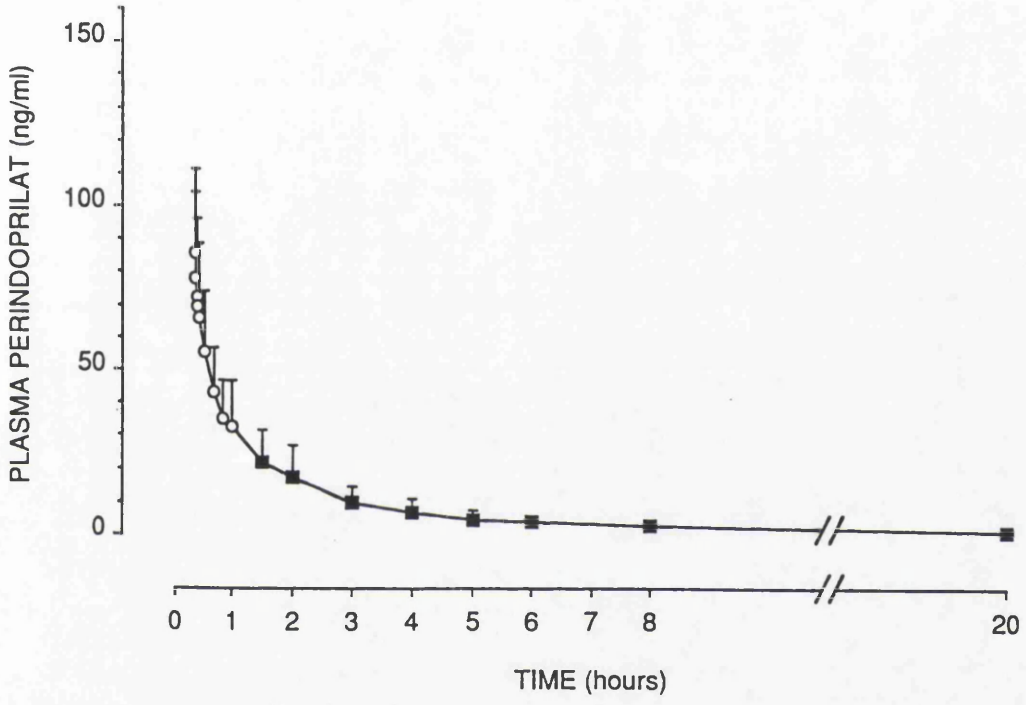


Figure 7.4: Drug elimination profile (mean±1 SD, n=10) in right heart plasma (○) and peripheral venous plasma (●)

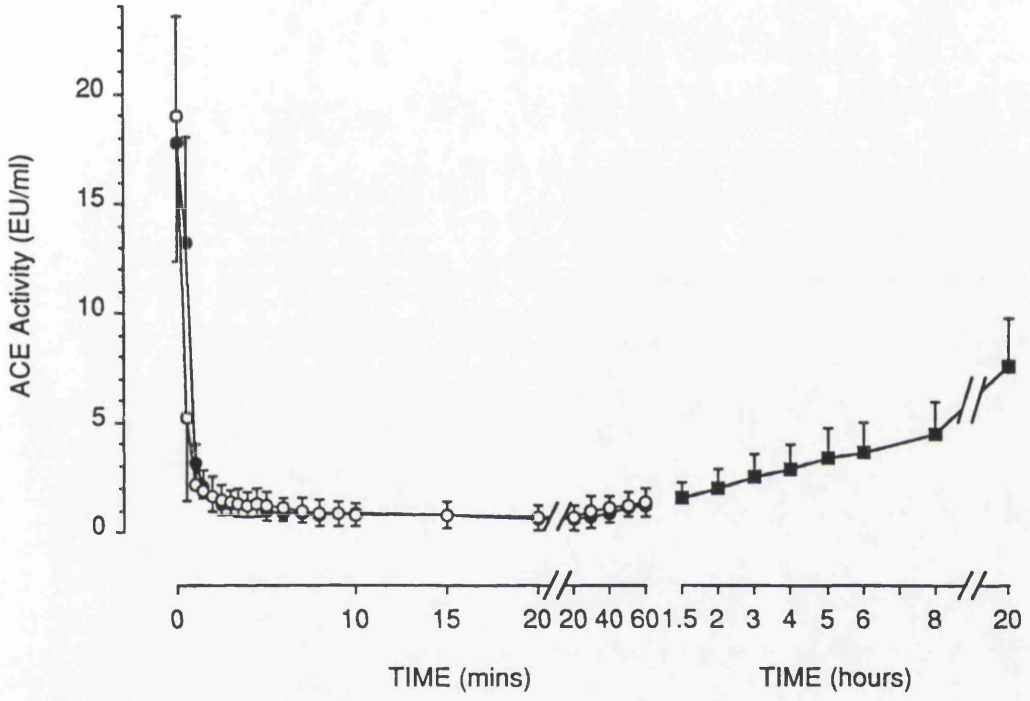


Figure 7.5: Mean ACE activity (±1 SD,n=10) in right (○) and left (●) heart plasma and peripheral venous plasma (■) during and following perindoprilat infusion.

D. Plasma Renin activity

Plasma renin activity determined from samples from the post-pulmonary circulation during transpulmonary catheterisation and subsequent peripheral venous sampling showed low basal levels prior to infusion and a delayed rise with a wide variation between individual patients (Figure 7.6).

E. Pharmacokinetic Modelling

The plasma concentration time profiles of perindoprilat were fitted simultaneously for all data sets. It did not prove possible to fit all data sets using a conventional 3 compartment linear model nor the non linear binding models which included terms for solely tissue binding or combined tissue and plasma binding. These were therefore rejected. Of the conventional models a 2 compartment zero order model provided a better fit for the observed data than 1 compartment as shown by the reduction in weighted residual sum of squares, F ratio and calculated Schwarz criterion (Table 7.2). This was not significantly improved by using a non linear binding model incorporating plasma binding terms.

It was clear from observing individual cases that the non-linear models did prove a much closer approximation to the observed data as had been previously observed (Lees et al,1989).

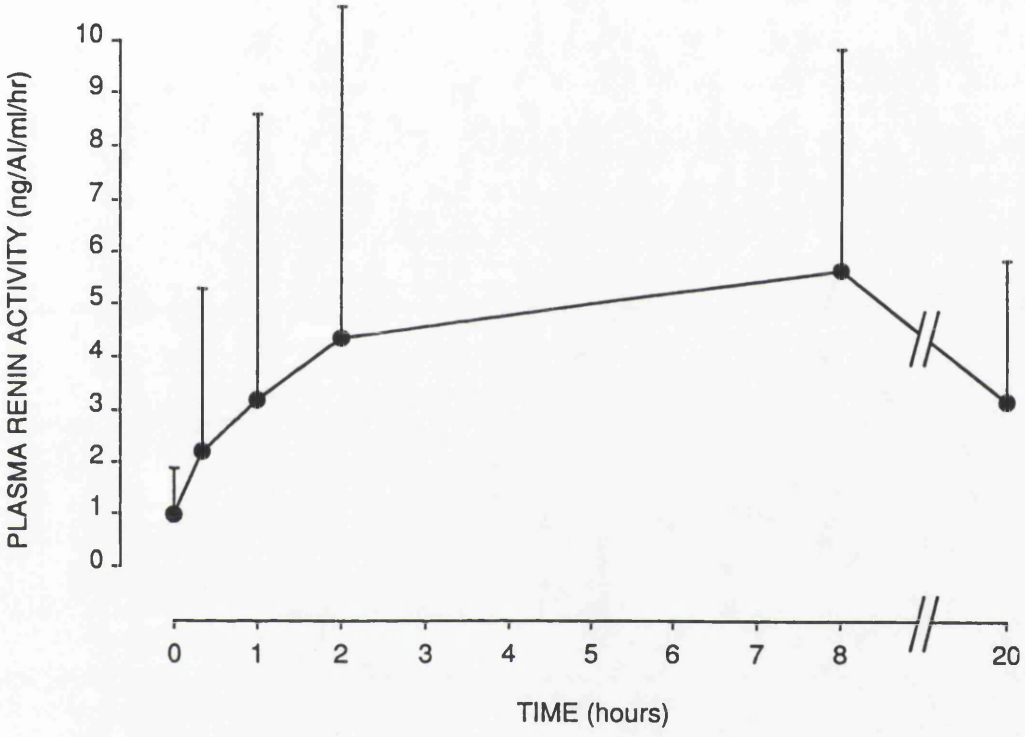


Figure 7.6: Mean supine plasma renin activity (±1 SD, n=10) before and following perindoprilat infusion (1 mg, 20 mins).

Table 7.2: Characterisation of concentration time profiles of perindoprilat (right heart and peripheral venous plasma) by a hierarchy of pharmacokinetic models.

	WEIGHTED RESIDUAL SUMS SQUARES MODEL			SCHWARZ CRITERION MODEL		
SUBJECT	A	B	E	A	B	E
1	101.5	78.9	79.7	146.7	142.3	142.6
2	146.8	56.5	57.6	178.1	148.3	149.0
3	44.4	26.7	26.5	136.3	122.0	121.8
4	91.8	91.8	70.8	161.7	165.3	156.3
5	105.7	35.3	34.7	166.6	131.8	131.3
6	111.1	50.9	51.1	168.4	144.7	144.8
7	71.6	31.7	31.7	153.0	128.0	128.1
8	53.0	41.9	41.9	142.5	137.9	137.9
9	56.5	23.0	22.7	144.7	116.9	116.4
10	99.3	95.7	95.5	164.5	166.8	166.7
RANK SUM				27	18	15
FREIDMAN				NS	NS	
(ANOVA)				P<0.05		

A = One compartment open, zero order input
B = Two compartment open, zero order input
E = One compartment open, zero order input, non-linear plasma binding.

7.2 PERINDOPRILAT INFUSION STUDIES IN SALT REPLETE MAN.

The intravenous route of administration has been proposed for use of ACE inhibiton in the management of acute heart failure (Flynn et al, 1988; Ahmad et al, 1990), the treatment of accelerated hypertension (Rutledge et al, 1988; Savi et al, 1990) and after acute myocardial infarction (Taylor et al, 1989; Nabel et al, 1991). Detailed experience with the intravenous route of administration is limited.

In this section it was sought to further test the observation that the pharmacokinetic disposition of ACE inhibitors may be influenced by specific and saturable binding to plasma ACE (Francis et al, 1987) and/or tissue ACE (Lees et al, 1989). From pharmacokinetic descriptions the contribution of tissue ACE to the haemodynamic effects of ACE inhibition in man might be achieved by indirect means. Specifically the lower end of the dose response relationship was of interest in keeping with the work in heart failure patients recieving low doses and other clinical populations eg peri-myocardial infarction where these might be relevant.

In the present study protracted low dose constant rate infusion schedules were employed in order to provide precise pharmacokinetic definition and also, despite the low likeliehood of significant response, from the standpoint of a basis for controlled dose titration and response (see section 6.2).

7.2.1. SUBJECTS AND METHODS

A. Design

A four-way, single-blind (subjects), crossover study was conducted in 8 normotensive male subjects. The subjects were randomised to receive a 3 hour infusion of saline placebo, or perindoprilat 1 mg by constant rate intravenous infusion over 1 hour, 3 hours or 6 hours. All subjects received all four treatments on separate occasions

at least 10 days apart.

B. Subjects

Eight healthy men (age 19-40 years; weight 65 - 89 kg) participated in a single blind, random treatment order study after screening by clinical history and physical examination, routine urinalysis, ECG, laboratory biochemistry and haematology (including serum ferritin).

C. Procedure

All subjects were free from concomitant medication for the duration of the study and followed their normal diet. No smoking or alcohol consumption was allowed for 12 hours before or during each of the four study days, which were conducted at least 10 days apart and commenced at around 0700 hour after a light breakfast at home. A single (subject) blinded randomised treatment design was employed whereby subjects received intravenous perindoprilat (1 mg in 30 ml normal saline) over 1, 3 or 6 hour or saline placebo (30 ml over 3 hour) via a peripheral venous cannula on the dorsum of the hand. A further heparinised cannula was inserted in an opposite antecubital vein for blood sampling purposes. The subjects were cannulated and rested supine for at least 30 minutes prior to commencing the study. Infusions were administered using a calibrated constant rate infusion pump (Braun Secura E; Melsungen, Germany) which was set up and running for at least 5 mins prior to being attached to the subject's cannula. At the end of the infusion the pump was switched off. The cannula remained attached and was removed at 6 hours without flushing. The subjects were unaware as to the significance of infusion duration and did not know that the placebo infusion would be 3 hours. A light snack was provided at 5 hour and 10 hour (no tea or coffee allowed). Blood pressure (BP) and heart rate were recorded supine and erect before and at frequent

intervals during the infusion and subsequently. Due to the sampling frequency no erect BP recordings were obtained for the 1 hour infusion. All values were recorded in triplicate.

Peripheral venous blood samples were drawn for the determination of plasma concentrations of perindoprilat and ACE activity. During the 1 hour infusion these were drawn at 0, 5, 10, 20, 30, 40, 50, 60, 65, 70, 80, 90, 100, 110 min and 2, 4, 6, 8, 10, 24, 32, 48 and 56 hour; during the 3 hour infusions (including placebo) at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.25, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 24, 32, 48 and 56 hour; during the 6 hour infusions at 0, 0.5, 1, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 13, 16, 24, 32, 48 and 56 hour. On each study day supine plasma renin activity was determined at 0 hour, at the end of the infusion, at the end of the study day and at 24 hours post study.

7.2.2. SPECIFIC DATA ANALYSIS

A. Blood pressure data

The timing of blood samples and blood pressure measurements was related to the duration of the infusion for each study day. For the purposes of comparison of blood pressure responses, only the common times of blood pressure recording were used. These were 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 hours from the commencement of the infusions. Mean data of triplicate values for these time points were compared using repeated measures analysis of variance (ANOVA) with Bonferroni correction (see Chapter 5). In an effort to generate a response record employing all available blood pressure time points each individual blood pressure record was subjected to a simple non-linear data smoothing technique (Velleman, 1980). Each subject then provided "smoothed" common time point blood pressure values which were again compared using repeated measures ANOVA for each phase as above. The blood pressure responses were further described by baseline corrected responses and by deriving the maximum fall

in systolic pressures for each individual during each phase. These were also compared using ANOVA.

B. Pharmacokinetic modelling

The concentration time profiles of perindoprilat using the different duration of infusions were characterised using a hierarchy of standard compartmental models and non-linear saturable binding models similar to those used in previous work whose derivation and validation has been described in detail (Lees et al, 1989). Briefly a range of multiexponential compartmental models (1 compartment (A), 2 compartment (B) and 3 compartment (C)) were fitted to the data using derivative-free, least squares, non-linear regression with the statistics package BMD-PAR on an ICL 3980 mainframe computer. Similarly, **simple** kinetic models (ie. one compartment, zero order input) with extra parameters to describe non linear, saturable tissue binding (D), plasma binding (E) or combined tissue and plasma binding (F), all with one compartment zero order input assumed, were fitted to the observed data. If a given model was unable to fit all data sets simultaneously then this led to rejection of that model. The "goodness of fit" of models for the observed data was compared using the general linear test and calculated Schwarz criterion where appropriate. This assess the statistical fit of the observed to predicted data for a range of model systems in the form of a comparative summary statistic. The calculation of the parameters derived from these models has been discussed elsewhere (Lees et al, 1989).

7.2.3. RESULTS

A: Haemodynamic Effects

The blood pressure response to intravenous infusion of perindoprilat is illustrated in Figure 7.7. During all treatments a rise in both supine and erect BP was seen between

5 and 7 hours after starting infusions which corresponded to the subjects' meal time. Supine systolic blood pressure fell with active infusions compared to the placebo control (Figure 7.7a). A similar pattern was observed with diastolic pressure. Similar changes were observed with erect blood pressures during the placebo, 3 hour and 6 hour active infusions (see Figure 7.7b). Statistically significant changes were seen between placebo and 6 hour infusion at the 6 and 10 hour time points. Smoothed erect systolic blood pressure falls with 6 hour infusions of perindoprilat were significantly greater than control between 3 and 6 hour after starting the infusion (see Figure 7.7c). There was no significant change in supine or erect heart rate compared to placebo.

B. Drug concentration profile

The observed drug accumulation and elimination profiles are illustrated in Figure 7.8. All three rates of infusion, but 3 and 6 hour infusions in particular, show a sigmoid accumulation profile with delay in the early accumulation of the measured drug in plasma. The observed mean maximal plasma concentrations of perindoprilat reflected the rate of infusion (1 hour 51.5±11.4 ng/ml; 3 hour 30.4±8.4 ng/ml; 6 hour 19.0±4.0 ng/ml) and were all significantly different from each other ($p = 0.013$, ANOVA).

C. Plasma ACE inhibition

Plasma ACE activity was rapidly inhibited by a 1 hour infusion of perindoprilat but less rapidly by a 3 or 6 hour infusion (Figure 7.9). The mean maximal observed inhibition was less with the slower rates of infusion (1 hour, 95.7±0.5% versus 3 hour, 92.3±2.7% and 6 hour, 87.4±5.1%; $p = 0.013$). After 48 hours there was no significant difference in ACE activity from placebo with any active infusion.

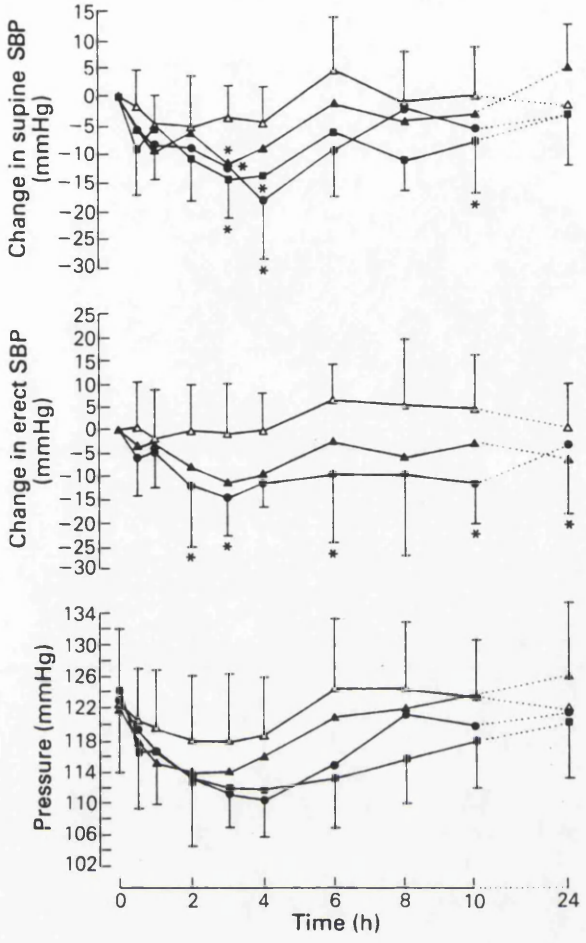


Figure 7.7: The effect of constant rate perindoprilat infusion (1 mg) over 1 (■), 3 (▲) or 6 (●) hours or placebo (△) over 3 hours on

(a) baseline corrected supine systolic pressure

(b) baseline corrected erect systolic pressure

or (c) smoothed erect systolic pressure (mean±1SD, n=8)

(* = $p < 0.05$ vrs placebo).

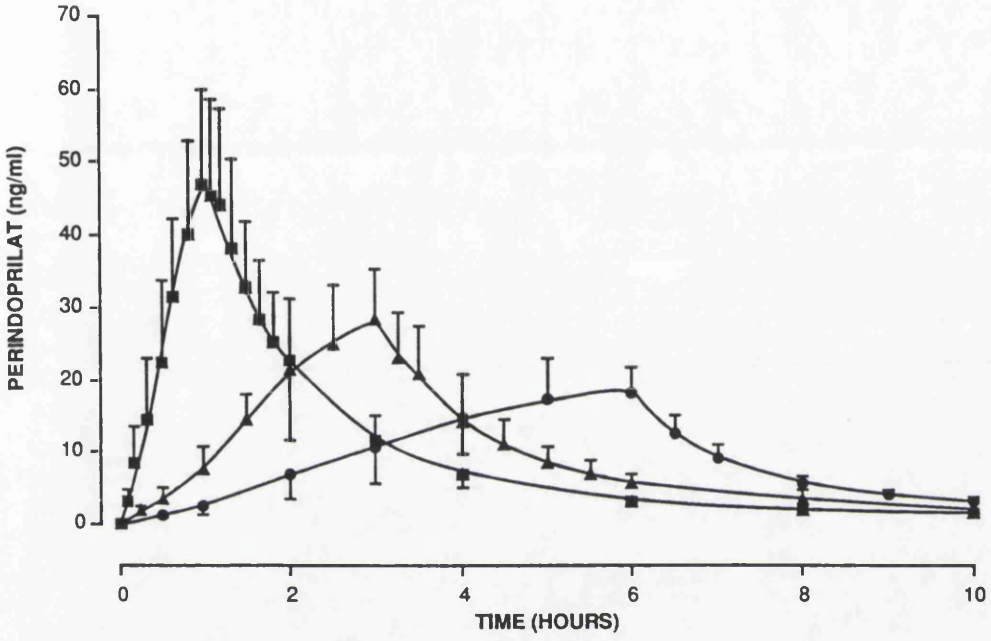


Figure 7.8: Concentration time profiles (mean±1SD, n=8) following constant rate intravenous infusion of perindoprilat (1 mg) over 1 (■), 3 (▲) or 6 hours (●).

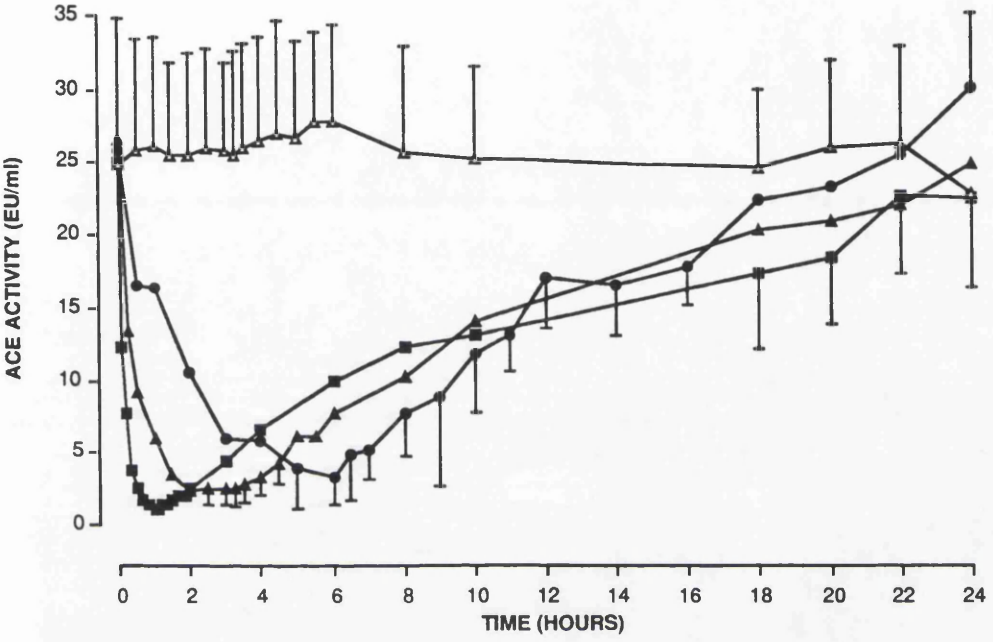


Figure 7.9: Plasma ACE activity (mean±1SD, n=8) following placebo (△) or perindoprilat (1 mg) by intravenous infusion over 1 hour (■), 3 hours (▲) or 6 hours (●).

D. Plasma renin activity

There appeared to be a greater reactive rise in plasma renin with the 6 hour infusion protocol (mean change in PRA, 2.4 ng AI/ml/hr) than that seen in response to 1 hour (0.5 ng AI/ml/hr) or 3 hour (1.8 ng AI/ml/hr) infusions of perindoprilat (see Figure 7.10), but these changes were not statistically significant ($p = 0.14$). Due to the small number of samples and different time points studied it is not possible to interpret the different profiles formally in greater detail.

E. Pharmacokinetic analysis

The pharmacokinetic data from the 1 hour infusion studies supported only a simple one compartment model. In contrast the 3 and 6 hour infusion studies were clearly better described by the models employing saturable non linear binding ($p < 0.0001$).

The best description of observed data was given by a model (F) incorporating elements to describe both tissue and plasma binding of the drug. The derived parameter estimates had acceptable coefficients of variation in most cases (Table 7.3). The mean (SD) volume of distribution for free drug was 12.1 (7.0) litres, with k_{el} for free drug of 1.24 h^{-1} (1.18). The mean estimated capacity of total binding sites for perindoprilat was 313 ng (211). Only 7.1 ng (3.9) of free drug (i.e. 163 ng in total) were estimated to produce 50% saturation of all binding sites. Ninety-one percent (7) of all binding sites appeared to be located in tissue compared to plasma.

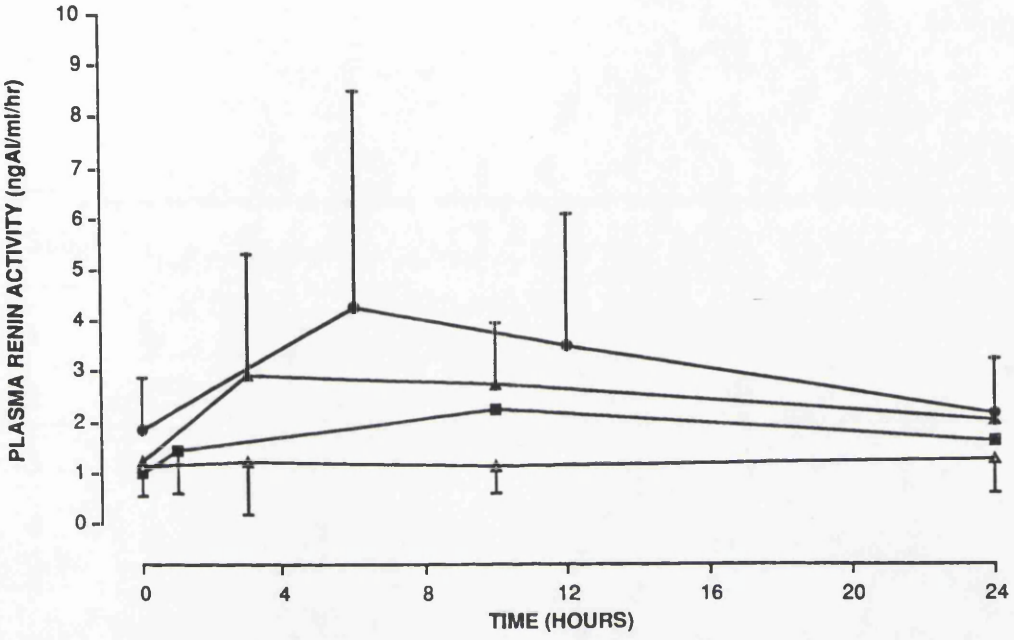


Figure 7.10: The effect of constant rate perindoprilat infusion (1 mg) over 1 (■), 3 (▲) or 6 (●) or placebo (△) over 3 hours on plasma renin activity (mean ± 1SD, n=8).

Table 7.3: Parameter estimates with coefficients of variation for model F, one compartment, open, zero order input non linear tissue and plasma binding, applied to 6 hour infusion profiles.

Subject	k_{el}	CV(%)	V_d	CV(%)	B_{max}	CV(%)	A_{u50}	CV(%)	F	CV(%)
1	0.66	10	12.2	9	179	12	4.3	38	0.87	3
2	4.09	123	2.7	100	789	7	2.5	90	1.00	1
3	1.3	37	5.8	39	381	16	13.6	26	0.99	1
4	0.82	8	9.9	8	147	9	4.9	28	0.89	2
5	0.92	16	10.5	16	241	13	9.5	27	0.94	2
6	0.50	13	23.6	13	201	17	4.5	68	0.89	4
7	1.1	30	11.4	30	371	14	11.1	26	0.95	2
8	0.6	16	21.0	14	191	20	6.4	49	0.78	7
Mean	1.24		12.1		313		7.1		0.91	
SD	1.18		7.0		211		3.9		0.07	

k_{el} = elimination rate constant
V_d = volume of distribution "plasma" compartment (l)
B_{max} = Total binding capacity (μg)
A_{u50} = Amount of free drug that produces 50% binding (μg)
F = Fraction of total binding sites in tissue.

7.3 ACE INHIBITION IN SALT DEPLETE MAN: ORAL ENALAPRIL IN A MODEL OF THE ACTIVATED RENIN ANGIOTENSIN SYSTEM.

To undertake the appropriately controlled and detailed studies of the response to blockade of the renin angiotensin system in patients with heart failure presents practical problems with concomitant medication, drug withdrawal, crossover designs and a variety of uncontrolled disease based factors which might influence response. The principal aim of this section was to establish a simple and reproducible human model suitable for detailed assessment of the responses to blockade of the renin angiotensin system and for differentiating drug effects. In the first instance the blood pressure and neurohormonal responses to low oral doses of enalapril were studied in young normotensive males in whom the renin angiotensin system had been acutely activated by a short programme of sodium depletion based on moderate dietary restriction and diuretic treatment.

7.3.1. SUBJECTS AND METHODS

Eight normotensive male volunteers (25.6±4.6 yrs; 70.5±6.1 kg) gave written consent for their participation in the study following confirmation of health by clinical history, physical examination, biochemical and haematological screen, 24 hour urinary sodium excretion (100-200 mmol) and electrocardiogram.

Salt depletion was implemented for three days prior to each of two study days. Volunteers were provided with all meals for a diet containing 40 mmol sodium per day. Dietary instruction by a qualified dietitian, and an accompanying diet sheet, defined allowed or disallowed supplemental foods. Fluid intake was encouraged but alcohol prohibited. Subjects continued on the dietary sodium restriction during the study day and until the return visit at 24 hrs following drug administration. During the three day pretreatment schedule the subjects took frusemide 40 mg (Antigen Pharmaceuticals,

UK), twice daily at 0800 hr and 1200 hrs. The last dose of diuretic was therefore administered approximately 20 hours before drug administration. Following instructions on technique, 24 hour urinary collections were performed at the screening visit, for the 24 hours preceding each of the two study days (day 3) and the 24 hours of each study day (day 4).

Volunteers attended the Clinical Pharmacology Research Unit (CPRU) after an overnight fast (10 hrs) on two separate occasions, 14 days apart, to receive enalapril 5 mg (Innovace, MSD, Hoddeson, UK) or placebo (vitamin C 50 mg, APS Pharma UK in an opaque gelatin capsule). Treatments were administered in a randomised, double blind, crossover design prepared by the Department of Pharmacy.

On attendance (0800 hr) subjects rested supine for at least 30 minutes following placement of a heparinised venous cannula in an antecubital vein for the purposes of blood sampling. Basal blood samples and blood pressure readings were collected supine prior to measurement of erect blood pressure and heart rate and bladder voiding. Blood pressure and heart rate were determined in triplicate using a semiautomatic device (Datascope Acutorr 3A, Paramus, New Jersey) at frequent intervals during the study day. Blood samples were drawn at intervals for the determination of serum drug concentration, serum electrolytes, plasma ACE activity and plasma renin activity. All subjects had been supine for at least 50 minutes prior to blood sampling for renin activity. Drugs were administered with 200 ml of fluid (0 hr), a further 200 ml was given with a light breakfast (2 hr), 400 ml with lunch (4 hr) and 200 ml with an evening snack (9 hr).

Serum and urinary electrolytes were determined using a routine autoanalyser by the hospital biochemistry laboratory.

7.3.2. RESULTS

A. Salt depletion

In general, the salt depletion regimen was well tolerated and no spontaneous symptoms were reported. Urinary volume was significantly increased on the third day (pre-study day), in comparison with the screening day, and this was associated with significant falls in urinary sodium excretion (Table 7.4). There was a non-significant trend towards a rise in urinary potassium excretion but urea and creatinine excretion were unchanged. Urine volumes were significantly lower during the two study days with a significantly greater degree of sodium loss following enalapril. Potassium excretion was not significantly altered but showed a trend to reduction following enalapril.

Salt depletion was associated with significant reductions in serum sodium (142.5±1.0 at screening, 138.9±0.8 and 139.6±1.3 $mmol.l^{-1}$ at baseline on the 2 study days) and in serum potassium (4.2±0.3 on screening, 3.8±0.2 and 3.7±0.2 $mmol.l^{-1}$ at baseline on the 2 study days). Changes in serum electrolytes during the study day are illustrated in Figure 7.11.

Screening blood pressure was reduced from 124/69±8/7 supine and 125/77±12/6 mmHg to 115/69±8/7 and 108/65±9/6 supine and 113/67±5/9 and 113/67±12/9 mmHg at baseline on the 2 study days.

B. Enalapril v placebo

Because supine blood pressure was slightly higher at baseline on the enalapril study day responses were individually corrected to the pretreatment (baseline) values on each study day. Baseline corrected changes in blood pressure are illustrated in Figure 7.12. Enalapril caused a mean maximal fall in supine systolic blood pressure of 18.9±7.6 mmHg at about 5 hours post dose, compared to 6.5±6.8 mmHg following placebo.

Similarly, enalapril caused a significantly greater fall in erect systolic blood

Table 7.4: Effect of salt depletion regimen and study treatments (placebo or enalapril 5 mg po) on urinary volume and electrolyte composition comparing screening baseline with day 3 of salt depletion and the treatment study day (day 4).

	Volume (ml)	Sodium mmol/vol	Potassium mmol/vol	Chloride mmol/vol	Urea mmol/vol	Creatinine mmol/vol
Baseline	1279 ±106	189 ± 69	72 ±16	150 ±17	429 ±106	19 ±4
Prestudy(d3) (Placebo)	1714 ± 628	98 ±39	87 ±12	103 ±35	375 ±91	17 ±2
Prestudy(d3) (Enalapril)	2101 ±1336	97 ±20	91 ±14	101 ±27	446 ±57	20 ±7
Placebo(d4)	1148 ±319	11 ±6	71 ±11	10 ±7	384 ±63	15 ±2
Enalapril(d4)	1003 ±284	25 ±7	59 ±15	21 ±14	435 ±56	15 ±2

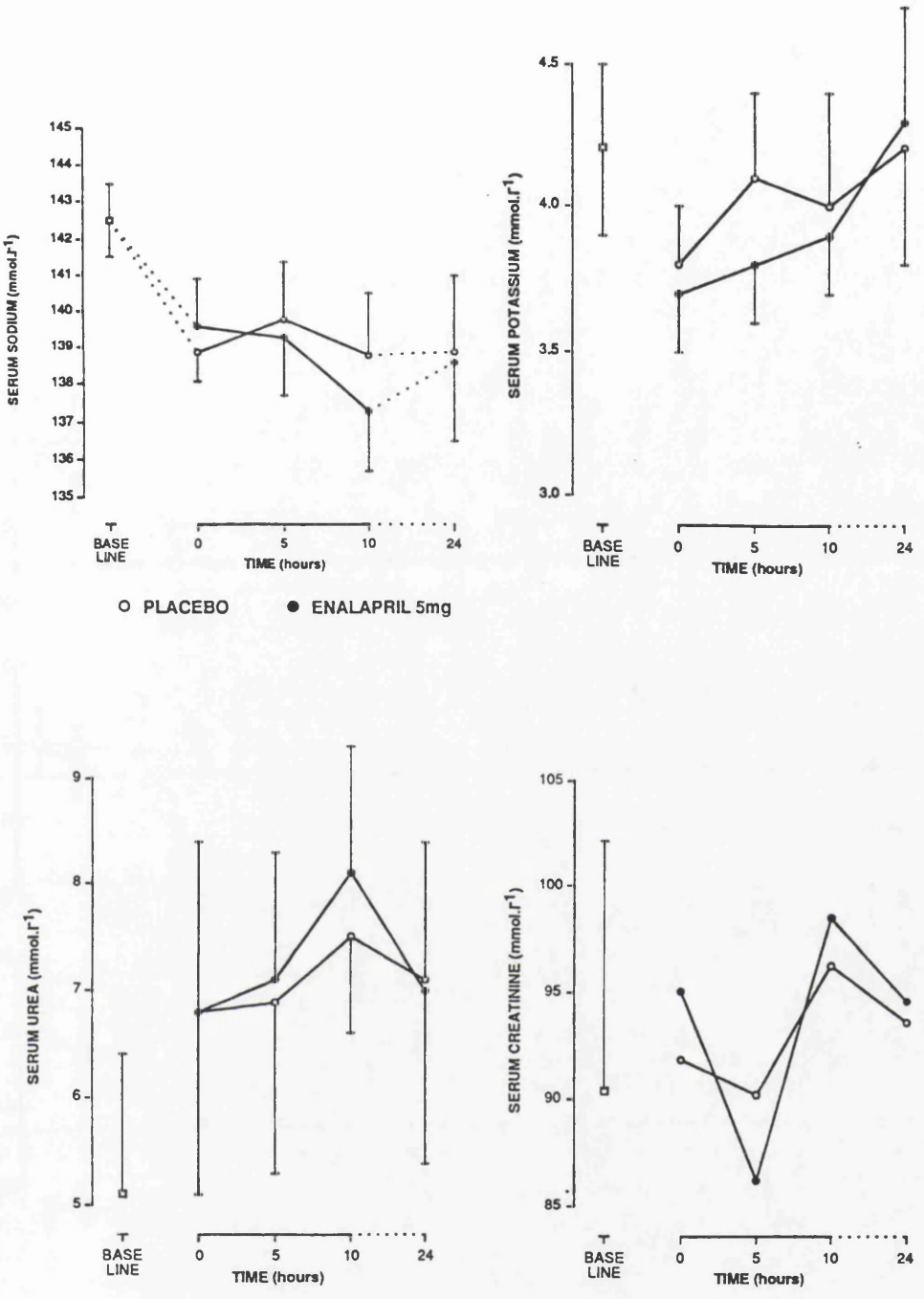


Figure 7.11: Changes in serum (a) sodium, (b) potassium,(c) urea, (d) creatinine (mean ± 1SD) from baseline, salt replete (screening) values and during the study day (○) placebo, (●) enalapril.

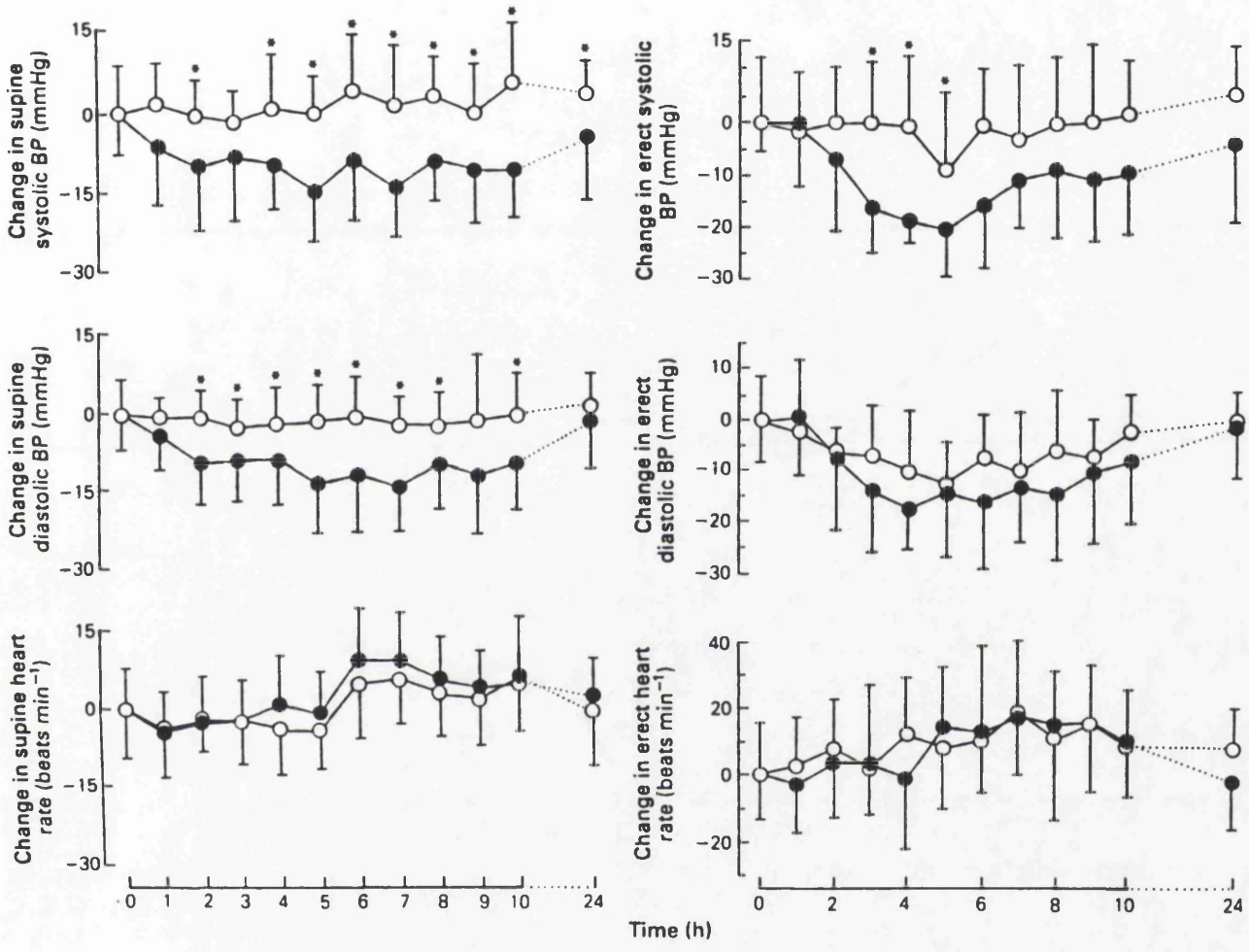


Figure 7.12: Baseline corrected changes (mean ± 1SD) in (a) supine and (b) erect blood pressures and heart rate following oral placebo (○) or enalapril (●).

rate was not significantly altered by either therapy but showed a tendency to rise during the latter part of the study day (following lunch).

C. Drug concentrations, ACE inhibition and plasma renin activity

Drug concentrations of enalapril and enalaprilat and plasma ACE inhibition are shown in Figure 7.13. The peak plasma enalapril concentration of 50±20.8 ng/ml was observed at 1.25±0.25 hours but the maximum concentrations of enalaprilat were not attained until 4-6 hours post dose. Near maximal plasma ACE inhibition was observed after about 2 hours and maximum inhibition of 78±5.7% occurred at 5.2±1.2 hrs.

Supine plasma renin activity was significantly elevated at baseline on each study day (6.0±3.2 on the placebo day and 5.2±2.1 ngAI.ml.hr^{-1} on the enalapril day) compared to salt replete pretreatment screening values (0.9±0.5 ngAI.ml.hr^{-1}). The profile of plasma renin activity showed a rise following enalapril but also during placebo therapy following food (Figure 7.14).

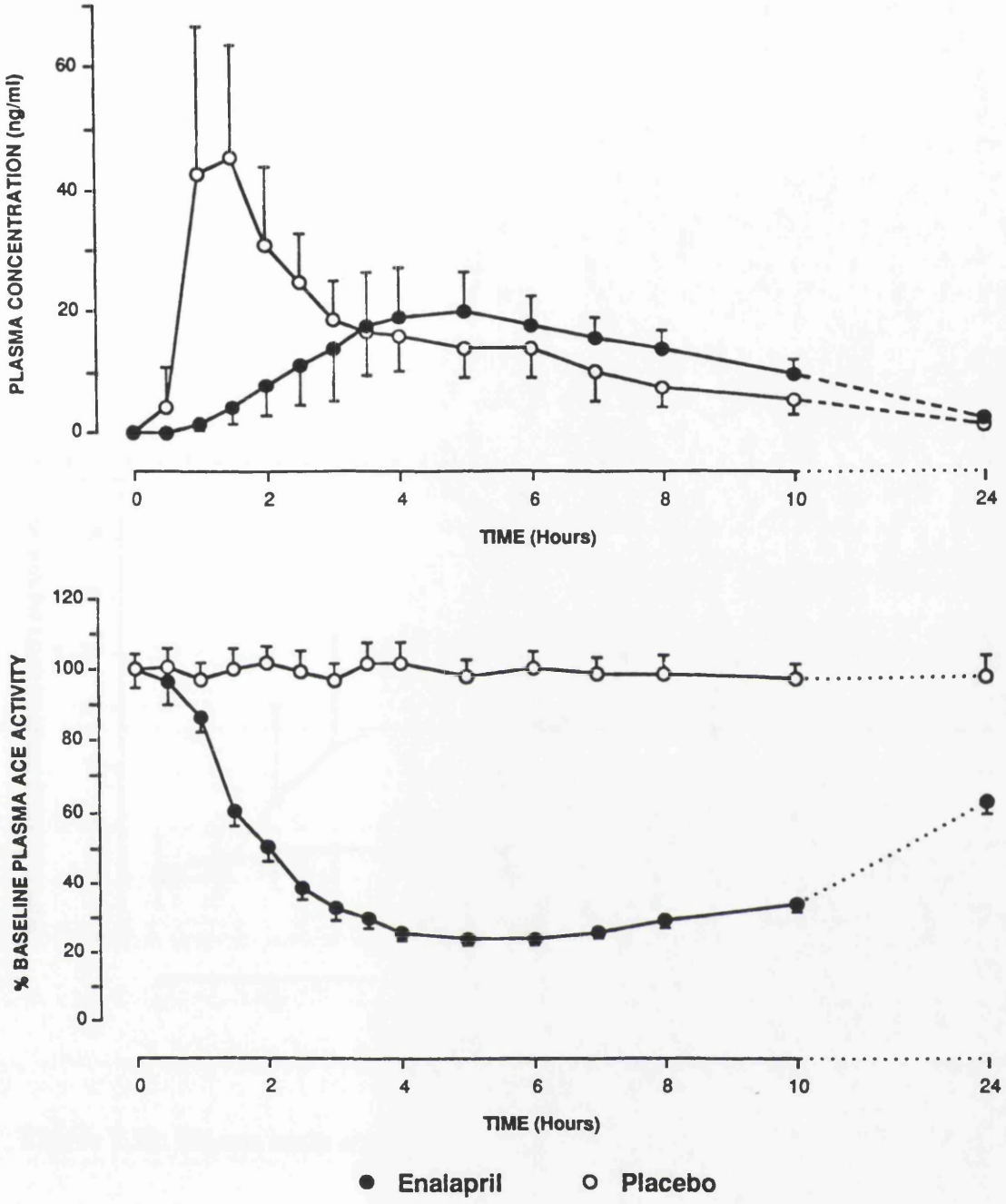


Figure 7.13: Drug concentration time profiles (mean ± 1SD) of enalapril (○) and enalaprilat (●) and plasma ACE activity.

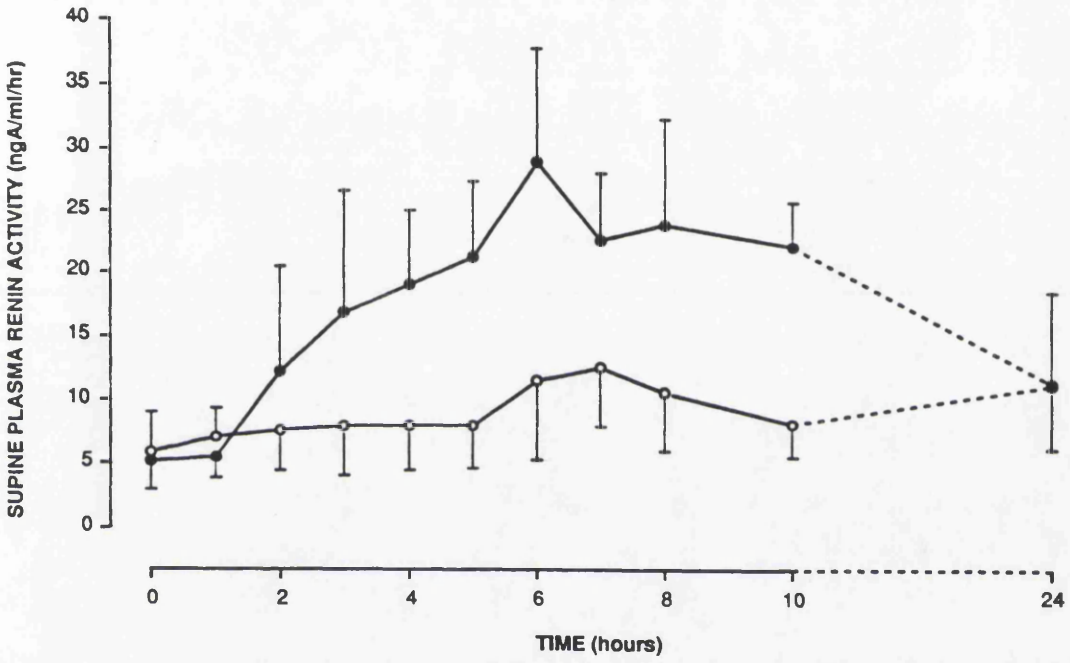


Figure 7.14: Plasma renin activity (mean ± 1SD) following enalapril (●) or placebo (○).

7.4 DISCUSSION

7.4.1 TRANSPULMONARY ACE INHIBITOR INFUSION

This study represents the first description of the transpulmonary pharmacokinetics of an ACE inhibitor in man.

Pulmonary ACE activity and the effects of ACE inhibitors on the enzyme at this site have been studied *in vitro, ex vivo* and in a limited number of *in vivo* animal experiments. The structure of the enzyme in pulmonary homogenates is suggested to be only marginally different from the circulating "soluble" enzyme and it has been suggested that the pulmonary circulation may be the source of circulating ACE activity (Das and Soffer, 1988). The enzyme has been localised on the vascular endothelium of the lung, in common with that found in a range of organs (Ryan et al, 1980, 1985), and has particular functions in the response to pulmonary hypoxia (Pitt et al, 1987; Oliver et al, 1989) or to chemical injury (Hollinger et al, 1980; Kelley, 1988). It is known to be present in association with alveolar macrophages (Friedland et al, 1977). In addition to its role as an exopeptidase contributing to localised AII generation, ACE has less clearly defined endopeptidase functions (Re and Rovigatti, 1988).

Studies in animals have shown temporal dissociation of plasma and pulmonary tissue ACE inhibition from the hypotensive response to ACE inhibition. It has been suggested that tissue ACE inhibition better describes the pattern of this response than the inhibition of ACE from a plasma sample (Cohen and Kurz, 1982; Chevillard et al, 1989; Kamei et al, 1989).

Previous animal work by Gillis and colleagues in rabbits had successfully defined pulmonary tissue ACE and its inhibition by captopril by employing single pass pharmacokinetic studies of the bolus injection of the synthetic ACE substrate, benzoyl-phenylalanyl-alanyl proline (BPAP) with the co-infusion of the intravascular marker dye ICG (Howell et al, 1984; Chen et al, 1984; Moalli et al, 1985; Linehan et al, 1985).

As the structure and function of ACE is likely to differ between species (Takada et al, 1982; Ibarra-Rubio et al, 1989) there has been difficulty defining the qualitative or quantitative aspects of tissue ACE inhibition in man. The relative importance of tissue ACE in generating the pharmacodynamic responses to ACE inhibitors in normal volunteers or patients remains difficult to assess. The use of a pharmacokinetic model to describe the concentration time profile of an ACE inhibitor may be a viable technique and this was studied in this and the subsequent section. This model incorporates elements describing a saturable competitive binding process to both plasma and tissue ACE (Lees et al, 1989)

Along similar lines to the studies of Gillis and colleagues in rabbits, this study was designed to use the pharmacokinetic profile of an ACE inhibitor in an attempt to mark tissue uptake, with the co-infusion of ICG to standardise for transpulmonary flow.

This study was conducted in a patient population who required central cardiac catheterisation as part of their on-going medical investigation. Both the dose and rate of infusion were chosen on the basis of previous studies in normal volunteers (Lees and Reid, 1987). The regimen was chosen to minimise the risk of adverse effects in a population with stable ischaemic heart disease and, as expected, the haemodynamic effects of infusion were minimal despite the presence of concomitant therapy in the population studied. Blood pressure data were collected primarily for reasons of safety and to exclude flow changes as a confounding variable in pharmacokinetic profile.

Care was taken in the study to eliminate errors due to inadequate pump priming by ensuring infusion prior to attachment to the peripheral venous cannulae. Furthermore, as ICG is known to aggregate in concentrated solution (Bjornsson et al, 1982) the infusate was prepared containing plasma protein solution to distribute the dye which was prepared along with the drug 5 - 10 minutes prior to commencing the study.

Previous studies using this dose and rate of infusion and peripheral venous

sampling had suggested that the early periods of accumulation were characterised by lower drug concentrations in peripheral venous blood suggestive of a sigmoid accumulation profile presumably due to the uptake of drug into the tissues during the process of arteriovenous passage (Lees et al, 1989). Despite a marked increase in the density of the sampling protocol the present study failed to find such a sigmoid accumulation profile in central venous samples taken from a main pulmonary artery.

Given the precautions taken with infusion the inhibition of circulating ACE activity in the initial samples was very marked. Clearly the available drug within 30 seconds of commencing infusion was sufficient to inhibit the majority of circulating ACE with a residual component available for tissue uptake. The concentrations in the central compartments would be subject to recirculation beyond the first 2-3 minutes.

There may be several reasons for the failure to demonstrate either a sigmoid pattern of accumulation or a significant early transpulmonary delay in drug concentration during this series of observations.

Although previous studies using this dose and rate of infusion had generated profiles which were suggestive of tissue binding of the drug influencing concentration time profiles a lower dose or rate of infusion might better define this type of profile given the marked ACE inhibition observed. In addition although the lungs have been regarded as the dominant source of ACE activity for the circulation this has had to be revised in the light of the widespread distribution of ACE activity at a variety of different tissue sites (Johnston et al, 1987). The relative proportion of accessible whole body ACE which is situated in the pulmonary circulation remains unknown. Previous studies involving peripheral venous sampling reflect exposure of the given dose to the "whole body" ACE pool and therefore the present study might not reflect exposure of the drug to a large enough pool of enzyme. In addition as an infusate containing plasma protein solution was employed, for reasons described above, this may have affected the

distribution of very small quantities of drug. In effect a slowed equilibration of drug between plasma and tissue ACE might not have been reflected in *ex vivo* ACE inhibition as a substantial time period occurred *ex vivo* prior to assay.

An arteriovenous concentration gradient would be expected from theoretical grounds with any drug which interacts with a tissue in order to generate its pharmacodynamic effect. However, such arteriovenous concentration time profiles are rarely defined (Chiou, 1989). In this setting the concentration of a drug at a given arterial or venous site is a reflection of both the blood flow at that site and the relative partition coefficient for the drug into the given tissue bed (Bischoff, 1986). Accordingly the flow rate across the lung is several hundred times greater than that for the peripheral sampling site and thus flow would be expected to dominate the partition of a drug such as an ACE inhibitor with its tissue binding sites which in this case would be predominantly tissue ACE. This element of site dependency in generation of a concentration time profile is probably the key element in our failure to describe a marked transpulmonary extraction of ACE inhibitors. It would seem unlikely that our patient population, predominantly with ischaemic heart disease, was associated with any particular reduction in pulmonary ACE sufficient to mask uptake. However, this aspect has not previously been studied.

Thus although unable to demonstrate a clear advantage of the non-linear saturable binding model over conventional multi-exponential pharmacokinetic models in this study individual data fits were often better described by the former structure. As a sum of exponentials or polynomial functions can always be formed to fit a series of observed data, the main problem is that these have no physical meaning and give no information about the primary system (Rescigno and Beck, 1987). It would be incorrect to suggest that the simple non linear models applied here are "physiological" but they may derive terms which are more descriptive of tissue binding and consequently better related to

pharmacodynamic effect. The observations in this section suggest that the most appropriate sampling site for such comparisons would be the traditional peripheral venous blood.

7.4.2 PERINDOPRILAT INFUSION STUDIES IN SALT REPLETE MAN.

An influence of protein binding on the pharmacokinetics of ACE inhibitors has been described by a number of groups. In particular, this binding is thought to occur to ACE in plasma and tissues (Francis et al, 1987; Lees et al, 1989) and for most ACE inhibitors this is a tight binding interaction at at least two binding sites in keeping with enzyme structure (Perich et al,1992). Present knowledge of the haemodynamic effects of ACE inhibitors stems from their use in clinical practice. The pharmacological studies which have been carried out offer very limited evidence of the effects which occur in response to low doses of these drugs. As a result, the minimum effective dose is poorly defined. This may be of particular importance where these drugs are given to individual patients who are at risk of a profound haemodynamic response.

The available information on the use of intravenous ACE inhibitors comes primarily from studies of pharmacokinetics and bioavailability (Reams et al, 1986; Nussberger et al, 1987; Evans et al, 1987; Creasey et al, 1988). A case for the clinical use of intravenous ACE inhibitors has been made both for accelerated hypertension (Rutledge et al, 1988; Savi et al, 1990) and for the management of acute heart failure (Flynn et al, 1988; Taylor et al, 1989) and preventing left ventricular dysfunction following myocardial infarction (Nabel et al, 1991; Sharpe et al, 1991; CONSENSUS II Study group,1992). These indications remain controversial (and indeed possibly detrimental) and are not approved by regulatory authorities. The available experience

with intravenous therapy is limited to bolus injection or infusion over periods as short as 2 to 10 minutes. In many of the studies rapid dose escalation has been employed and this has hampered the description of the dose-response relationship in that neither haemodynamic, primary or seconary hormonal equilibrium is possible or in many cases documented.

When the approximate oral bioavailability of the drugs and the known effects on inhibition of circulating plasma ACE activity are taken into account, the intravenous doses which have been administered are likely to have been maximal or in most instances supramaximal. This would make an adequate description of the concentration response relationship impossible and more importantly limit dose titration and control over the fall in blood pressure.

In this study a clear haemodynamic response was documented in young salt replete normal volunteers after a low dose of the diacid ACE inhibitor perindoprilat. In addition, by using slow constant rate infusion it is evident that the slower rates of infusion are at least as effective in lowering BP as faster rates with equivalent total dosage. This is despite a correspondingly reduced C_{max} and reduced maximal plasma ACE inhibition. The larger doses of ACE inhibitor given acutely or in this study by more rapid infusion, result in a high free fraction of drug which is readily eliminated by the kidney. Smaller doses or slower administration result in a lower free fraction. The ACE inhibitors are almost unique, in that the free fraction is **not** the determinant of drug effect and it is the bound fraction which is inhibiting ACE, predominantly that present at tissue based sites which results in the haemodynamic response. Thus, the drug is less "efficient" in generating its haemodynamic response when greater concentrations are achieved through rapid infusion. A similar pattern of response has recently been documented for the calcium channel-blocking drug, felodipine (Cohen et al, 1990).

The earlier work with perindoprilat by Lees suggested that the accumulation

phase of the pharmacokinetic profile might provide further information on the tissue uptake of ACE inhibitors. The pharmacokinetic profile was best described by a non-linear saturable binding relationship and the mathematical description of this profile provided estimates of the maximal binding and the extent of inhibition of "tissue" ACE (Lees et al, 1989). This study provided more detailed confirmation of a sigmoid drug accumulation profile, most notably with the 6 hour infusion. It has reaffirmed the superiority of the non-linear saturable binding models for description of ACE inhibitor disposition. The parameter estimates agree closely with those previously reported (Lees et al, 1989).

The individual parameter estimates which can be obtained from pharmacokinetic analysis may reflect overall tissue binding and ACE inhibition. These estimates can be obtained from drug concentration data in peripheral venous blood. They now need to be related to the haemodynamic response. In normal subjects blood pressure changes are definable but limited as seen in this study. A setting of enhanced response is requred to relate concentration to haemodynamics.

Whilst low dose constant rate intravenous infusions of ACE inhibitors are not currently employed in clinical practice. These results reaffirm the findings in heart failure (section 6.2). If intravenous treatment is indicated, constant rate low dose infusion should be considered. Large falls in blood pressure with intravenous administration are likely to be due simply to the supramaximal doses employed. The temporal dissociation of blood pressure response to short infusions of perindoprilat (1 mg) and the high peak plasma drug concentration reaffirm this message. Slow constant rate infusion should allow better control over blood pressure by discontinuation of infusion where marked sensitivity is encountered.

7.4.3 ACE INHIBITION IN SALT DEPLETE MAN: ORAL ENALAPRIL IN A MODEL OF THE ACTIVATED RENIN ANGIOTENSIN SYSTEM.

The primary aim of this study was a basic description of a human model for the detailed study of ACE inhibitor responses, using readily acceptable means of inducing activation of the renin system. Although dietary salt restriction has been widely used in this context, a very low sodium diet (< 10 mmol/Na) has usually been employed and controlled studies with modern renin analysis are rare (Hollenberg et al,1981; Navis et al,1987). Such low salt diets are barely palatable for unselected volunteers; difficult to control under outpatient circumstances; require careful preparation; and are liable to non-compliance. In this respect, where controlled studies involving salt repletion and depletion are required over prolonged or repeated periods the nature and composition of the diet becomes even more important to the individual volunteers. We therefore chose moderate sodium restriction (40 mmol) with dietary provision and instruction over the four day study periods. In order to produce further sodium loss and to achieve activation of renin angiotensin system oral frusemide (40 mg b.d.) was administered on each of the three pre-study days. The pharmacodynamic response to frusemide has been extensively characterised (Hammarlund-Udenaes&Benet,1989; Boles-Ponto & Schoenwald,1990). To prevent overt fluid depletion, which might predispose to reduced intravascular volume, a high fluid intake was specifically encouraged during the pre-study period and similarly, the fluid intake on the study day was carefully controlled. Volunteers tolerated the pretreatment well and, in particular, none spontaneously commented on marked diuresis at any point in the study.

Both the serum and urinary electrolyte data on the third day of baseline and the pretreatment values on the study day suggest moderate salt depletion and renin angiotensin system activation. The programme of frusemide pretreatment, with the gap

between the last oral dose and the study day avoids any acute haemodynamic effects which follow frusemide treatment (Johnston et al,1984) and, as predicted, produced a persistent degree of hyper-reninaemia (Tuck et al,1982).

Significant reductions in pretreatment serum sodium and potassium were achieved without overt hyponatraemia or hypokalaemia. Similarly, urinary urea or creatinine values were not significantly altered from baseline values and this provides further reassurance that overt fluid depletion was not caused and additionally that urinary collections were reasonably complete. In keeping with the moderate but persisting activation of the renin angiotensin system during the study day, there appeared to be some degree of antinatriuresis although there continued to be some urinary sodium loss from the 40 mmol dietary provision.

Although not universally accepted, measurable falls in blood pressure have been demonstrated in controlled studies in salt replete volunteers (Kiowski et al,1992; Lees & Reid,1991) but the magnitude of the response is small and may limit the value of such studies, at least with regard to blood pressure, as a means of assessing the response to renin angiotensin system blockade. In this study, despite the low dose of enalapril, there were considerable reductions in blood pressure.

The role of baseline renin angiotensin system activation in the blood pressure response to ACE inhibitor treatment has practical as well as theoretical importance. Since the introduction of ACE inhibitor into the treatment of hypertension and heart failure there has been widespread clinical concern over the so called "first dose hypotensive response" which occasionally is symptomatic and rarely is associated with renal, cerebral and cardiac hypoperfusion damage. The precise origin of this profound blood pressure fall is unclear but many factors may play a role: salt and fluid balance, the status and setting of baroreceptor reflexes, concurrent or temporarily discontinued drugs such as diuretics, vasodilators or digitalis and more general factors such as drug

dose, age, diurnal blood pressure patterns, metabolism and elimination pathways and individual subject sensitivity. Using a model of renin angiotensin system activation such as that described here it will be possible to address in controlled double blind studies the contribution of the particular drug, the dosage selected and the level of activation of the renin angiotensin system to the overall pattern of response in terms of blood pressure, heart rate, regional perfusion, etc. Similarly, the differential effects of alternative means of renin angiotensin system blockade based on orally active drugs blocking the rate limiting enzyme, renin or angiotensin receptor blocking drugs. The baseline level of circulating renin seen in our study is easily comparable with those seen in renovascular hypertension (Hodsman et al,1982) or heart failure (Anand et al,1989) but derived in more controlled circumstances.

Double blind studies can readily be performed by providing oral salt repletion in addition to the standard 40 mmol sodium diet using coated tablets and placebo frusemide. In this setting active salt depletion is produced by matched placebo salt tablets and active frusemide. Such controlled observations are not common in the assessment of ACE inhibitor responses despite 15 years of clinical study. The newer agents for renin angiotensin system blockade could also benefit from such assessment.

In summary, this study has described a simple and well tolerated model of the activated renin angiotensin system in man based on moderate dietary sodium restriction and acute diuretic therapy. This model is suitable for initial dose ranging and dose-response studies with a variety of agents which act via the renin angiotensin system, for studies of the role of renin angiotensin system activation in individual response and for more detailed concentration effect studies integrating secondary neurohormonal changes with treatment.

CHAPTER 8

CONCLUDING REMARKS

The studies on angiotensin converting enzyme inhibition presented here demonstrate, in controlled circumstances, that the haemodynamic profile of apparently similar agents can be differentiated at least at low doses in heart failure patients. This is the first occasion that such differentiation has been noted in any clinical or experimental study. Regardless of the practical significance of first dose hypotension, which remains very much an anecdotal phenomenon, this differentiation is of considerable pharmacological importance and may have some clinical application in avoiding excessive haemodynamic effects in some circumstances.

The observations with oral prodrugs and intravenous diacids may suggest that a differential interaction with extravascular renin angiotensin systems as one explanation for this observation. Clearly this requires verification and further analysis with direct measurements of the tissue based system being the most logical step. This would most easily be achieved in animal studies but, as was explored in chapter 7, a pharmacokinetic approach may be one alternative and has the considerable advantage of being viable in intact man.

The controlled low dose infusion studies in heart failure conducted over relatively protracted periods were shown, albeit in a stable population of heart failure patients, to give good control over the extent of the blood pressure response. In practical terms this might lend itself to further use of this technique in more unstable patients should therapy with these drugs be required for example in the period around acute myocardial infarction or in the management of acute heart failure of an appropriate cause.

The tentative description of a transient pressor response in these subjects was an

unexpected finding. Again it emerged largely due to the controlled setting of the observations and regular recordings. Although likely to be of less therapeutic significance it may prove, on further examination, to be a further reflection of the tissue based renin system and its activation in these patients. Exploration of this phenomenon should probably be aimed at initially confirming the effect and detailed measurements of the circulating concentrations of angiotensin I and angiotensin II peptides. This could test the possibility of a peptide distribution phenomenon based on transient intravascular ACE inhibition by the poorly lipid soluble diacid drugs at the low concentrations seen during the early stages of constant rate infusion.

The studies in normal and patient volunteers summarised in chapter 7 focus on the pharmacokinetic description of tissue distribution of diacid ACE inhibitor drugs in the first two sections. This is the essential first stage to any attempt to link drug concentration and effect (eg haemodynamic effect) in individual subjects using a concentration effect modelling technique.

The transpulmonary studies were entirely novel in man but unfortunately failed to reveal the first pass extraction of drug across the pulmonary circulation that was expected. The possible reasons for this relate most probably to the dose of drug and rate of infusion. It did not prove possible to repeat the study with a lower dose or rate of infusion or indeed to study the effect on a lipid soluble agent such as one of the prodrug inhibitors.

The description of diacid pharmacokinetics on protracted infusion confirmed the efficacy of a simple non linear binding model incorporating terms for tissue and plasma binding parameters as the optimal technique of the range considered. This was not adequately described in terms of drug concentration profile during the infusion stage in the only previous study. The non linear binding model allows parameter estimates to be derived for individual subjects which might be related to patterns of haemodynamic

response through a more complex concentration effect model. Although small blood pressure changes were noted in this controlled study it is likely that, at the very least, activation of the renin system would be required to approximate the setting in heart failure and make the correlation of the kinetic and dynamic response feasible.

The third section in chapter 7 clearly established an outline of salt depletion based on a practical schedule of diuretic treatment and low salt diet which was well tolerated, easily administered and controlled. The responses observed with a standard ACE inhibitor, enalapril given orally, were clear cut and activation of the renin angiotensin system confirmed at a number of levels.

This series of studies can now progress to define the pharmacokinetic and pharmacodynamic responses to ACE inhibition during double blind salt repletion or salt depletion and relate tissue binding parameters to the observed blood pressure response in individual subjects.

In conclusion a variety of studies are presented directed at the investigation of blockade of the renin angiotensin system in the setting of common disease, heart failure, where this system is activated. Detailed analysis of response can pay dividends both at the level of pathophysiology, drug differentiation and optimisation of treatment, in this instance with relevance to the initiation of therapy.

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