CLINICAL PHARMACOLOGY
OF
CALCIUM ANTAGONISTS

Studies on the pharmacodynamics and the pharmacokinetics of calcium antagonists in man

A THESIS PRESENTED
FOR THE DEGREE OF
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BY

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PREFACE

This thesis describes research undertaken in the Department of Materia Medica, University of Glasgow, at Stobhill General Hospital.

While the primary responsibility of all the research work was my own, many of the studies have been done in collaboration with Dr. Henry Elliott and Dr. Peter Meredith. The study on renal blood flow was done with the collaboration of Dr. David Sumner, Department of Nuclear Medicine. The study on platelets was done with Dr. Richard Jones, who carried out the aggregation tests.

The writing of this thesis is my own work.

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SUMMARY

This thesis has investigated several aspects of the clinical pharmacology of calcium antagonists in man.

The first study in normal volunteers was designed to compare the acute pharmacodynamics and pharmacokinetics of a new dihydropyridine analogue, nisoldipine, with those of the established drug, nifedipine. The results with both drugs showed only minor changes in blood pressure and heart rate, both drugs had similar terminal elimination half-lifes and there were no major adverse effects.

The antihypertensive potential of nisoldipine was, therefore, next assessed in a group of patients with essential hypertension following both single and multiple dosing. The efficacy of the drug was demonstrated after acute dosing and was maintained during a one month period with nisoldipine as monotherapy.

For many antihypertensive drugs it has proved difficult to describe correlations between plasma concentration and therapeutic effect and accordingly this was one of the specific aims of a subsequent study of the acute and chronic effects of the calcium antagonist verapamil in a further group of hypertensive patients. To investigate this relationship in detail in individual patients concentration effect analysis was applied and similar relationships between the fall in blood pressure and the plasma concentration of verapamil were found after both acute and

chronic treatment.

Verapamil and nisoldipine were then used in a comparative study to investigate the inter-relationship between alpha adrenergic receptors and calcium channels. The effects of each drug on the pressor responses to adrenergic and non adrenergic vasoconstriction were assessed in a group of normotensive subjects. Intravenous incremental infusions of phenylephrine and alpha-methylnoradrenaline were used to measure the effects on peripheral vascular responsiveness, mediated via $alpha_1$ and $alpha_2$ adrenoceptors respectively, and angiotensin II was similarly used to assess non adrenergic responsiveness. Despite the theoretical differences in the relative peripheral vascular actions of these two drugs a comparable attenuation of all pressor responses was observed after both acute and repeated administration. Additionally, there was no evidence from this study in man to substantiate the finding in some animal experiments that calcium antagonists preferentially antagonised responses mediated via $alpha_2$ adrenoceptors.

The inter-relationship between alpha adrenoceptors and calcium channels was also assessed in two further studies. With both verapamil and nisoldipine, during 4 days oral dosing, there was significant inhibition of platelet aggregation. This effect, however, was only in part due to an inhibition of $alpha_2$ adrenoceptor mediated aggregation. The combined effects of calcium channel blockade and alpha

adrenoceptor antagonism were then assessed in a study with verapamil and prazosin.

The therapeutic potential of this combination was investigated in normotensive subjects to evaluate the possible dynamic and kinetic interactions between the two drugs. The combination showed an earlier, longer and greater hypotensive effect than either drug alone. In part this increased effect was due to changes in prazosin pharmacokinetics with increases in peak concentration and AUC indicating an enhancement of prazosin bioavailability.

Changes in liver blood flow, as assessed by indocyanine green clearance, were thought to have a role in the interaction between verapamil and prazosin. Both verapamil and nisoldipine were shown to cause acute increases in apparent liver blood flow, in normotensive subjects. Similar changes in renal function were observed. With verapamil there were transitory increases in effective renal plasma flow whereas nisoldipine significantly increased glomerular filtration rate and urinary sodium excretion in addition to effective renal plasma flow. The changes returned towards placebo values following repeated administration.

In the final part of the thesis the effects of calcium antagonists on the release of various hormones were studied. Nicardipine, following intravenous infusion and oral administration in a group of normotensive subjects, attenuated the pressor response produced by exogenous

angiotensin II but did not inhibit significantly the secretion of aldosterone. In a clinical study in hypertensive patients, nifedipine did not show significant changes in insulin secretion and glucose tolerance in response to an intravenous load. Thus, although there is experimental evidence for the central role of calcium ions on hormone release, these studies failed to demonstrate a clinically significant effect attributable to calcium antagonist drugs.

In conclusion the results of these studies indicate that the antihypertensive effects of calcium antagonist drugs result primarily from their activity as peripheral vasodilators. This effect includes an action on adrenergic mediated vasoconstriction but there was no evidence of an additional antihypertensive component related to interference with aldosterone release. The relaxant effect on smooth muscle was not confined to the peripheral vasculature but also caused transitory alterations of renal and liver blood flow. These changes, particularly if they directly influence drug clearance, may be important for the interactions with co-administered drugs. More research is still required to define the clinical pharmacology of calcium antagonists and their role in the treatment of hypertension.

CHAPTER 1

INTRODUCTION

1.1. HISTORICAL REVIEW

Dan Shen is the name of a traditional Chinese remedy used for the treatment of coronary disorders.

It is derived from the roots of "Salvia Miltiorrhiza Bunge" and the alcoholic extract, "tanshinone II A sulphonate", which is the active principal, has been shown to have calcium entry blocking properties. These are likely to be responsible for its clinical efficacy (Patmore and Whiting, 1982).

In the early 1960s the first calcium antagonist, verapamil, was introduced into clinical practice in Germany as an antianginal agent but originally it was classified as a beta adrenoceptor blocker (Melville and Benfey, 1965). Further research showed that verapamil also possessed local anaesthetic properties and important antiarrhythmic activity (Melville et al, 1964; Schmid and Hannah 1967; Garvey, 1969).

In 1969 Fleckenstein classified a heterogenous group of drugs and called them "calcium antagonists" to indicate their common property of inhibition of the influx of calcium into cardiac and vascular smooth muscle cells (Fleckenstein et al, 1969). The mechanism of action of verapamil was thus elucidated (Fleckenstein et al, 1972; Fleckenstein, 1977) and it represented the prototype of a class of antiarrhythmic agents (Singh et al, 1972) different from beta adrenoceptor blockers and quinidine-like drugs. In the 1970s verapamil began to receive consideration also in

the United States (Singh et al, 1978).

The other landmark of the calcium antagonist story was the synthesis of nifedipine, a dihydropyridine derivative, in 1971. This substance was first used in Germany and Japan for treatment of angina. Since then the concept of calcium antagonism has become more widely accepted and calcium antagonists have become useful therapeutic agents in the treatment of angina pectoris and cardiac arrhythmias (Opie, 1980; MacLean and Feely, 1983).

It was further realised that the vasodilator properties of these substances were not specific for the coronary artery and that they have powerful peripheral arterial vasodilator and afterload reducing properties (Wartlier et al, 1981; Kahan et al, 1981; Aoki et al, 1982a). Over the last few years several experimental and clinical studies have focused attention on the value of drugs of this class in the treatment of arterial hypertension. By decreasing the entry of calcium ions into vascular smooth muscle cells, calcium antagonists effectively decrease the strength of contraction causing a vasodilation. As most hypertensive patients show increased peripheral vascular resistance, the use of calcium antagonists as a therapeutic approach to high blood pressure seemed most appropriate, and early results were very encouraging (Guazzi et al, 1977; Bartorelli et al, 1978). It is noteworthy that since 1969, research undertaken in Germany and elsewhere had shown the efficacy of the calcium antagonist verapamil in the treatment of

hypertensive crisis (Brittinger et al, 1969; Bender, 1970). More clinical experience has demonstrated that calcium antagonists are effective not only in severe, but also in mild to moderate hypertension, giving a blood pressure reduction both at rest and during exercise (Midtbo et al, 1982; Klein et al, 1983; Murphy et al, 1983). Nifedipine and verapamil are the most widely used drugs of this class in the treatment of hypertension and have been joined by diltiazem. Recently other dihydropyridines including nitrendipine and nicardipine have been actively tested. There is now real prospect of development of newer compounds with greater selectivity of action but their role in the treatment of hypertension remains to be established.

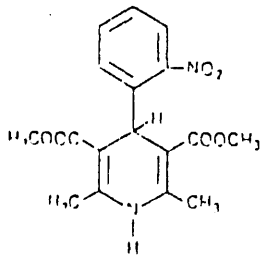
Further therapeutic possibilities in the control of a variety of cardiocirculatory disorders have been proposed. For example, the reported efficacy of nimodipine, a nifedipine analogue, in the treatment of cerebral vasospasm associated with subarachnoid haemorrhage (Allen et al, 1983) and the haemodynamic improvement following diltiazem in patients with pulmonary hypertension (Crevey et al, 1982).

1.2. CLINICAL PHARMACOLOGY OF CALCIUM ANTAGONISTS

The organic substances used as calcium antagonist drugs can be divided into several distinct groups (Figure 1.1a, b and c).

1) Papaverine derivatives: such as verapamil, gallopamil, tiapamil

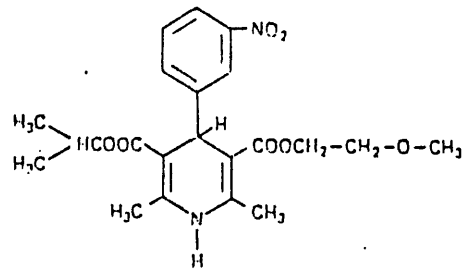
DIHYDROPYRIDINE DERIVATIVES



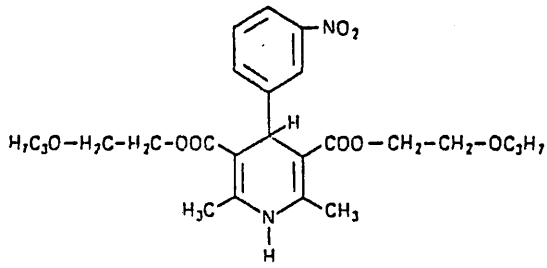
nifedipine

Fig.1.1.a

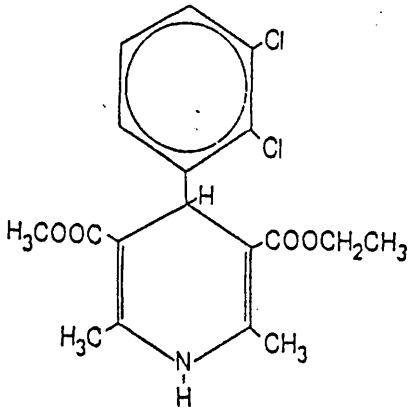
Structural formulae of calcium antagonist drugs



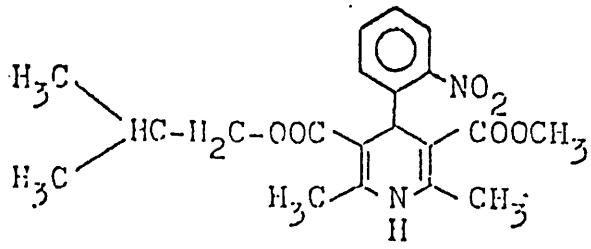
nimodipine



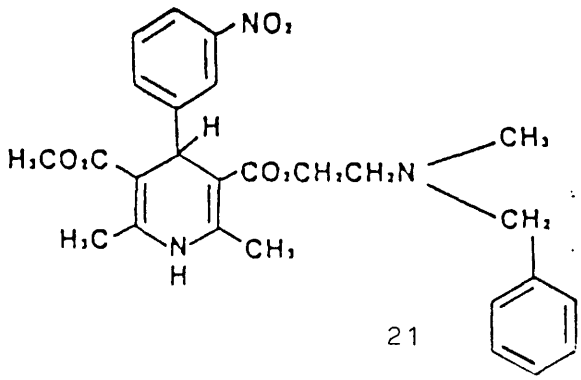
niludipine



felodipine



nisoldipine



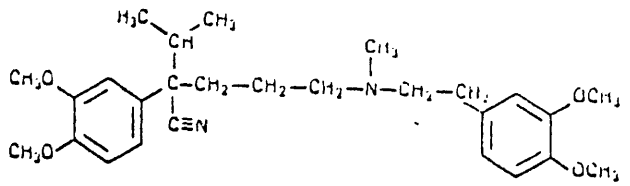
• HCl

nicardipine HCl

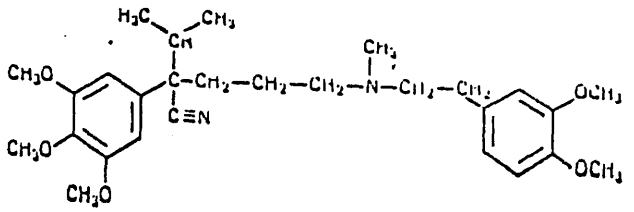
PAPAVERINE DERIVATIVES

Fig.1.1.b

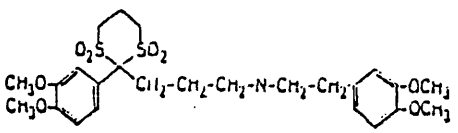
Structural formulae



verapamil

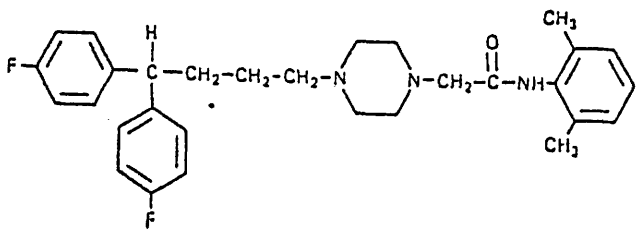


gallopamil

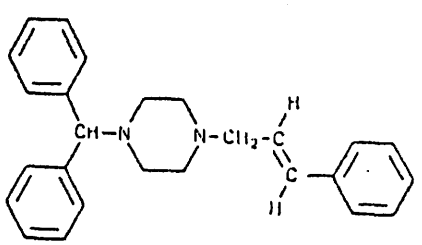


tiapamil

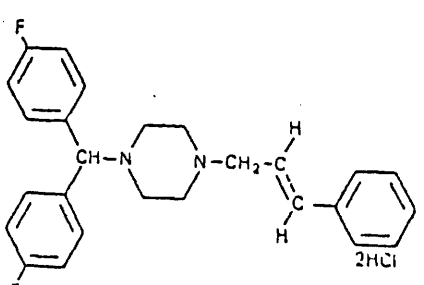
PIPERAZINE DERIVATIVES



lidoflazine



cinnarizine

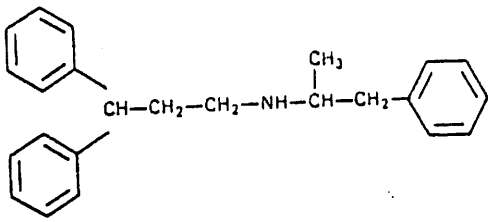


flunarizine

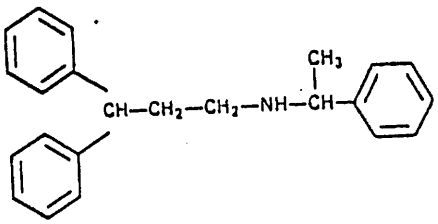
Fig.1.1.c

Structural formulae

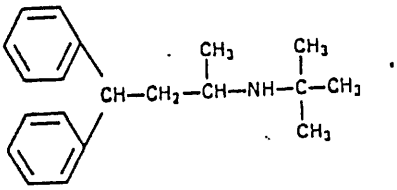
OTHERS



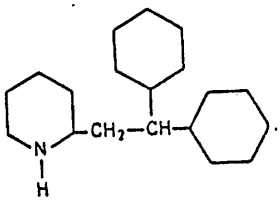
prenylamine



fendiline

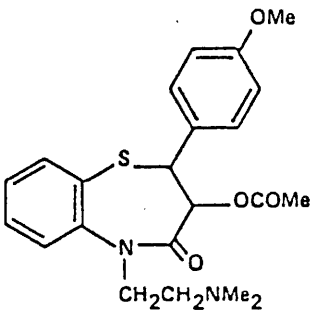


terodiline



perhexiline

BENZOTHIAZEPINE DERIVATIVE



diltiazem

2) Dihydropyridine derivatives: felodipine nicardipine, nifedipine, niludipine, nimodipine, nisoldipine.

3) Piperazine derivatives: cinnarizine, flunarizine, lidoflazine

4) Benzothiazepine derivates: diltiazem

5) Others: prenylamine, fendiline, terodiline, perhexiline.

The action common to all groups is inhibition of the inward movement of Ca^{++} through the cell membrane of heart muscle and vascular smooth muscle. There are, however, important cellular differences to be considered. In the myocardial cell depolarisation is initiated by a rapid influx of sodium ions via the fast inward channel. When the transmembrane potential has fallen a second inward current develops. It is mainly due to Ca^{++} ions and it determines the plateau phase of the cardiac potential. This slow influx of Ca^{++} is believed to pass through channels anatomically different from those of the fast current (Triggle and Swamy, 1983). A further mechanism of calcium entry involves Na^{+}/Ca^{+} exchange (Blaustein, 1977). All these events lead to an increase of intracellular Ca^{++} which stimulates release of more Ca^{++} from intracellular sites in the sarcoplasmic reticulum. The high intracellular Ca^{++} concentration activates a cascade of events to allow the interaction between actin and myosin and the shortening of sarcomere (Braunwald, 1982).

In the smooth muscle cell contraction is initiated by the activation of the slow channels (Zelis and Flaim, 1981). Two distinct types of calcium channels have been postulated: a potential dependent calcium channel and a receptor operated channel (Figure 1.2.). The influx of Ca^{++} through the latter can also release Ca^{++} from intracellular stores. Intracellular Ca^{++} links to calmodulin, a calcium modulating protein, which activates a myosin light-chain kinase (Tomlison et al, 1984). The sequence of events will lead to the interaction between actin and myosin to produce contraction. A connection between alpha adrenergic receptors and calcium channels has been recently established. In vascular smooth muscle, $alpha_1$ adrenergic stimulation releases Ca^{++} from an intracellular source resulting in an early rapid phase of contraction followed by the late slow phase related to the influx of Ca^{++} across the membrane (Deth and Van Breemen, 1974). $Alpha_2$ adrenoceptors also appear to modulate other calcium channels (Van Zwieten and Timmermans, 1983) (Figure 1.3.).

From a therapeutic point of view the most interesting drugs are verapamil and nifedipine. In vitro experiments have shown that the two drugs depress electrical excitability, slow atrioventricular conduction and are negative inotropic agents. On vascular smooth muscle they act as a vasodilator but the relative potency of verapamil is less than that of the dihydropyridine derivatives. Some new agents have shown greater vasodilator effects on

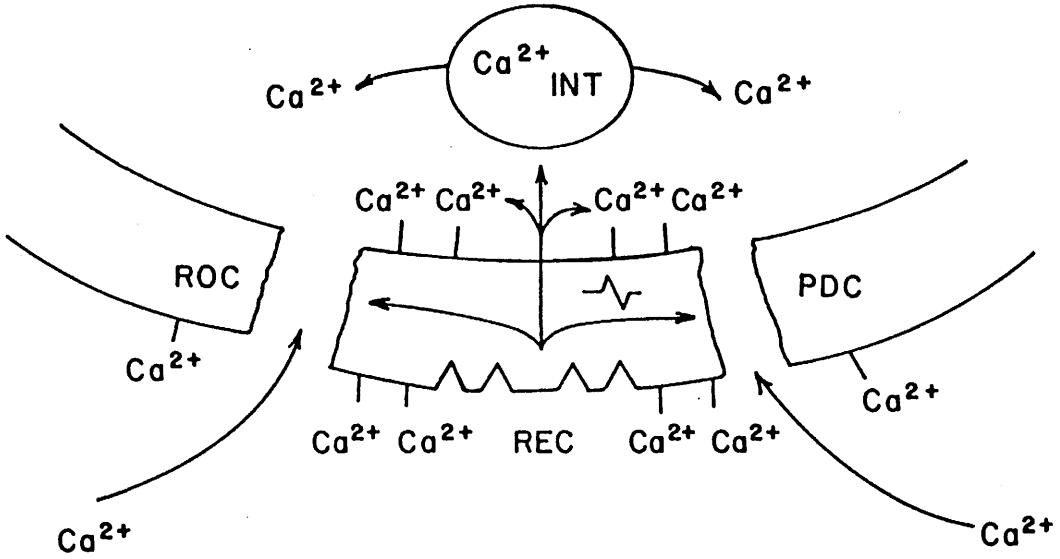


Fig. 1.2. Ca^{++} influx through receptor operated (ROC) and potential dependant channel (PDC).

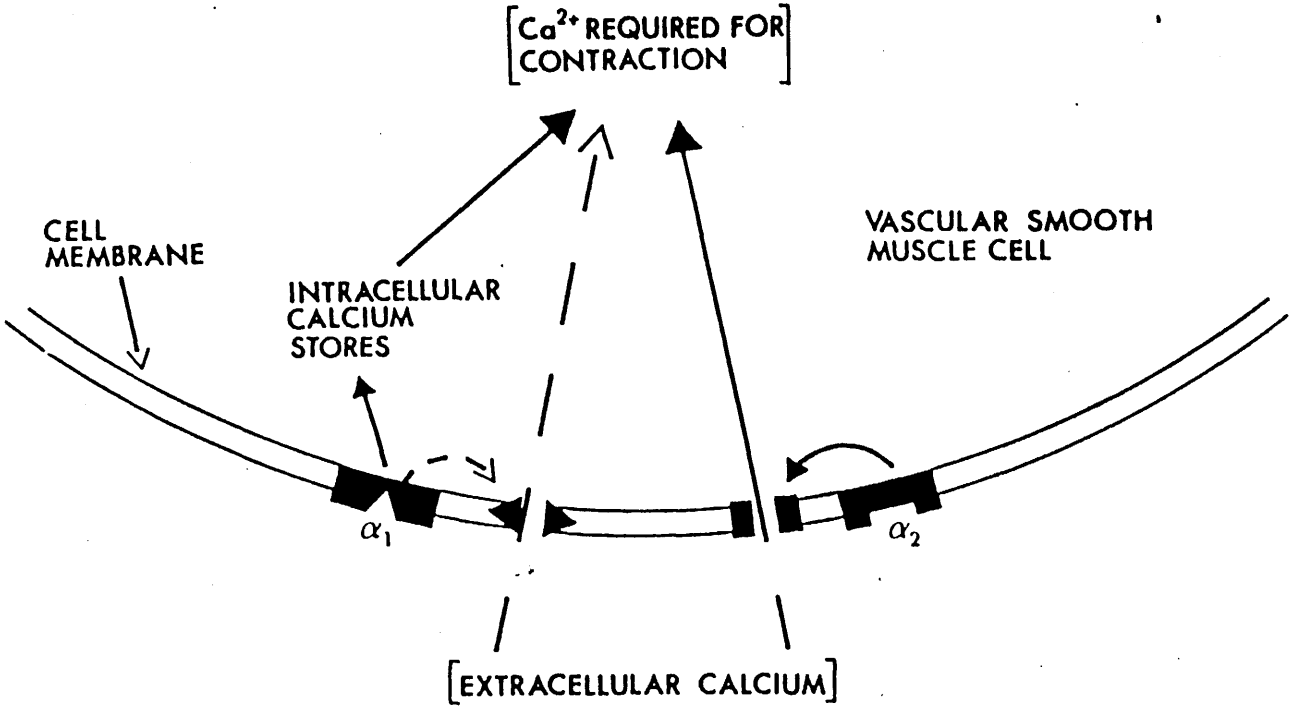


Fig. 1.3. Possible relationship between the post-synaptic alpha-1 and alpha-2 adrenoceptors and the membrane calcium channels.

coronary arteries and peripheral vessels. In clinical practice, at therapeutic doses they do not depress heart contractility and are particularly suitable for the treatment of hypertension (Table 1.1.).

Nifedipine is well absorbed after oral or buccal administration. An intravenous formulation has not been widely available because of poor solubility and photolability. Peak plasma concentrations of drug occur one or two hours after oral dosing. Nifedipine is extensively protein bound in plasma and is metabolised to yield lactone and "free acid" pyridine derivatives (Waller et al, 1984). The elimination half life of nifedipine has been reported as between two and five hours. It has been measured by GLC electron capture methods although some of these methods have not distinguished the parent drug from the metabolites. A study on the antihypertensive effects of nifedipine revealed very wide interpatient variation in plasma levels relative to oral dosage. However, if individual correlations are examined, instead of groups, significant correlation between plasma nifedipine levels and the magnitude of the individual hypotensive response has been shown by several authors (Lederballe Pedersen et al, 1980a; Aoki et al, 1982b; Pasanisi and Reid, 1983). The side effects caused by nifedipine and most dihydropyridine derivatives are facial flushing, headache, early in treatment, and ankle oedema with more prolonged therapy (Kiowski et al, 1983a; Brennan et al, 1983).

TABLE 1.1.

RELATIVE PHARMACODYNAMIC EFFECTS OF CALCIUM ANTAGONISTS IN MAN

	NIFEDIPINE	VERAPAMIL
Coronary arterial dilatation	++	++
Peripheral arterial dilatation	+++	++
Negative inotropism	+	++
Hypotension	++	+
Reflex beta-adrenergic activity	++	+
AV nodal conduction disturbance	0	+++

TABLE 1.2.

PHARMACOKINETICS OF CALCIUM ANTAGONISTS

	NIFEDIPINE	VERAPAMIL
Oral absorption	> 90%	> 90%
Bioavailability	30-60%	10-20%
Onset of action (oral)	< 20 min	< 30 min
Protein binding	> 90%	> 90%
Elimination half-life (single dose)	2-6 h	3-7 h
Route of elimination	Hepatic	Hepatic
Active metabolite	No	Yes

More pharmacokinetic information is available for verapamil (Table 1.2.). It is widely available for both oral and intravenous administration. Verapamil is well absorbed after oral administration but undergoes extensive first pass metabolism. It is approximately 90% plasma protein bound (McAllister and Kirsten, 1980, 1982). A large number of metabolites have been identified using HPLC assay but only its N-demethylated metabolite, norverapamil appears to exert significant haemodynamic effects. Non linear pharmacokinetics appear to apply to the orally administered drug. A tendency for accumulation of verapamil has been shown during chronic dosing with a significant prolongation of the drug's elimination half life. There was also evidence for accumulation of norverapamil. This effect has been claimed to be due to limited capacity for hepatic metabolism of the drug. The clearance of the drug also appears to depend on hepatic blood flow and is markedly impaired in patients with liver disease (Eichelbaum et al, 1981; Semplicini et al, 1982; Weiner et al, 1984).

A wide range of side effects of the various slow channel blocking drugs have been described. After long term oral administration verapamil may cause constipation. Other side effects are gastric irritation, vertigo, agitation and headache. Overdose of verapamil may lead to atrioventricular dissociation and severe hypotension has occurred. The former is more likely if a beta blocker has

been given concurrently.

1.3. CALCIUM ANTAGONISTS IN HYPERTENSION

It is generally agreed that an increase in cytosolic calcium concentration represents the signal that initiates the contractile process in vascular smooth muscle. There is also experimental evidence that calcium handling by smooth muscle cells is abnormal in most forms of hypertension (Wei et al, 1976; Zsoter et al, 1977; Daniel and Kwan, 1981). The development, therefore, of drugs that interfere with the entry of Ca^{++} ions into cells has provided the clinicians with important new therapeutic agents for use in the treatment of systemic arterial hypertension.

The available clinical data suggest a potentially important role for the calcium channel blockers in the future. Their clinical utility has not, however, been fully established, particularly with respect to adverse effects during long term therapy. To date studies of the calcium antagonists have mainly examined the acute and chronic (months) treatment of hypertension in uncontrolled studies. Preliminary information is available regarding the combination of calcium antagonists with other antihypertensive drugs, but such combinations need further evaluation.

Several reports demonstrating the efficacy of nifedipine in patients with hypertensive emergencies have been published (Ueda et al, 1979; Guazzi et al, 1977). After sublingual administration of nifedipine mean arterial

pressure decreased significantly. The onset of action was seen within minutes and the main side effect was facial flushing (Beer et al, 1981; Ueda et al, 1979). Acute reduction in blood pressure was often (Aoki et al, 1978; Lederballe Pedersen et al, 1980a) but not always (Corea et al, 1980) accompanied by an increase in heart rate, probably due to baroreceptor mediated reflex stimulation.

The effect is dose related (Lederballe Pedersen et al, 1980b) and the higher the blood pressure before treatment, the greater the fall in pressure that follows the drug (Ekelund et al, 1979; Guazzi et al, 1980). Young normotensive subjects do not usually drop their blood pressure in response to nifedipine although transient tachycardia is seen.

There are fewer studies concerning the usefulness of calcium antagonists as single agents in chronic hypertension (Lederballe Pedersen and Mikkelsen 1978; Buhler, 1983).

The efficacy of nifedipine appears to be dose-related, with a moderate decrease in arterial pressure following doses of 10 mg of the capsule formulation (Ueda et al, 1979; Levenson et al, 1983) and more dramatic reductions in pressure occurring with 30 mg doses (Aoki et al, 1976). The duration of antihypertensive action is also dose-related (Lederballe Pedersen and Mikkelsen, 1978). During chronic oral therapy, nifedipine 10 mg has an antihypertensive effect which lasts 8-12 hours. Administration every 6 hours significantly reduces blood pressure throughout the

day. This drug also causes an acute increase in urinary output and sodium excretion (Klutsch et al, 1972) and raises plasma renin activity and noradrenaline concentration in the short term (Aoki et al, 1978; Corea et al, 1979, 1980). However plasma aldosterone does not change (Lederballe et al, 1979). Chronic treatment did not result in any consistent change in plasma renin activity (Lederballe et al, 1979; Corea et al, 1980). These results suggested that nifedipine may be useful as a single agent in the control of hypertension.

Verapamil has been successfully used intravenously during acute hypertensive crises (Bender, 1980). As for nifedipine, oral administration of verapamil has little or no effect on blood pressure of normotensive subjects but lowered blood pressure of hypertensives with no significant change in heart rate and plasma renin activity (Leonetti et al, 1980; Corea et al, 1981). Verapamil is also effective in the long term treatment of mild to moderate essential hypertension in daily doses ranging from 240 to 720 mg (Leonetti et al, 1980; Midtbo and Hals, 1980; Anavekar et al, 1981; Corea et al, 1981). Conflicting results, however, have been reported by other groups. Its hypotensive effect was not sustained in one trial of 320-640 mg daily over a seven week period (Lederballe, 1978). In a later study, using a continuous intra-arterial pressure monitoring, before and after six weeks of oral verapamil treatment (120-160 mg b.d.) the drug was demonstrated to

produce a significant reduction in blood pressure (Gould et al, 1982). The wide individual variation in the response to verapamil has been explained on the basis of high first pass metabolism (Midtbo, 1980).

When verapamil and nifedipine have been directly compared a similar antihypertensive activity has been demonstrated (Gould et al, 1982; Leonetti et al, 1982; Muiesan et al, 1982).

There is clearly a need for further controlled studies of calcium antagonists alone and in combination with other agents in patients with hypertension.

1.4. SCOPE OF THE THESIS

Calcium ions have a central role in a variety of processes particularly in the contraction of muscle cells in the heart and the peripheral vasculature. This forms the theoretical basis for the potential usefulness of substances antagonising calcium influx into the cell in a wide range of cardiovascular disorders. With respect to the clinical use of calcium antagonists, the review of the literature has revealed a lack of detailed information on aspects of the pharmacokinetics and the pharmacodynamics, particularly of the dihydropyridine derivatives.

In this thesis detailed studies have been undertaken with both verapamil and nifedipine, and additionally with the analogues nisoldipine and nicardipine. These studies have addressed several themes. For example the clinical

haemodynamic effects of nifedipine have been compared with the new dihydropyridine nisoldipine in normal volunteers and subsequently the new drug was tested in hypertensive patients. An evaluation of the relationship between plasma concentration of verapamil and blood pressure reduction was also undertaken in hypertensives. The mechanisms underlying the peripheral vascular effects of calcium antagonists have been assessed by investigating the relationship between calcium channels and alpha adrenoceptors in the regulation of vascular tone. A study of the pressor responses to different agonists in man and an evaluation of platelet aggregation during administration of calcium antagonist drugs were undertaken. Detailed studies of the interaction between a calcium antagonist and a selective $alpha_1$ adrenoceptor blocker are also described. Further pharmacodynamic and pharmacokinetic issues concerning the effects of acute and prolonged dosing with calcium antagonist drugs on renal and hepatic function have been studied. This has possible implications for the kinetics during long term administration and differences in the acute and chronic effect. Finally, the effects of nicardipine and nifedipine on hormone secretion has been evaluated in normotensive and hypertensive subjects.

This thesis represents an attempt to clarify some aspects of the clinical pharmacology of calcium antagonist drugs.

CHAPTER 2

METHODS

2.1. General Procedure

All the protocols of the clinical studies described in this thesis were approved by the Research and Ethical Committee of the Northern District of the Greater Glasgow Health Board. All subjects gave their informed written witnessed consent prior to participation. Studies were performed in a quiet room at constant temperature in the Clinical Pharmacology Research Unit of the Department of Materia Medica. A clinical examination, full biochemical, haematological and ECG screening was undertaken before the study and repeated at the end.

Subjects attended the CPRU at approximately 8.30 in the morning of the study having avoided all drugs for at least one week before each study day and abstained from alcohol, caffeine-containing beverages and cigarette smoking from 22.00 hours the day before the study. A light breakfast of orange juice and toast was allowed between 7 and 7.30 on the morning of the study unless otherwise specified. A blood pressure cuff was applied and an indwelling cannula (Venflon, Viggo) inserted into an antecubital vein for blood sampling and for the infusion of pressor substances. Cannulae were kept patent by intermittent flushing with heparinised saline solution. During the infusions standard ECG leads were applied and the ECG displayed on a Grass Polygraph (Grass, Polygraph).

Following a period of 30 minutes quiet recumbency in

bed after insertion of the cannula, baseline readings of blood pressure and heart rate by semiautomatic recorder (Sentron, Bard Biomedical) were taken and blood samples collected for baseline (time 0) drug or hormone levels. Thereafter subjects received their treatments. Subsequent blood pressure readings were taken at frequent intervals, in the supine position after at least 10 minutes recumbancy and on standing after 2 and 5 minutes, or until the systolic pressure fell to 80 mmHg or less, or until orthostatic symptoms were experienced by the subjects. Blood samples were also taken throughout the day at corresponding times.

Observations were typically made over a period of 10 hours and side effects were carefully recorded.

Full resuscitation equipment was available at all times.

2.2. Determination of plasma noradrenaline concentration

Plasma noradrenaline concentrations were estimated by the radioenzymatic assay of Henry et al (1975). Venous blood was withdrawn from an indwelling cannula inserted in the forearm and collected into lithium heparin tubes. Plasma was separated by centrifugation at $4^{o}C$ for 15 minutes at 3000 rpm and stored at $-70^{o}C$ until assay.

The method utilizes the conversion of norepinephrine (NE) to tritiated epinephrine (E) by partially purified

bovine adrenal phenylethanolamine-N-methyl-transferase (PNMT) and tritiated S-adenosyl-methionine (SAME).

The first stage of the assay is the concentration phase. To 1 ml of plasma was added 100 µl of sodium metabisulphite, 5 mg 100 ul noradrenaline as internal standard, 50 mg of alumina, 0.5 ml of tris buffer (1.0 M tris HCl containing 2 g/100 ml EDTA: pH 8.6. The mixture was agitated and washed with water several times, the supernatant removed and 100 µl of it added to 100 ul of 0.01 M H:Cl. The external standard was prepared with 1 ng noradrenaline in a volume of 100 ul and 100 ul 0.1 M HCl). The blank contained 100 µl of 0.01 M HCl and 100 ul 0.1 HCl.

The reaction mixture was made up dissolving 1 mg DTT into 10 ml tris buffer (2.0 M tris HCl containing 5 g/100 ml EDTA: pH 8.6) to give a final concentration of 0.1 mg/ml. To 1.5 ml of the mixture were added 0.1 ml tritiated-S-adenosyl methionine (H-SAMe) and 0.4 ml PNMT.

Fifty ul of the mixture were added to each sample and incubated for 1 hour at 37°C in an agitated water bath. The reaction was terminated by addition of 2 ml tris phosphate buffer 2.0 M tris H/Cl containing 0.5 Na phosphate + H_2O, 5 g/100 ml EDTA: pH 8.6). In the final stage 50 mg of alumina were added and after vortexing supernatant was removed. One ml 0.1 M perchloric acid, 200 µl phosphotungstic acid and 100 µl non tritiated SAMe were added.

After vortexing, refrigeration and centrifugation 1 ml

was taken and added to 1 ml potassium phosphate in a plastic tube. Upper organic phase was separated by freezing lower aqueous layer in acetone/dry ice. A vial containing 0.4 ml permafluor was used for counting in a liquid scintillation counter (PACKARD TRICARB). The intra assay coefficient of variation was 10% and the interassay coefficient of variation was 12-15%.

2.3. Determination of plasma renin activity

Plasma renin concentration was measured by incubation of plasma with sheep renin substrate (angiotensinogen) at 37°C by the method of Skinner (1967). Angiotensin I generated during the incubation was assayed by radioimmunoassay. Venous blood was withdrawn from an indwelling cannula inserted in the forearm and collected into potassium EDTA tubes. Plasma was separated by centrifugation at 4°C for 15 minutes at 3000 rpm and stored at -20°C until assay. The first step of the assay consisted in the incubation of renin with angiotensinogen to generate angiotensin I. Fifty ul of plasma were added to 50 µl of fresh buffer inhibition mixture (45 ml phosphate buffer pH 7.5 + 1 ml PMSF solution + 2 ml trasylol + 2 ml 8 OHQ) and to 100 µl sheep renin substrate (containing 0.1% neomycin sulphate). Two sets of samples were taken: one was incubated for 1.3 hours at 37°C and the other at 4°C for the same time. Two hundred ul of tris acetate buffer (tris base 0.1 µl/l + neomycin 0.2% w/v + bovine serum albumin

0.35% + lysomycine 0.1%) were added to stop the enzymatic reaction. Samples were then stored at -20°C. The second step of the assay is the radioimmunoassay of generated angiotensin I. Ninety µl of renin antibody were taken and dilute to 100 ml tris acetate buffer at 4°C. One ampoule of I^{125} angiotensin I (New England Nuclear) was dissolved in 5 ml tris acetate buffer. This solution was diluted so that 50 µl gave counts of 10,000-12,000 cpm.

Fifty ul of the I^{125} angiotensin I solution were added to 50 µl of the generated angiotensin I samples or standards and to 500 µl of the antibody solution for each sample. Samples were incubated at 4°C for 18-24 hours. After separation of free and bound antigen by charcoal separation and followed by centrifugation samples were counted using a gamma counter (BERTHOLD LB 2100, MULTI CRYSTAL GAMMA COUNTER).

The intra-assay coefficient of variation was 5.5%. The inter-assay coefficient of variation was 10%.

2.4. Determination of Plasma Aldosterone Concentration

Plasma aldosterone concentrations were estimated by radioimmunoassay of De Man et al (1980) and using materials supplied in kit form by CIS International (St. Quentin, France).

Venous blood was withdrawn from an indwelling cannula inserted in the forearm and collected into lithium heparin tubes. Plasma was separated by centrifugation at 4°C for

15 minutes at 3000 rpm and stored at -20°C until assay. The principle of the assay is based on the competition between the I^{125} labelled aldosterone and aldosterone contained in standards or specimens to be assayed, for a fixed and limited number of antibody binding sites. After the incubation, the amount of labelled aldosterone bound to the antibody is inversely related to the amount of unlabelled aldosterone present in the sample. In the commercial preparation the anti-aldosterone serum is bound to the inner surface of the tube so that separation of free and antibody-bound ligand is achieved by decanting, whereas a dextran charcoal separation with dissolved antibody is described by De Man. The assay of each plasma sample was performed in duplicate. The radioactivity of all tubes were measured using a gamma counter (BERTHOLD LB 2100, MULTI CRYSTAL GAMMA COUNTER).

The intra-assay coefficient of variation was 7.3% and the inter-assay coefficient of variation was 10%.

2.5. Determination of Serum Insulin Concentration

Serum insulin concentrations were estimated by radioimmunoassay of Zick et al (1982) and using materials supplied by kit from Behringweeke AG (Marburg, W. Germany). Serum from blood samples was separated after blood collection and stored at -20°C. 100 µl standard or serum and 200 µl distilled water were added to the test tubes for the insulin assay. The contents of test tube were dissolved

and mixed thoroughly on a vortex. Samples were covered and incubated for 24 hours at 20°C. 1 ml of polyethylene glycol solution was added to each test tube to effect the separation of antibody-bound and free insulin. The solution was homogenised on a mixer before centrifugation of the turbid solution for 15 minutes at 1,500 rpm.

The supernatant fluid was drawn off and radioactivity of the precipitate of the tube was evaluated for one minute in the ^{125}I-channel of a gamma scintillation counter.

The intra-assay coefficient of variation was 10% and the inter-assay coefficient of variation was 15%.

2.6. Determination of Serum C-Peptide Concentration

C-peptide concentrations were estimated by radioimmunoassay using material supplied in kit from ByK-Mallinckrodt (Dietzenbach, W. Germany). 100 µl of C-peptide standard or serum were added to test tubes plus 100 µl ^{125}I C-peptide solution. 100 µl of C-peptide antiserum were also added to each test tube and mixed on vortex. Test tubes were incubated for 24 hours at 20°C. 500 ul goat-anti-rabbit-gamma globulin solution was added to each test tube and mixed on vortex. Test tubes were incubated for 30 minutes at 20°C then centrifuged at 2000 rpm for 20 minutes. Supernatant was removed and remaining activity counted with a gamma-counter.

The intra-assay coefficient of variation was 10% and the inter-assay coefficient of variation was 15%.

2.7. Determination of verapamil and norverapamil

The method used is based on the direct high-performance liquid chromatographic (HPLC) analysis of an extract of a relatively small sample volume, and permits the simultaneous measurement of verapamil and norverapamil in the presence of the two remaining principal metabolites. The metabolite D 517 was used as internal standard (Cole et al, 1981).

The solvent delivery system was a constant-flow reciprocating pump (Applied Chromatography Systems, Model 750/04) and sample injection was performed using a Rheodyne Model 7125 syringe-loading valve fitted with a 100 ul sample loop. Stainless steel tubing (0.25 mm I.D.) was used to connect the outlet port of the valve to the analytical column, a stainless steel tube 125 x 5 mm I.D. packed with Spherisorb 5 silica (Hichrom. Woodley, Great Britain), which was used at ambient temperature (normally 22°C). The column effluent was monitored using a Schoeffel Model FS 970 fluorescence detector, with an excitation wavelength of 203 nm, no emission filter and a time constant of 0.5 sec. The mobile phase was a solution of potassium bromide (3 mM) and perchloric acid (0.37 mM, equivalent to 0.004%, v/v) in methanol, and was helium-degassed before use. The flow rate was 2.0 ml/min, maintained by a pressure of approximately 60 bar.

The chromatography on this system of a methanolic solution containing verapamil and the three metabolites under study, together with the internal standard, is

illustrated in Figure 2.1. The retention times, measured relative to the internal standard, of verapamil, the metabolites under study and some additional compounds are given in Table 2.1.

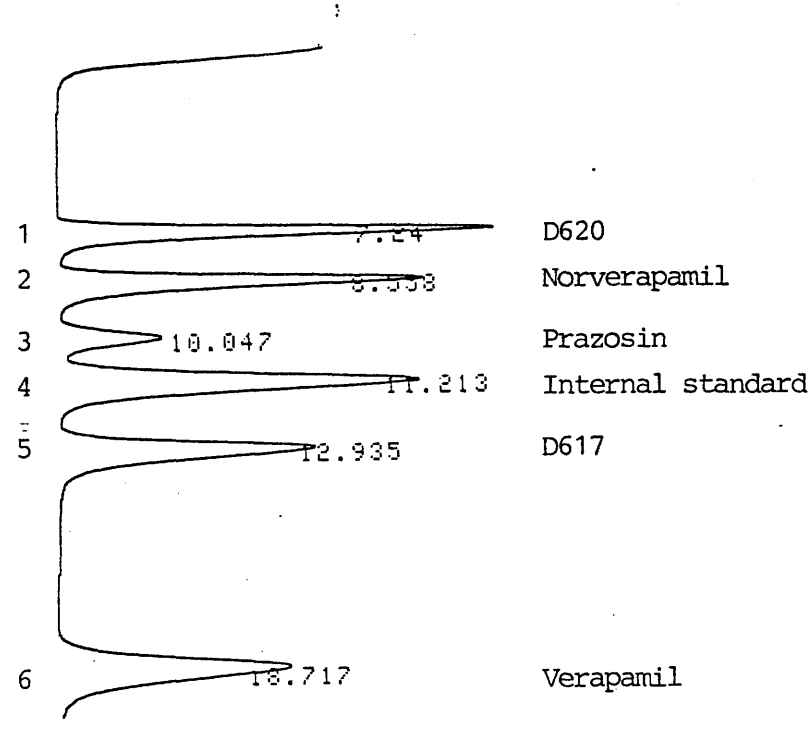
Plasma or serum (100 µl) was pipetted into a small (Dreyer) test tube (Poulton, Selfe and Lee, Wickford, Great Britain). Internal standard solution (50 µl), sodium hydroxide solution (50 µl) and methyl tert.-butyl ether (200 µl) were added using Hamilton repeating mechanisms fitted with Hamilton gas-tight luer-fitting glass syringes and stainless steel needles. The contents of the tube were vortex-mixed for 30 sec and centrifuged at 9950 g for 2 min in an Eppendorf centrifuge 5412 (Anderman, East Molesey, Great Britain). Subsequently, a portion (approximately 110 µl) of the extract was taken and used to fill the sample loop of the injection valve.

Duplicate sample analyses were performed, and the mean result taken.

The intra-assay and the inter-assay coefficients of variation were less than 5% for both verapamil and norverapamil. The limit of detection of the assay was 2 µg/L using a 100 µl sample.

2.8. Determination of Prazosin

The assay was performed according to the method of Yee et al, 1979. Whole blood or plasma 0.1 - 1.0 ml, was added to an 8 ml capacity culture tube fitted with a PTFE-lined



CHROMATOPAC C-R3A

SAMPLE NO	0	FILE	3
REPORT NO	108	METHOD	1043
IS WT	100	SAMPLE WT	100

PKNO	TIME	HIGHT	MK	IDNO	CONC	NAME
1	7.24	3502		2		D620
2	8.558	2941	V	3		NV
3	10.047	848	V	4		P
4	11.213	2914	V	1		IS
5	12.935	2070	V	5		D617
6	18.717	1848		6		VERAP
	TOTAL	14124			0	

Fig. 2.1. Standard HPLC chromatogram.
Simoultaneous determination of verapamil and prazosin.

Tab. 2.1. Characteristics of the peaks.

screw cap and containing 54 ng of the internal standard added to 100 µl of water. Water was added to samples with less than 1.0 ml, so that all tubes had an equal volume of aqueous phase. The blood was alkalinized with 200 µl of 2 N sodium hydroxide and extracted immediately with 5 ml diethyl ether to prevent the formation of solid aggregates. Samples were mixed on a Labquake for 10 min and then centrifuged for 10 min. The aqueous phase was frozen by placing the tube in an acetone-dry ice bath and the organic phase decanted into an 8 ml tube with an elongated cone at its base, of approximately 30 µl capacity, containing 20 µl of 0.1 N sulfuric acid. The sample was extracted with a Vortex mixer for 1 min, the tubes chilled in the refrigerator for 10 min and then centrifuged for 5 min. All or part of the dilute sulfuric acid, sampled through the diethyl ether with a 25 µl syringe, was injected into the high pressure liquid chromatograph.

A Varian model 8500 dual pump gradient elution high pressure liquid chromatograph fitted with a varian micro-Pak MCH-10 (monomeric C_{18} bonded) reversed-phase column (25 cm x 2.0 mm I.D.) was used for the analysis. One pump contained a 0.01 M solution of pentane sodium sulfate in water adjusted to pH 3.4 with glacial acetic acid (solvent A). The other pump contained the same concentrations of pentane sodium sulfate and acetic acid as solvent A in methanol (solvent B). Both solvents were filtered before use. An isocratic mixture of 49% solvent B and 51% solvent A was

used with daily minor adjustments in solvent composition (1-2%) to maintain optimum baseline separation of prazosin and the internal standard. The flow rate of the solvent mixture was 40 ml/h, with a column input pressure of 150 atm (2300 p.s.i.). The column was insulated with sponge rubber in order to minimise baseline noise. A Varian Fluorichrom with a deuterium lamp and Baird Atomic 20 nm bandpass filters, 253.1 nm for excitation and 390 nm for emission was used. A 0.5 μm porosity stainless-steel frit was placed on the efferent side of the detector to maintain the detector pressure thereby preventing formation of bubbles. A Varian A25 dual pen recorder was employed with one pen set at 1 mV full scale deflection and the other varied between 2 mV-50 mV depending on the expected concentration of the sample.

A standard chromatogram is shown in Figure 2.2.

The assay was calibrated by adding known amounts of prazosin (0.2 ng-50 ng) and internal standard (54ng) to 1 ml of whole blood or plasma which was then analysed. The peak height ratio (PHR) of prazosin to the internal standard was plotted versus the amount of prazosin added. To determine the accuracy and precision of each set of unknown samples a calibration curve consisting of 0.2, 0.5, 1, 2, 5, 10, 20 and 50 ng of prazosin was assayed along with the unknown samples. The PHR of the standard samples were divided by the amount of the prazosin added to derive the amount of prazosin in the unknown samples and the coefficient of variation provides an estimate of the

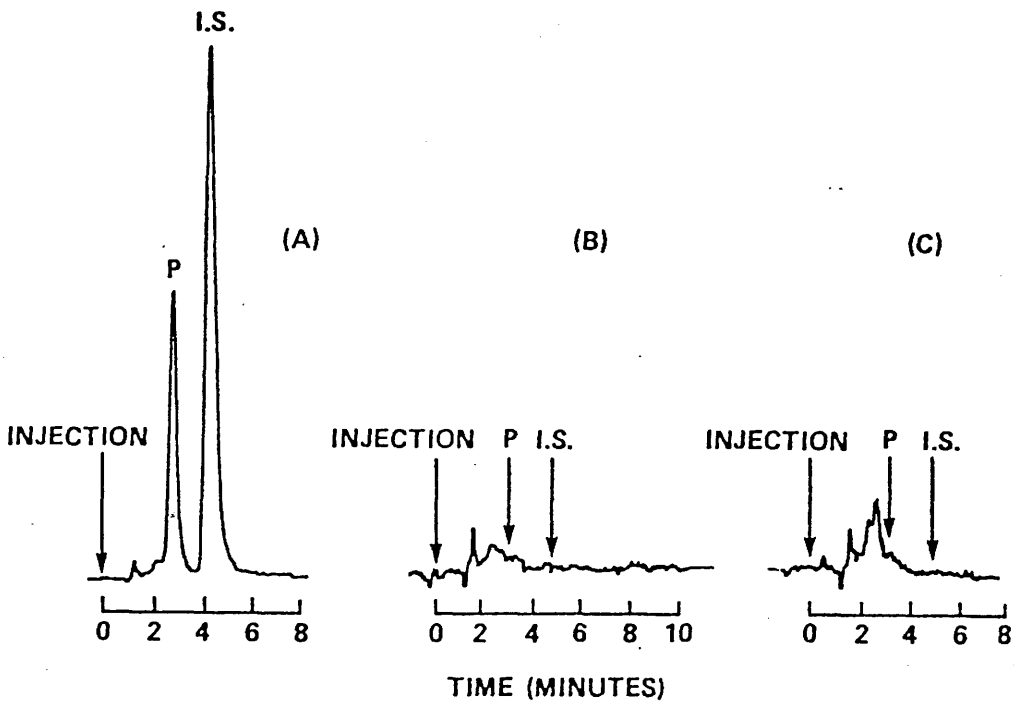


Fig.2.2. HPLC of prazosin.

(A) chromatogram of extracted whole blood containing 5ng of prazosin (peak P,3 min) and 54 ng of internal standard (I.S.,4.5min)with attenuation at 5 mV full scale deflection.

(B) and (C) chromatograms of extracted control whole blood and plasma,respectively. No peaks are seen corresponding to prazosin (P) and internal standard (I.S.),with attenuation at 2 mV full scale deflection.

accuracy of the method over the range of standard samples.

The reproducibility of the method was investigated by analysing five replicate samples in whole blood and ten replicate samples in plasma of 2 ng and 20 ng concentrations of prazosin. The effect of variable sample size was studied using 0.2-2.0 ml of whole blood or plasma without the addition of water, keeping constant the volume of internal standard solution, sodium hydroxide, and diethyl ether. The recovery of prazosin was determined by comparing the peak heights of extracted known concentrations of prazosin injected directly into the chromatograph.

The stability of prazosin in heparinized whole blood was investigated by assaying samples after they had been frozen at -20° for 6 months, refrigerated for up to 5 days or left at room temperature for 1h, 2h, 3h or 5h. The internal standard was added at the time of analysis. Concentrations in each stored set were calculated from the normalized PHR of a freshly prepared calibration curve analysed on the same day.

2.9. Determination of Nifedipine

Plasma nifedipine concentrations were determined after extraction under basic conditions into toluene and volumes of 2 µl were injected directly into a gas liquid chromatograph equipped with an OV-101 column and ^{63}Ni electron capture detector (Hamann and McAllister, 1983).

Nitrendipine was used as internal standard. The limit

of detection is 1-2 ng/ml. The intra-assay coefficient of variation of the method, given a plasma nifedipine concentration of 50 μg/l is 6% and the inter-assay coefficient of variation is 7%.

2.10. Determination of Nisoldipine

Nisoldipine plasma levels were assessed by the method of Ramsch (unpublished data) using gas liquid chromatography with an electron capture detector. The limit of detection is 2 μg/l. The assay was performed by Bayer AG, Wuppertal, Germany.

2.11. Determination of liver blood flow by Indocyanine green

Indocyanine green (ICG) is a water soluble, tricarbocyanine dye with a peak spectral absorption at 800-810 nm in plasma or blood. Following intravenous injection, ICG is rapidly bound to plasma proteins of which albumin is the principal carrier (95%) Indocyanine green undergoes no significant extrahepatic or enterohepatic circulation and is taken up from the plasma almost exclusively by the hepatic parenchymal cells and is secreted entirely into the bile. Its rate of elimination in normal individuals is highly dependent on hepatic blood flow, consequently ICG has been used to estimate liver blood flow (Caesar et al, 1961). Subjects were studied in a fasting basal state in the supine position. Subjects were weighed and

the dosage calculated on the basis of 0.5 mg/kg of body weight. Fifty mg ICG powder was dissolved with 10 ml sterile distilled water giving 5 mg of dye per ml of solution. The appropriate amount of dye was injected into an indwelling cannula inserted in a forearm vein as rapidly as possible as a bolus. Six ml of venous blood were collected prior to injecting ICG, for a serum blank and standard curve construction, and then further samples were collected into lithium heparinised tubes at 3, 6, 9, 12, 15, 18 and 21 minutes after administration. Samples were centrifuged and the plasma was separated and stored at -20°C until analyis. Indocyanine green plasma concentrations were determined after the precipitation of proteins by the addition of 1 ml cold (4°C) acetone to 1 ml plasma. After mixing by vortex (x 10 secs) samples were centrifuged at 1000 rpm for 20 minutes. The absorbance of the supernatant was measured at 786 nm with a double beam spectrophotometer equipped with a red sensitive photomultiplier as described by Svensson et al (1983). Calibration curves were obtained in each subject's plasma on each study day. $AUC_{0\rightarrow\infty}$ was estimated by the log trapezoidal rule. VDss was calculated by the following equation

$$VD = DOSE/Co$$

where Co is the concentration at zero time determined by extrapolation. Indocyanine green plasma clearance was

calculated from dose divided by $AUC_{0\rightarrow\infty}$, and this was converted to blood clearance by the following equation:

$$Cl_B = \text{plasma clearance} \times 1/1\text{-haematocrit}$$

Haematocrits were estimated in each subject on the morning of each study day. A one compartment model has been found appropriate to determine ICG clearance (Figure 2.3.).

2.12. Determination of Glomerular Filtration Rate and Effective renal plasma flow using ^{51}Cr EDTA and I^{125} PAH

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by evaluation of the plasma clearances of ^{51}Cr EDTA and I^{125} PAH given by intravenous injection (Harries et al, 1972). Subjects were studied in a fasting basal state in the supine position. A total volume of 5 ml containing 2 M Bq of ^{51}Cr-EDTA and 1 M Bq of I^{125} PAH was injected into a venous cannula inserted in the forearm vein, 1 hour after oral administration of either drug or placebo.

A 3 ml blood sample was taken before the injection and further 3 ml samples were collected from the other arm at 2, 7, 17, 30, 40, 75, 100, 120, 150, 180 minutes after injection in plastic heparinised tubes. The exact time at which the injection was given and the samples were taken was carefully recorded. A pooled

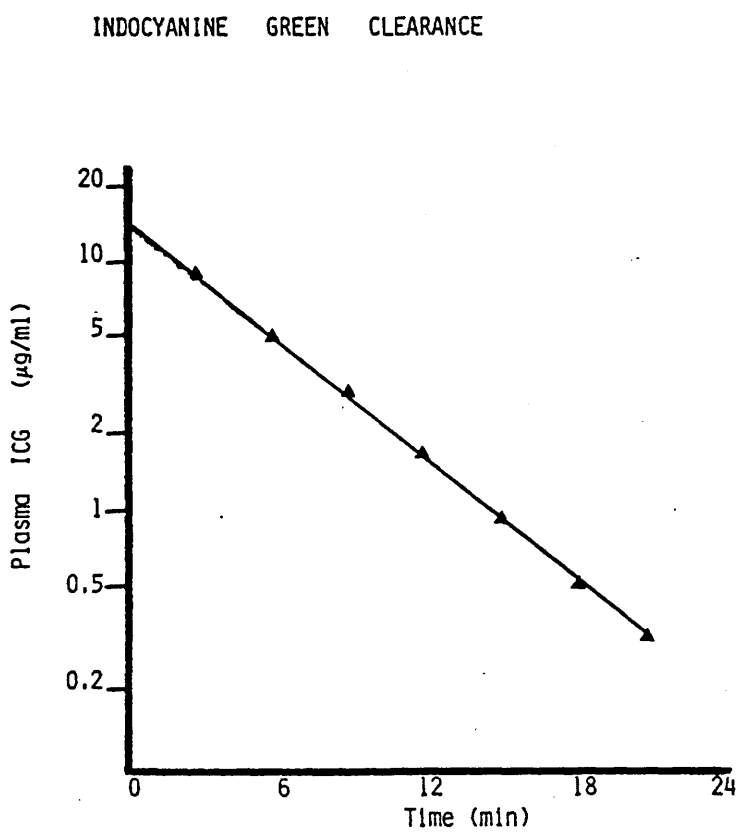


Fig 2.3. ICG plasma concentration vs. time. One compartment model.

urine collection 3 hours after the injection was taken and the volume was estimated. One ml sample of plasma urine and the standard solution were simultaneously counted in a gamma counter (LKB-WALLAC). ^{51}Cr has a gamma ray emission of 323 KeV and ^{125}I of 35 KeV.

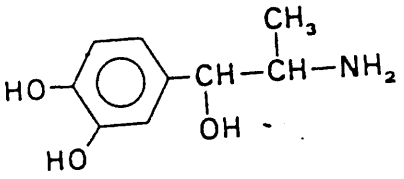
The clearance of the substances was calculated according to the following equation:

$$\frac{\% \text{ dose excreted in urine over 3 hours}}{AUC_{0-3 \text{ hours}}}$$

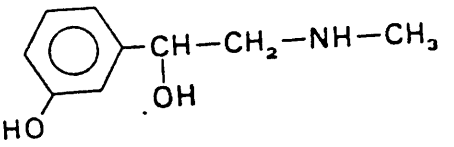
The $AUC_{0-3 \text{ hours}}$ represents the area under the % plasma concentration of tracer from time of injection to 3 hours.

2.13. Analysis of pressor responses

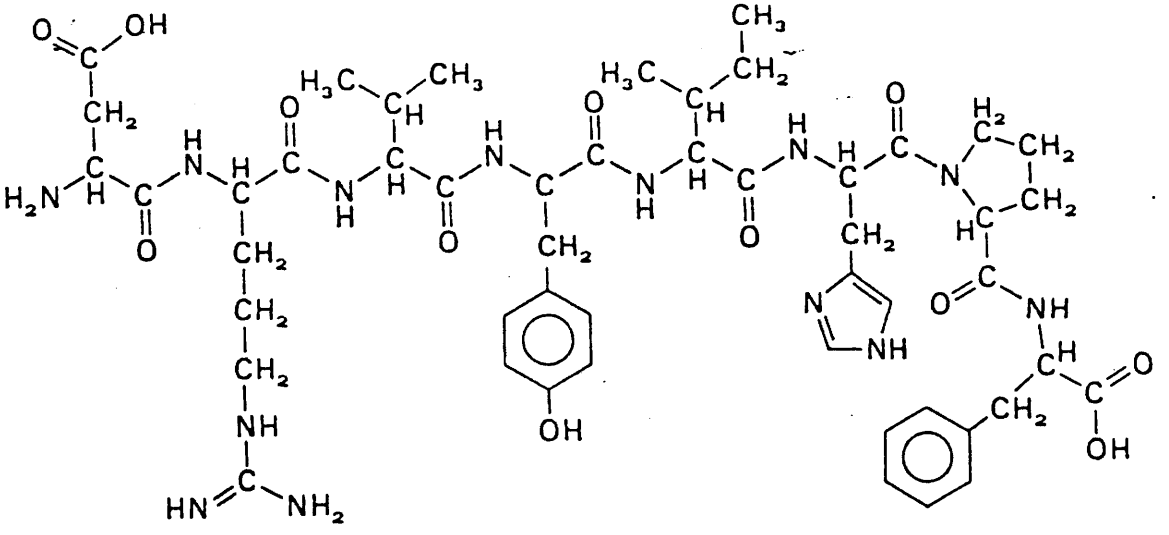
Pressor substances such as phenylephrine, alphamethylnoradrenaline and angiotensin II (Figure 2.4.) are injected intravenously in 0.9% sodium chloride solution (total infusion volume 40 to 160 ml) with a Braun Perfusor IV continuous infusion pump that has a series of predetermined dose rates (Sumner et al, 1982). Each infusion dose is maintained for not less than 5 minutes while recordings are made of blood pressure and heart rate by automatic recorder (Sentron, Bard) at minute intervals. A steady state response should be achieved at each infusion rate, after which the infusion rate is increased to the next level. The infusion is stopped when an increase of not more than 45 mmHg



α-Methylnoradrenaline



Phenylephrine



Asp—Arg—Val—Tyr—Ileu—His—Pro
Phe

N-terminal

C-terminal

Angiotensin II

Fig.2.4. Structural formulae of pressor substances.

systolic pressure or 30 mmHg diastolic pressure is achieved. Heart rate is monitored continuously by ECG. Thus the systolic and diastolic blood pressures are measured at each infusion dose rate and the response is calculated by subtracting the baseline values.

A line or curve is drawn through the set of points representing the blood pressure responses at particular doses of agonist. The dose of agonist required to raise the blood pressure by 20 mmHg (PD_{20}) is obtained by interpolation.

In clinical studies, the linear and the lower portion of the sigmoid dose-response curve can be obtained.

A satisfactory fit to the curve is obtained by the use of a quadratic function of the form:

$$AX^2 + BX + C$$

where X = log (dose) for the pressor-response curve.

2.14. Statistical Analysis

Where applicable the results were calculated and expressed as mean $\pm$ standard deviation (S.D.), the standard deviation being calculated from the expression:-

$$S.D. = \sqrt{\sum \frac{(x - \bar{x})^2}{n - 1}}$$

where x was any of the values measured, $\bar{x}$ the mean value,

and n the number of observations.

The significance of the results was calculated by Student's t test using the formula:-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where s was an estimate of the combined standard deviation of both groups calculated from:-

$$S^2 = \frac{\sum(x - \bar{x}_1)^2 + \sum(x - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

the degrees of freedom $F = n_1 + n_2 - 2$

$\bar{x}_1$ and $\bar{x}_2$ were the means of both groups and n_1 and n_2 the number of observations in each group.

The significance of paired sets of data was analysed by a paired Student's t test using the formula:-

$$t = \frac{\bar{x}}{S\sqrt{n}}$$

where $\bar{x}$ was the mean of the differences between the paired samples and S was calculated from:-

$$S = \sqrt{\sum \frac{(x - \bar{x})^2}{n - 1}}$$

The degrees of freedom F = n-1.

CHAPTER 3

STUDIES ON THE EFFECTS OF CALCIUM ANTAGONIST DRUGS IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

3.1. INTRODUCTION

Calcium antagonist drugs are now widely used in the treatment of ischaemic heart disease and hypertension. The dihydropyridine nifedipine has an established place as second or third line drug particularly in combination with beta blockers (Murphy et al, 1983; Kendall et al, 1984; LeJeune et al, 1985). Recent observations suggest that calcium antagonists may also be used successfully as monotherapy in some groups of patients, particularly in the elderly and in those with chronic respiratory disease, peripheral vascular disease or other absolute or relative contraindications to beta blockers (Buhler et al, 1982). Several experimental and clinical reports indicate differences between calcium antagonists as far as their tissue specificity is concerned (Kahan et al, 1981; Crevey et al, 1982; Allen et al, 1983). These findings have promoted interest in the development of more selective and specific drugs.

The dihydropyridine compound, nisoldipine, is an example of such development. Compared to nifedipine it has similar effects on the heart but exerts a more potent inhibition of vascular contraction in vitro. In dogs nisoldipine has been found to decrease total peripheral resistance by dilatation of the peripheral arterial system (Maxwell et al, 1982). In addition, it appears the first calcium antagonist for which an effect on the venous system has been demonstrated at therapeutic concentrations.

The portal vein also seems to be sensitive to nisoldipine in vitro (Kadza et al, 1980). Intravenous administration of nisoldipine to patients undergoing cardiac catheterisation caused an immediate blood pressure fall and heart rate increase with a marked reduction of total peripheral resistances (Vogt et al, 1980) showing that nisoldipine was a rapid and powerful peripheral vasodilator with its actions apparently restricted to the peripheral vasculature. On account of its relatively modest direct effect on cardiac muscle and its relative peripheral vascular specificity and potency, nisoldipine has potential therapeutic advantages over nifedipine in hypertension. This study investigates the effects of oral nisoldipine on blood pressure, heart rate and circulating hormones in both normotensive subjects and patients with essential hypertension.

3.2. METHODS

3.2.1. Study of normotensive subjects

A double blind, double dummy, random order comparison, using a Latin square design, was made of three single dose treatments:

1) Nifedipine 20 mg retard tablet and nisoldipine placebo
2) Nisoldipine 10 mg tablet and nifedipine placebo
3) Nifedipine placebo and nisoldipine placebo.

The study was undertaken in nine healthy male volunteers, aged 20-29 years (mean age 23.5 ± 3 years; mean

weight 68.3 ± 6 kg) on three study days at least one week apart. Blood pressure and heart rate were measured by automatic recorder (Sentron) at intervals up to eight hours after dosing. From an indwelling cannula (Venflon) inserted in a forearm vein blood for drug level measurement was collected at time 0, .5, 1, 1.5, 2, 3, 4, 6 and 8 hours after administration.

Samples for plasma noradrenaline, aldosterone and renin activity were taken at time 0 and 2, 4 and 8 hours after dosing. In view of the potential photolability of the dihydropyridines blood samples were wrapped in aluminium foil and after centrifugation plasma was separated under a sodium lamp. Plasma nifedipine and nisoldipine concentrations were measured by GLC with electron capture (Hamann and McAllister, 1983).

Plasma drug concentration-time data were most appropriately fitted to a one compartment open model. By application of the general linear (F ratio) test to a hierarchy of pharmacokinetic models this model was deemed most appropriate for both drugs.

3.2.2. Study of hypertensive patients

Eight patients, 3 males and 5 females, aged 40-60 years (mean age 54.5 ± years; weight 67.4 ± 15 kg) with essential hypertension (blood pressure > 150/100 and < 240/125 on two occasions one week apart) on no treatment for at least two weeks, entered the study. All patients had normal renal

function and no clinical evidence of secondary hypertension. Three patients had been previously treated with beta blockers, two with diuretics, two with calcium antagonists and one had had no previous treatment. After a two week placebo period they were treated with nisoldipine 10 mg tablets twice daily as monotherapy. Patients were asked to avoid taking any other drugs during the study period. Patients were studied on three 8-hour study days:

1) after at least two weeks on placebo
2) after the first dose of nisoldipine 10 mg (acute)
3) after four weeks treatment with nisoldipine 10 mg twice daily (chronic).

In all studies blood pressure and heart rate were measured, using an automatic recorder (Sentron) in the supine position, after a minimum of 10 minutes rest, and after 2 and 5 minutes standing. Readings were taken before and 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours after dosing. Blood samples for plasma noradrenaline aldosterone and renin activity were collected at baseline, 2, 4 and 8 hours. Side effects were assessed both by spontaneous comment and by direct enquiry from a standard list of questions. The hypertensive patients attended the outpatient clinic at weekly intervals for review of blood pressure and side effects for four weeks.

Both protocols were aproved by the Ethical Committee and all subjects, volunteers and patients, gave written witnessed informed consent for participation in the studies.

Routine biochemical and haematological measurements were undertaken before commencing the studies and repeated at the end. A 12 lead ECG was recorded on each study day. Statistical analysis of pharmacodynamic data was by repeated measures analysis of variance.

3.3. RESULTS

3.3.1. Study of normotensive subjects

In the supine posture there were no significant differences in blood pressure between treatments. A significant increase in supine heart rate was observed after nifedipine ($p < 0.05$) with a maximum heart rate of 70.2 ± 11.8 beats/min at 6 hours after dosing compared to 64 ± 7 after placebo. On standing for 5 minutes nisoldipine caused a significant fall in systolic blood pressure, maximal at 3 hours with a nadir of 95.6 ± 7.4 mmHg compared to 109 ± 6 with placebo ($p < 0.05$) and 103 ± 9 with nifedipine. However, with nifedipine the fall in blood pressure did not attain statistical significance. Both drugs caused a slight but not significant increase in standing heart rate when compared to placebo, maximum changes were achieved 6 hours after dosing with 89 ± 12 beats/min on placebo, 96 ± 17 on nifedipine and 97 ± 12 on nisoldipine (Figure 3.1.).

Changes in plasma renin activity, aldosterone and noradrenaline were not statistically significant for either drug compared with placebo (Table 3.1.-3.2.).

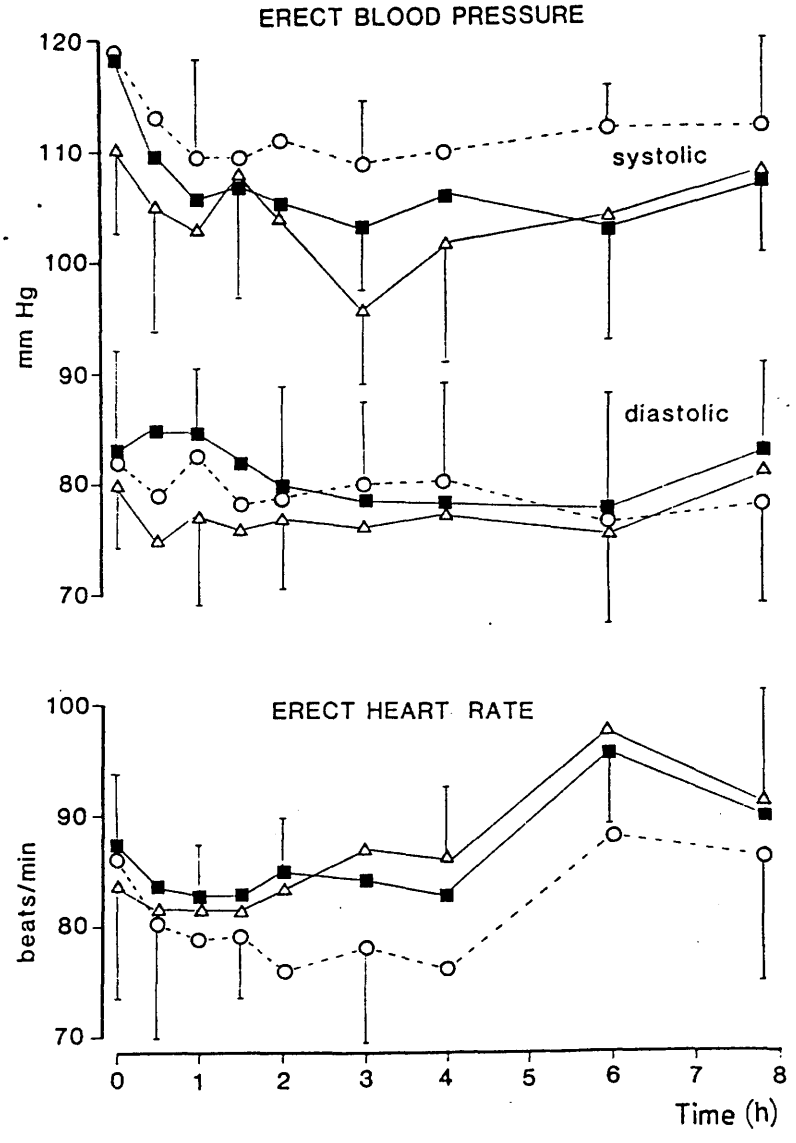


Fig. 3.1. Erect blood pressure and heart rate (mean ± SD) after nisoldipine 10 mg (▵—▵), nifedipine 20 mg (▮—▮), or placebo (o——o) in 9 normotensives.

TABLE 3.1.

PRA AND ALDOSTERONE CONCENTRATIONS FOLLOWING ORAL ADMINISTRATION OF NIFEDIPINE (20 mg) AND NISOLDIPINE (10 mg) IN 9 NORMOTENSIVE SUBJECTS

PLASMA RENIN ACTIVITY (MEAN ± SD) ngAI/ml/hr

Treatment	TIME AFTER DOSING 0	2h	4h
Nifedipine	2.01 ± 1.0	2.12 ± 1.3	1.58 ± 0.7
Nisoldipine	2.13 ± 1.09	2.36 ± 1.4	1.5 ± 1.0
Placebo	1.89 ± 1.0	1.31 ± 0.5	1.39 ± 0.5

PLASMA ALDOSTERONE (MEAN ± SD) pg/ml

Treatment	TIME AFTER DOSING 0	2h	4h
Nifedipine	170.1 ± 57.1	116.1 ± 25.7	130.1 ± 35.7
Nisoldipine	121.8 ± 27.0	116.1 ± 37.7	133.6 ± 56.7
Placebo	134.4 ± 53.1	116.1 ± 41.1	121.7 ± 49.9

Table 3.2. Noradrenaline plasma concentration following oral administration of nisoldipine and nifedipine in 9 normotensive subjects.

SUPINE PLASMA NORADRENALINE (MEAN ± SD) nm/ l

	TIME AFTER DOSING (hrs)		
Treatment	0	2	4
Nifedipine	1.73 ± 1.13	1.42 ± 0.38	1.61 ± 0.41
Nisoldipine	1.30 ± 0.40	1.45 ± 0.63	1.46 ± 0.74
Placebo	1.28 ± 0.58	1.17 ± 0.38	1.22 ± 0.49

ERECT (5 min) PLASMA NORADRENALINE (MEAN ± SD) nm/ l

	TIME AFTER DOSING (hrs)		
Treatment	0	2	4
Nifedipine	2.18 ± 0.86	2.83 ± 0.73	3.51 ± 1.50
Nisoldipine	2.12 ± 0.81	3.37 ± 0.73	3.02 ± 0.71
Placebo	2.21 ± 0.45	3.04 ± 2.48	2.33 ± 0.93

Pharmacokinetic parameters in young normotensive subjects are shown in Table 3.3. and in Figures 3.2.-3.3. The terminal elimination half lifes of both drugs were similar (127.3 ± 27 min for nifedipine and 124.2 ± 42 mins for nisoldipine) while the low peak concentrations and the area under the curve for nisoldipine reflect its very high first pass metabolism compared to nifedipine.

The only side effect experienced by the subjects was a mild frontal headache lasting 2-4 hours but this was less frequent with nisoldipine compared to nifedipine (4 out of 9 compared to 7 out of 9); one subject had a mild headache after placebo.

3.3.2. Study of hypertensive patients

On the first day of treatment with 10 mg nisoldipine the blood pressure fell between 1 and 6 hours from 172/97 ± 17/7 mmHg at baseline to a nadir of 149/85 ± 16/9 at 2 hours after dosing in supine position; and from 177/101 ± 16/13 at baseline to 144/87 ± 19/10 at 2 hours after 5 minutes standing. On the placebo day blood pressure was 180/97 ± 19/9 at baseline and 178/98 ± 16/8 at 2 hours in supine position; baseline standing blood pressure was 172/103 ± 19/8 and 174/103 ± 19/10 at 2 hours.

The overall supine heart rate was significantly increased compared to placebo ($p < 0.05$) and at two hours was 71 ± 10 on the placebo day and 78 ± 12 on the acute

TABLE 3.3.

Pharmacokinetic parameters of nisoldipine 10 mg orally in normotensives.

	Peak con. ng/ml	Time to peak min	t.1/2 min	AUC ng min/ml
Mean	1.78	100.2	124.2	340.2
SD	0.88	36	42	168

Pharmacokinetic parameters of oral nifedipine 20mg (Adalat Retard) in normotensives

	Peak con. ng/ml	Time to peak min	t.1/2 min	AUC ng min/ml
Mean	60.2	163.3	127.3	19,793
SD	21.7	65.5	26.8	5,642

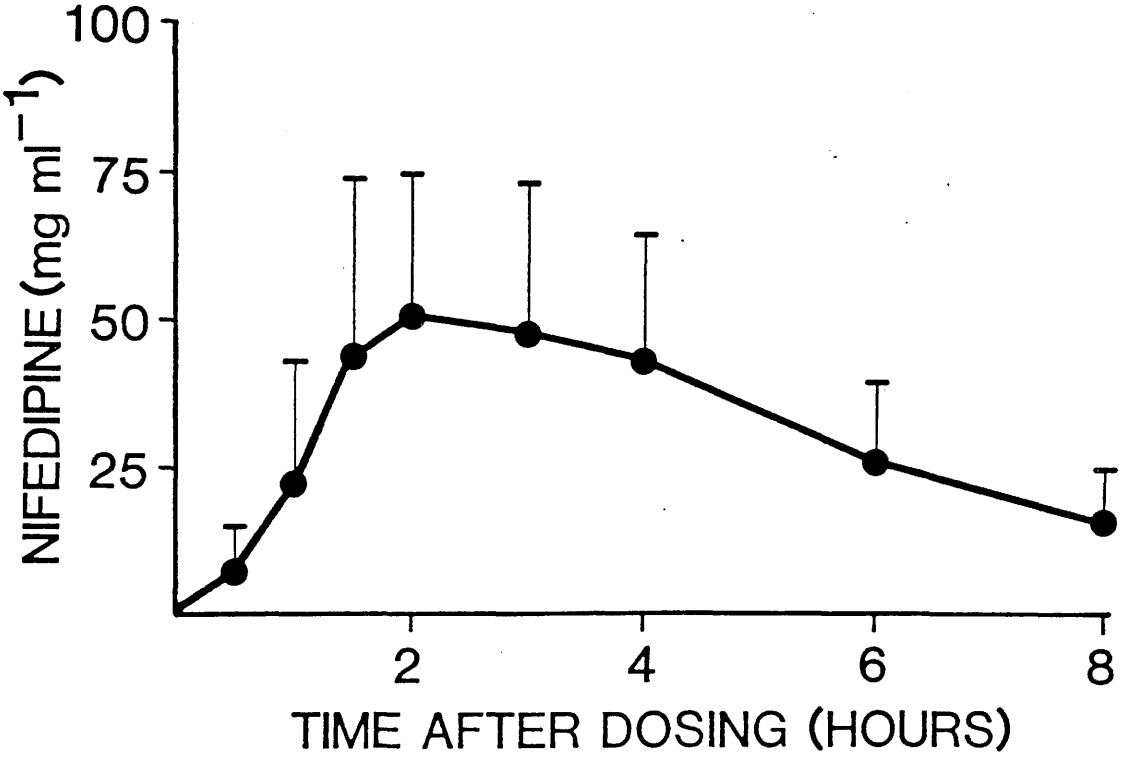


Fig. 3.2. Concentration vs time curve following oral administration of nifedipine 20 mg in 9 normotensives (mean $\pm$ SD).

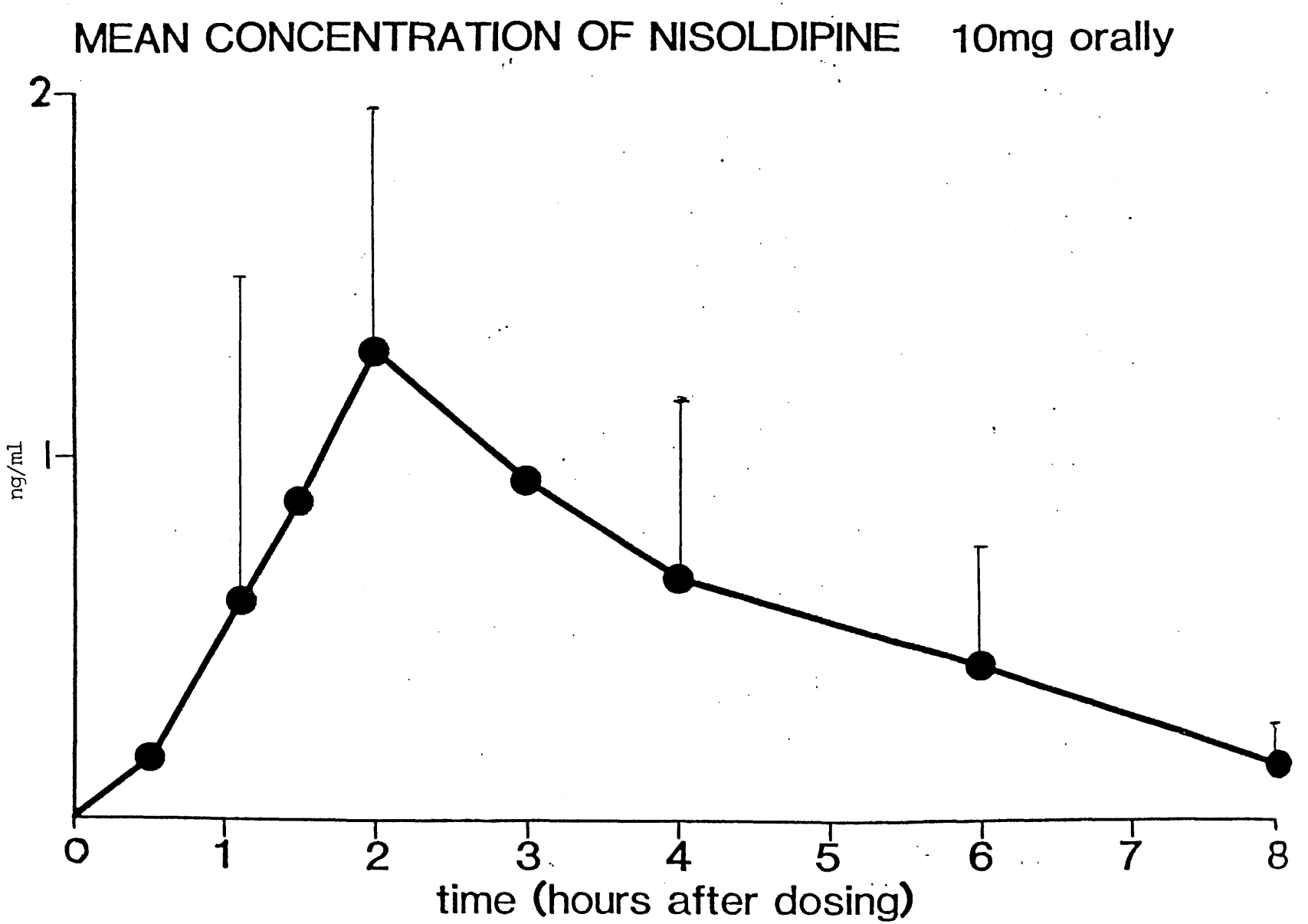


Fig. 3.3. Nisoldipine plasma concentration in normotensive subjects. (n=9) (mean±SD).

treatment day.

At the weekly review in the outpatient clinic supine blood pressure showed a significant fall from 186/105 ± 15/8 at entry to 162/90 ± 15/9 mmHg ($p < 0.01$) after 1 week and 161/90 ± 9/7 after 2 weeks of treatment. Corresponding heart rate showed a slight increase from 85 ± 9 to 93 ± 9 beats/min after 1 week and was 91 ± 8 at two weeks. Standing blood pressure was 179/105 ± 16/9 at entry and fell to 160/90 ± 13/9 mmHg after 1 week ($p < 0.01$) and 154/89 ± 12/9 at 2 weeks. Corresponding heart rates were 91 ± 9 and 99 ± 8 beats/min after 1 week and 92 ± 9 at 2 weeks (Figure 3.4.).

After 4 weeks of treatment the mean arterial pressure at baseline, i.e. 12 hours after the previous dose of 10 mg, was significantly lower than the baseline values on the placebo day ($p < 0.05$). On the last day of treatment blood pressure showed a further fall from pre dosing levels of 160/93 ± 11/8 to 143/83 ± 11/9 (supine) and from 152/97 ± 12/6 to 132/85 ± 15/11 (standing) at 6 hours after dosing (Figure 3.5.). The overall blood pressure fall was statistically significant compared to placebo ($p < 0.05$). The profiles of heart rate in the erect position were significantly elevated when compared to placebo following both acute and 4 weeks of treatment (Figure 3.6.). Plasma noradrenaline levels showed no significant change after 4 weeks of treatment (Table 3.4.). Plasma renin activity and plasma aldosterone did not show significant changes after

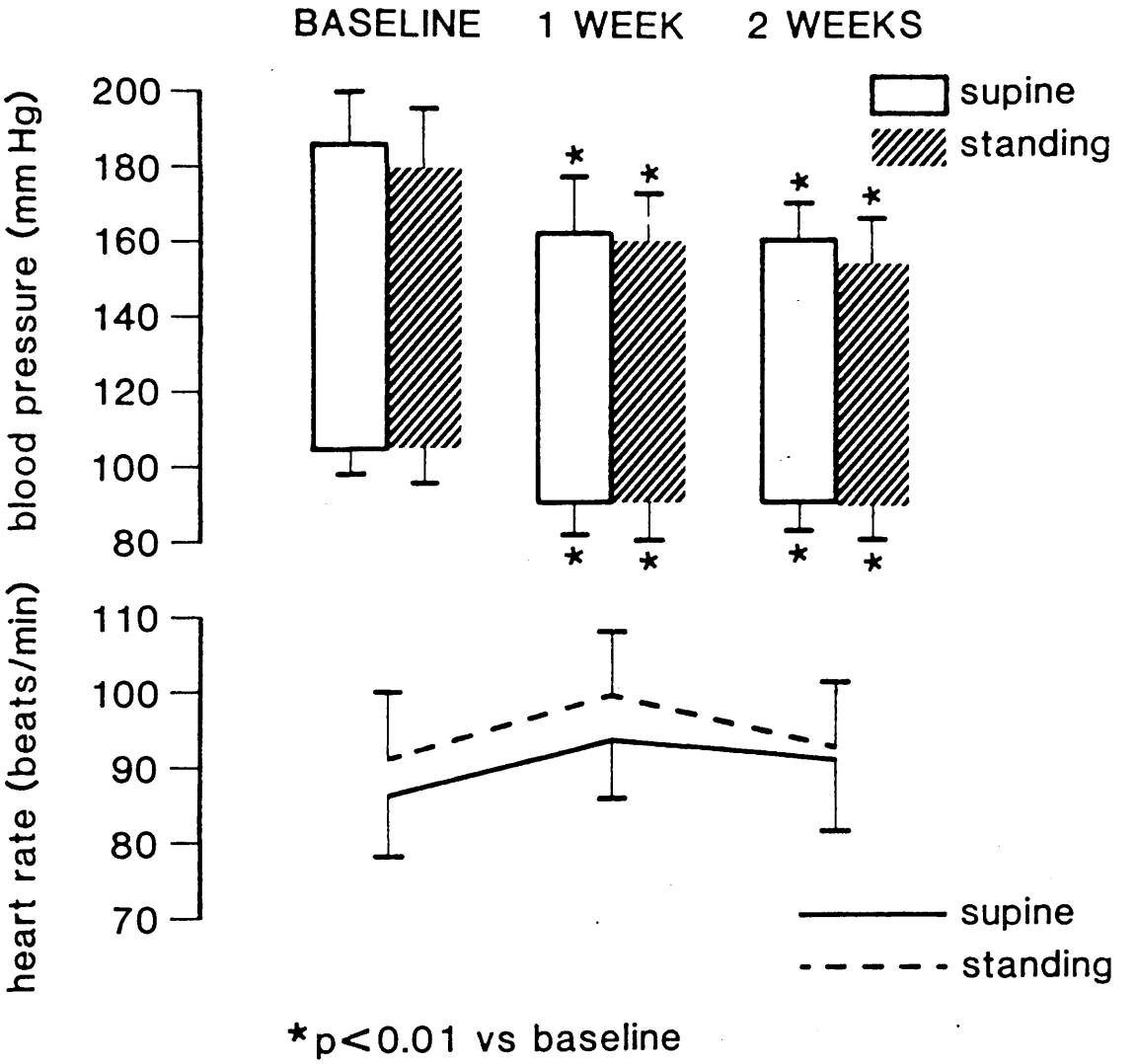


Fig. 3.4. Supine and standing blood pressure and heart rate during antihypertensive treatment with nisoldipine 10 mg twice daily (mean ± SD). (n=8).

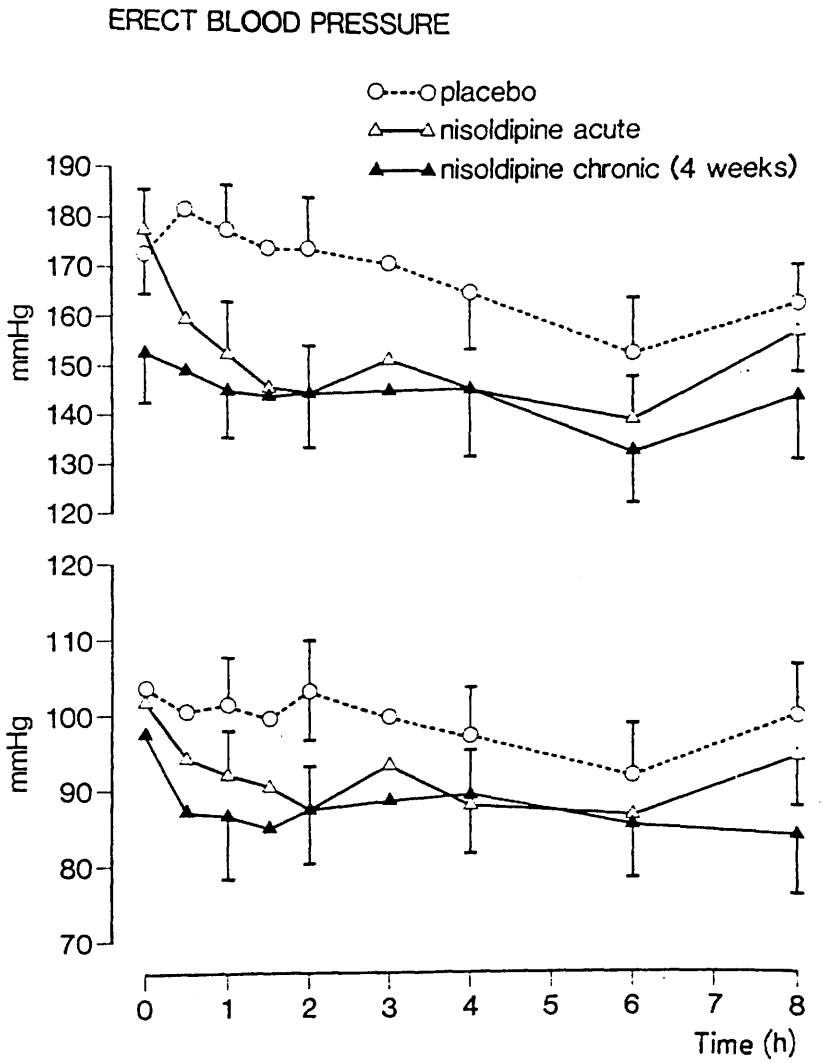


Fig. 3.5. Erect systolic and diastolic blood pressure in 8 hypertensives. (mean±SD).

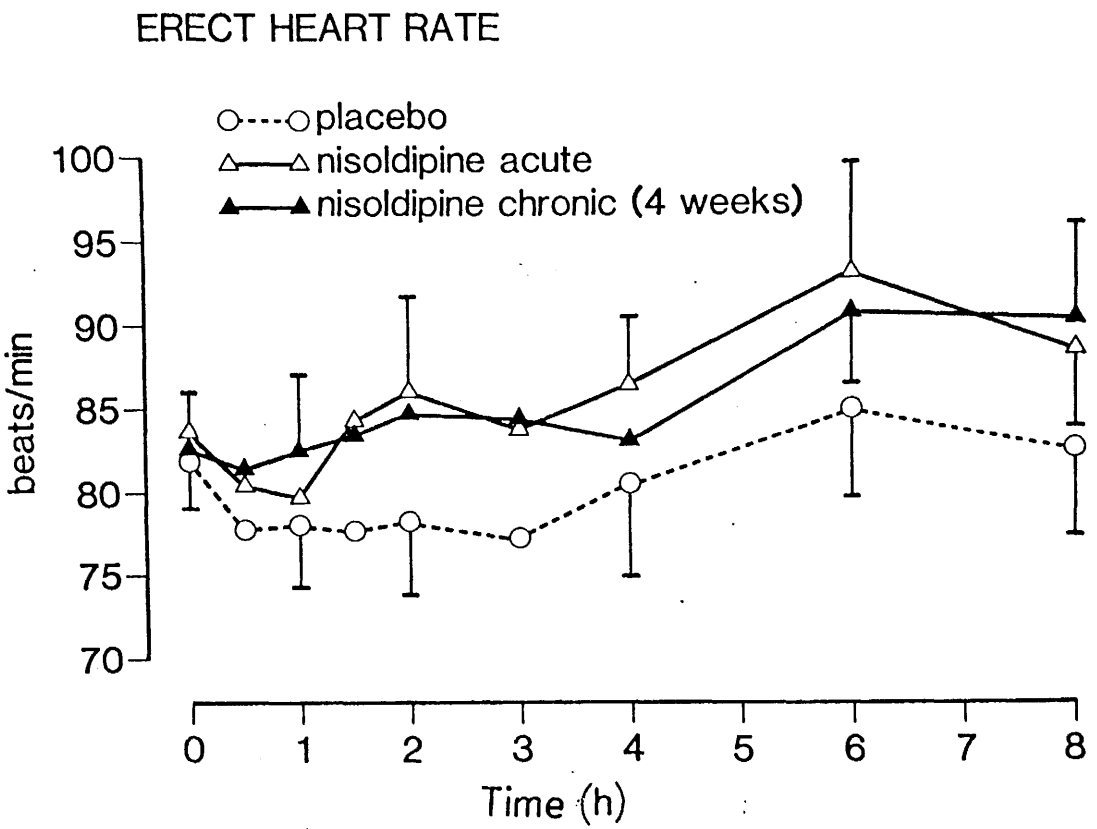


Fig. 3.6. Erect heart rate in 8 hypertensives (mean±SD).

TABLE 3.4.

Plasma noradrenaline (Mean ± SD) (nm/L) in essential hypertensives after placebo or nisoldipine 10 mg orally.

	TIME AFTER DOSING, (hrs)							
	0		2		4		8	
Treatment								
Placebo	2.23	0.77	2.03	0.71	1.72	0.74	1.71	0.52
Nisoldipine acute	2.53	1.25	2.57	1.09	1.92	0.86	2.53	0.85
Nisoldipine chronic (4 weeks)	3.31	2.46	3.05	1.59	2.7	2.1	2.52	1.29

TABLE 3.5.

PRA AND ALDOSTERONE CONCENTRATIONS IN 8 HYPERTENSIVES DURING NISOLDIPINE TREATMENT

PLASMA RENIN ACTIVITY (MEAN ± SD) ng AI/ml/hr

	HOURS AFTER DOSING			
Treatment	0	2	4	8
Placebo	1.53 ± 1.8	1.69 ± 1.8	1.64 ± 2.06	1.76 ± 1.36
Nisoldipine Acute	1.75 ± 1.4	1.42 ± 1.36	1.74 ± 0.97	1.28 ± 0.7
Nisoldipine Chronic	1.48 ± 1.53	1.72 ± 1.67	1.4 ± 1.07	1.76 ± 1.55

PLASMA ALDOSTERONE (MEAN ± SD) pg/ml

	HOURS AFTER DOSING			
Treatment	0	2	4	8
Placebo	92.5 ± 54.5	88.1 ± 42.2	71.8 ± 43.0	89.6 ± 45.3
Nisoldipine Acute	87.8 ± 52.6	72.6 ± 35.5	52.7 ± 25.3	70.4 ± 45.6
Nisoldipine Chronic	82.3 ± 56.6	56.1 ± 22.6	65.2 ± 38.0	84.1 ± 45.0

acute or chronic dosing when compared to placebo (Table 3.5.).

Two patients experienced mild frontal headache on the placebo treatment day, two had headache after the first dose and one on the last study day. Four patients complained of facial flushing within two hours of dosing during the first week of treatment; this was not apparent after 10-14 days and did not require dose reduction or withdrawal from the study. Four patients developed mild ankle oedema between two and four weeks of treatment; one of them showed a moderate weight gain of 2 kg. No patient complained of orthostatic symptoms after acute or chronic dosing. There were no significant changes in haematological or biochemical measurements or on the 12 standard lead E.C.G.

3.4. DISCUSSION

In this group of young normotensive subjects nisoldipine caused a significant fall in erect systolic blood pressure, confirming its activity as a blood pressure lowering agent. The fall in blood pressure appeared to be longer lasting than the modest short lived falls previously described in some other studies with nifedipine (Millar et al, 1983). Indeed in the present study nifedipine did not lower blood pressure significantly in this group of young subjects but evidence of haemodynamic activity was provided by the significant increase in supine heart rate. This is not inconsistent with previous reports that nifedipine does

not significantly reduce blood pressure in healthy young subjects (Corea et al, 1980; MacGregor et al, 1982). The modest effects which calcium antagonists have on the blood pressure in normal subjects has been contrasted with other antihypertensive drugs, for example the beta adrenoceptor antagonist propranolol and the A.C.E. inhibitor captopril (MacGregor et al, 1982), which produce similar blood pressure falls in both normotensives and hypertensives. This has been interpreted as evidence of a cellular abnormality, with excessive availability of intracellular Ca^{++} in hypertensives which can be "corrected" by calcium antagonist drugs (Buhler, 1983). In addition to the presence or the absence of hypertension, age and sodium status or plasma renin activity are other factors which have been implicated as determinants of the acute blood pressure response to nifedipine. In the volunteer study all subjects were aged less than 30 years and were not on a sodium restricted diet. The effect of dietary sodium on blood pressure is mediated by the activity of the renin-angiotensin system as well as the sympathetic nervous system (Vollmer, 1984) and so may well affect the response to antihypertensive treatment (Buhler et al, 1984).

The antihypertensive efficacy of nisoldipine was demonstrated in a group of patients with essential hypertension following both acute dosing and continued treatment over 4 weeks. In fact recordings of blood pressure 12 hours after the evening tablet showed the

persistence of a significant antihypertensive effect in both the supine and erect postures. There were larger increases in heart rate after the first dose than after the last day of treatment despite lower blood pressure levels. This is consistent with previous reports in the literature of tolerance to the acute reflex responses to dihydropyridine calcium antagonists probably secondary to resetting of the baroreceptors (Littler et al, 1983; Young et al, 1984). The side effects observed in these patients were similar to those reported for other calcium antagonists like nifedipine (Lederballe Pedersen et al, 1979). The intensity of headache and flushing seemed to be less after the first week of treatment. Ankle oedema independent of weight gain was observed relatively frequently.

The pharmacokinetic analysis demonstrated that the plasma half-life of nisoldipine following single doses is not significantly longer than that of nifedipine although nisoldipine appeared to have a longer duration of action. Previous pharmacokinetic studies with nifedipine have shown great interindividual variability in its plasma concentrations following oral administration, reflecting wide differences in oral bioavailability and first pass metabolism (Raemsch and Somner, 1983).

The present results confirm that the disposition of the retard formulation of nifedipine differs from that reported for the capsule formulation (Banzet et al, 1983). The side effects of nifedipine capsules have been correlated with

high plasma concentrations immediately after dosing. In addition it has been previously observed that the fall in blood pressure during chronic dosing with nifedipine is closely related to plasma levels at least in individual hypertensive patients (Pasanisi & Reid, 1983). A possible explanation for the relatively long duration of action for nisoldipine in hypertensives is that active metabolites may contribute to its hypotensive effect but there are no data to support this hypothesis.

The study of nisoldipine in hypertensive patients was an open study and lacked placebo control but it showed encouraging antihypertensive effects of nisoldipine which deserved further investigation.

CHAPTER 4

VERAPAMIL IN ESSENTIAL HYPERTENSION

KINETICS, DYNAMICS AND CONCENTRATION-EFFECT RELATIONSHIP

4.1. INTRODUCTION

The relationship between blood concentration and effect for drugs used in the treatment of hypertension has received limited consideration. This may be related to the lack of a clear dose-response relationship for some of the commonly used drugs, for example, beta blockers (Collste et al, 1976; Von Bahr et al, 1976).

As the clinical response (i.e. fall in blood pressure) is readily detectable, little attempt is made to rationalise treatment prospectively because the dosage can be adjusted retrospectively. Thus, unlike antiarrhythmic (Meffin et al, 1977) and bronchodilator drugs (Whiting et al, 1984), clinical pharmacokinetics have been seldom applied to improve drug use in hypertension. As a consequence the variation in responsiveness to antihypertensive drugs has been related only to factors such as age or ethnic origin. It has been suggested, for example, that the response of hypertensive patients to calcium antagonists is not only quantitatively but qualitatively different from normotensives, implicating abnormalities of intracellular calcium handling as a primary pathogenic mechanism in hypertension (Buhler et al, 1982). These claims have often been based on inadequate data with observations being made of responses to different doses at different times and with no account taken of interindividual and time-related differences in plasma drug levels.

In the case of nifedipine a correlation between plasma

concentrations and fall in blood pressure has not been found in a group of patients (Pedersen et al, 1980a) whereas a significant correlation was demonstrated by others (Aoki et al, 1982b).

In recent years concentration-effect analysis has been developed by different authors (Sheiner et al, 1979; Whiting and Kelman, 1980). This "modelling" technique which seeks to explain pharmacological response in terms of the time course of a drug in the body (as reflected by blood concentration measurements) depends on having sufficient data to characterise the response profile associated with simultaneously observed drug concentrations. This approach has been successfully used to investigate in normotensive subjects the effects of acute dosing with alpha adrenoceptor antagonists such as doxazosin (Vincent et al, 1983), trimazosin (Meredith et al, 1983) and labetalol (Elliott et al, 1984).

Considering the inter-relationship between drug concentration and effect for a calcium antagonist such as verapamil, presents more of a problem. The role of this drug in the treatment of hypertension is well established (Corea et al, 1981; Buhler et al, 1982) but its pharmacokinetics are known to change with continued drug administration compared to acute dosing (Freedman et al, 1981; Shand et al, 1981) and there is the possibility that the major metabolite of verapamil, norverapamil, may contribute to the overall pharmacodynamic profile

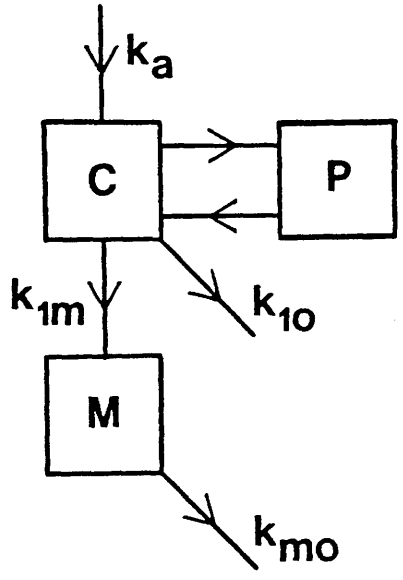
(Neugebauer, 1978). The concentration effect relationships following acute and chronic dosing with verapamil in the control of essential hypertension were investigated in an attempt to further elucidate the factors decreasing the response to this calcium antagonist in individual patients.

4.2. PATIENTS AND METHODS

Six mild-moderate essential hypertensive men (age 54 ± 7 years) were studied. They received no antihypertensive therapy for at least four weeks prior to the study. Patients were studied on three days, following placebo administration, after acute oral dosing of verapamil 80 mg (subject 4 received 160 mg) and then were established on a regimen of 80 mg twice daily verapamil (subject 4 received 160 mg twice daily) for one month. At this time they were studied again with the same single dose of verapamil 80 mg (subject 4, 160 mg). On each study day blood pressure and heart rate were measured supine and standing and blood samples were withdrawn at frequent intervals for 8 hours after dosing for the determination of plasma levels of verapamil and norverapamil according to the procedure described in Chapter 2.

The pharmacokinetics of verapamil and its metabolite were studied using an integrated model with two compartments describing drug disposition and a third compartment for the metabolite (Figure 4.1.). Pharmacodynamic profiles were initially analysed by application of the trapezoidal rule to

PHARMACOKINETIC ANALYSIS



$$C(d) = A\, e^{-\alpha(t)} + B\, e^{-\beta(t)} - (A + B)e^{-ka(t)}$$

$$C(m) = k_{1m} \frac{Vc}{Vm} \left[\frac{A}{(k_{mo}-\alpha)} (e^{-\alpha t} - e^{-k_{mo}t}) + \frac{B}{(k_{mo}-\beta)} (e^{-\beta t} - e^{-k_{mo}t}) - \frac{A + B}{(ka-k_{mo})} (e^{-ka t} - e^{-k_{mo}t}) \right]$$

Fig. 4.1. Three compartment model used for evaluation of verapamil and norverapamil kinetics. Details of equations are given in chapter 7.

derive to the area under the effect time profiles. Concentration-effect analysis was applied to integrate the pharmacokinetic and pharmacodynamic profiles (Meredith et al, 1983).

The pharmacokinetic data were related to the fall in systolic blood pressure corrected for any placebo day response (i.e. the difference in the systolic blood pressure after 5 minutes of standing following active treatment and placebo administration). The standard pharmacokinetic model is augmented by an effect compartment that is deemed small enough not to influence the pharmacokinetics and is governed by first-order processes. The measured effect (fall in blood pressure) is then related to the concentration of drug in the effect compartment by non linear least squares fitting with equal weighting of the points. The three parameters derived from this procedure are $\underline{m}$, $\underline{i}$ and $\underline{K}_{\underline{eq}}$; where $\underline{m}$ is the slope which represents the sensitivity to the drug (i.e. responsiveness = effect or change in blood pressure per unit of increase in drug concentration in the effect compartment), $\underline{i}$ is the intercept term from the equation relating drug concentrations to effect, and $\underline{K}_{\underline{eq}}$ is the first-order rate constant which characterises the concentration-effect disequilibrium. It is an integral part of this "modelling" approach that, at steady state, drug concentrations in plasma are directly related to the pharmacological response and that this steady state relationship can be identified by extrapolation from

analysis of the response to the initial dose of the drug.

4.2.1. Statistical analysis

Blood pressure and heart rate changes were evaluated by analysis of variance.

Comparison of kinetic results was made by paired t test.

4.3. RESULTS

4.3.1. Pharmacodynamics

On the first day of treatment with verapamil supine blood pressure fell significantly between 1 and 6 hours from 168/96 ± 15/9 mmHg at baseline to a nadir of 136/77 ± 15/9 at 5 hours after dosing; and from 162/98 ± 14/10 at baseline to 152/89 ± 21/10 at 5 hours in the standing position.

Corresponding supine heart rate was 78 ± 7 beats/min at baseline and 69 ± 7 at 5 hours; standing heart rate was 80 ± 7 at baseline and 76 ± 13 after 5 hours (N.S.). After 1 month treatment predosing supine blood pressure was 162/92 ± 17/5 and fell to 140/77 ± 15/13 at 5 hours and was 154/79 ± 21/9 at 8 hours after dosing. Standing blood pressure was 170/97 ± 17/5 predosing and fell to 150/87 ± 20/12 at 5 hours. The overall blood pressure fall was statistically significant compared to placebo ($p < 0.05$). Pre-dosing heart rate was 69 ± 10 and 63 ± 7 at 5 hours in supine position. Standing heart rate was 75 ± 12 and 72 ± 12

respectively (N.S.). The profile of systolic blood pressure and heart rate standing is shown in Figure 4.2. Placebo corrected standing systolic blood pressure showed a sustained fall throughout the day, maximal at 2 hours (Figure 4.3.).

Application of the trapezoidal rule to the individual placebo corrected effect-time profiles showed a mean fall in standing systolic blood pressure of 44 ± 10 mmHg.h after acute administration and 49 ± 8 after chronic treatment (N.S.) (Table 4.1.).

4.3.2. Pharmacokinetics

Mean peak plasma concentration was 121 ± 45 ng/ml after acute administration of verapamil and increased significantly to 201 ± 69 after 1 month treatment ($p < 0.01$) (Figure 4.4.). Mean time to peak concentration was 1.2 ± 0.6 h after acute dosing and 0.8 ± 0.7 after chronic dosing (N.S.). Individual data are shown in Table 4.2.

The pharmacokinetic parameters obtained in fitting acute and chronic concentration data to a three compartment model are given in Tables 4.3. and 4.4. The derived pharmacokinetic parameters showed a significant increase ($p < 0.001$) in the area under the concentration time curve (AUC dose) for verapamil from 645 ± 304 acutely to 1314 ± 391 ng.h.ml following chronic dosing. There was also a significant increase in terminal elimination half-life for the drug (t1/2) from 4.20 ± 1.48 h to 10.6 ± 6.2 h.

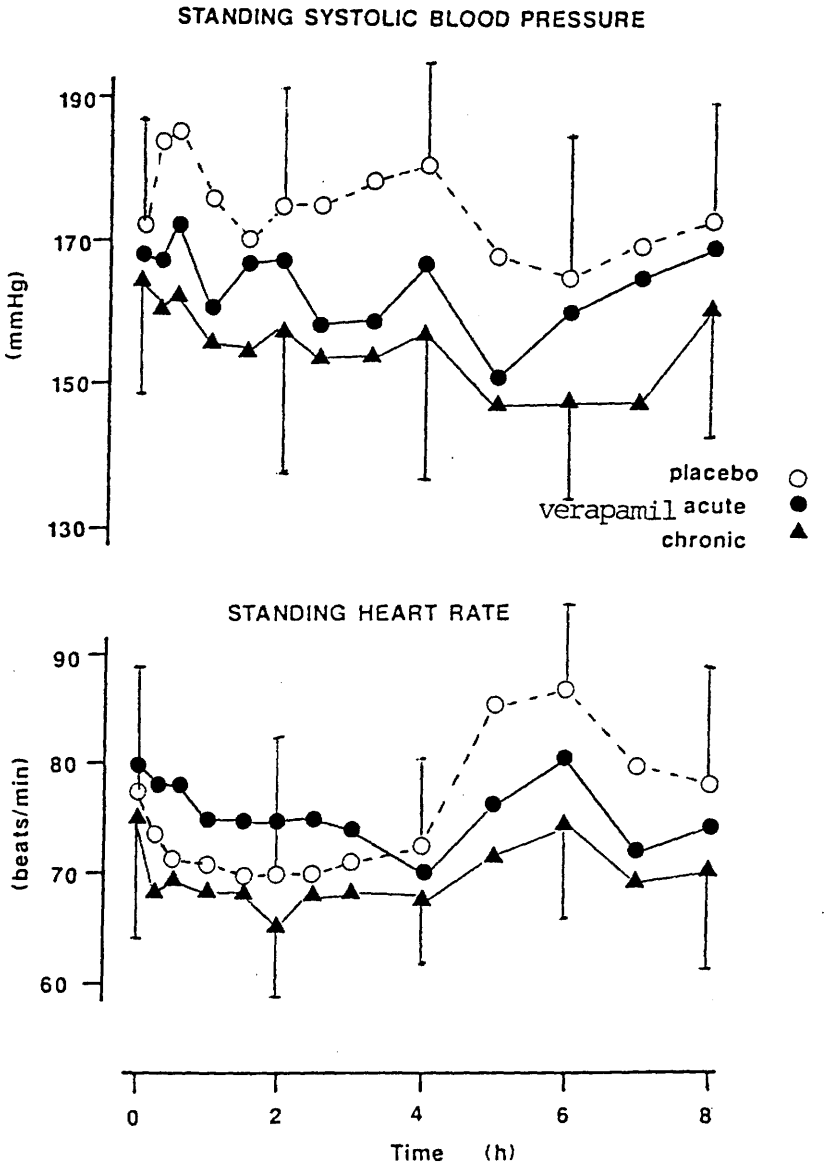


Fig.4.2. Mean standing systolic blood pressure and heart rate during the study day after placebo, acute and chronic administration of verapamil in 6 hypertensives.

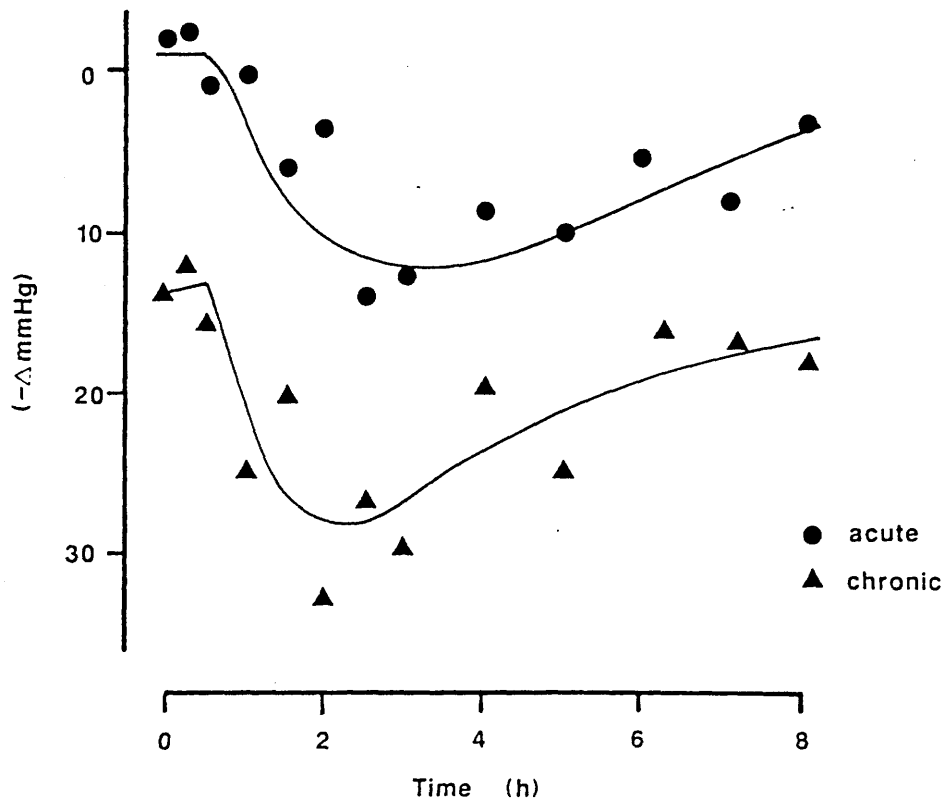


Fig 4.3. Placebo corrected standing systolic blood pressure fall after acute and chronic administration of verapamil 80mg in a representative hypertensive subject. Computer derived best fit of the points.

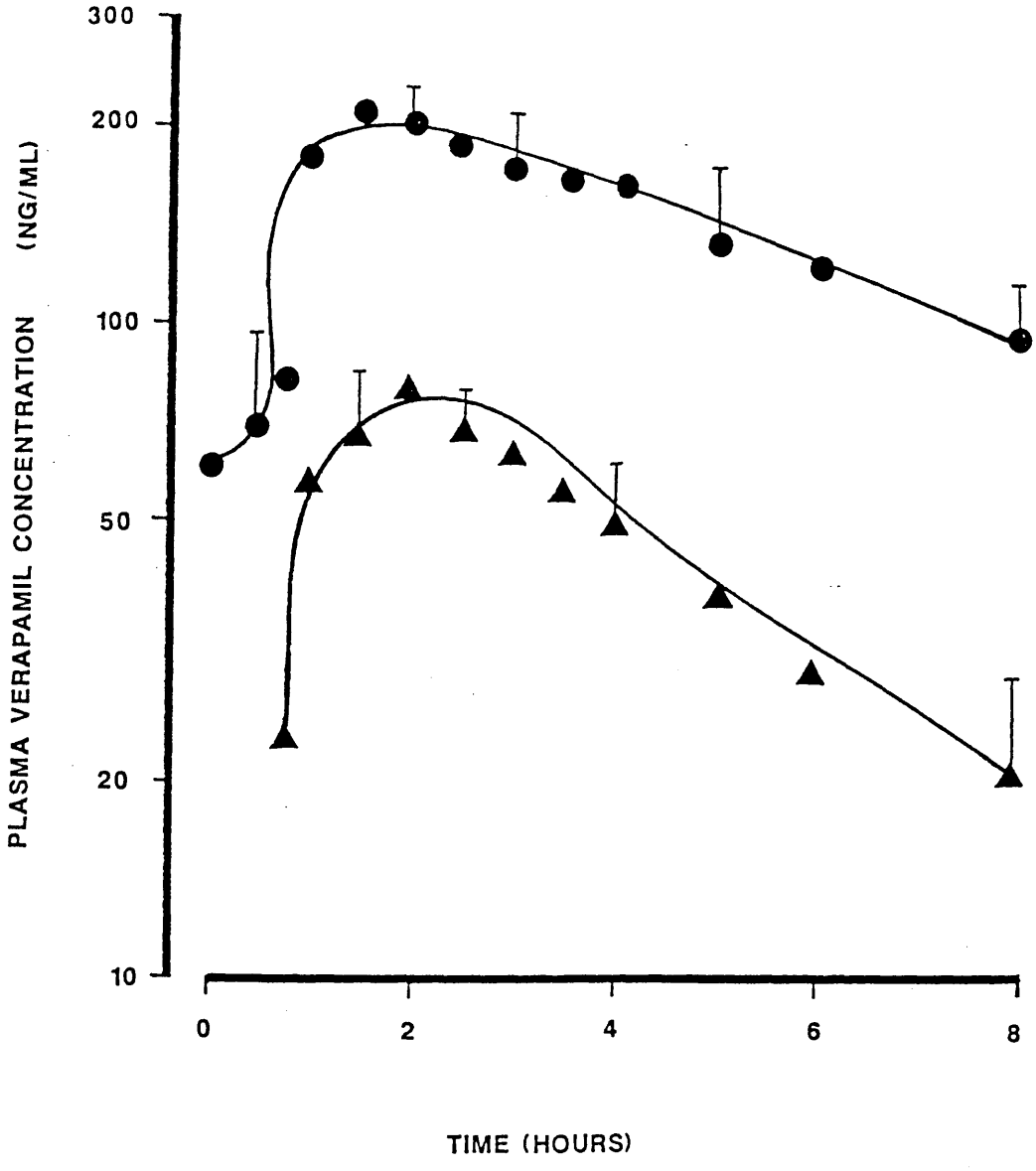


Fig.4.4. Mean pharmacokinetic profiles following acute (▲) and chronic (●) administration of verapamil in 6 hypertensive patients. Computer derived best fit of the points.

TABLE 4.1.

AREA UNDER THE PLACEBO CORRECTED FALL IN SYSTOLIC BLOOD PRESSURE PROFILE 0-8 h, AS DERIVED BY THE TRAPEZOIDAL RULE, AFTER VERAPAMIL ADMINISTRATION IN HYPERTENSIVE PATIENTS

	$AUC_{0-8\ h}$ (mmHg.h)	
Patient	Acute	Chronic
1	-34.10	-40.5
2	-61.5	-64.9
3	-50.4	-49.3
4	-42.9	-48.0
5	-33.5	-48.3
6	-47.3	-45.0
Mean ± S.D.	-44.9 ± 10.6	-49.3 ± 8.3
	NS	

TABLE 4.2.

PEAK VERAPAMIL CONCENTRATIONS AND TIME TO PEAK CONCENTRATIONS FOLLOWING ACUTE AND CHRONIC VERAPAMIL TREATMENT IN 6 HYPERTENSIVE PATIENTS

Patient	Peak Verapamil Concentration (ng/ml)		Time to Peak (-tlag) (h)	
	Acute	Chronic	Acute	Chronic
1	87.5	265.2	1.50	0.62
2	156.1	192.3	0.46	0.48
3	151.8	215.0	0.55	0.50
4	89.5	268.2	1.22	0.58
5	66.2	78.3	2.03	2.30
6	176.0	191.3	1.85	0.62
Mean ± S.D.	121.1 ± 45.4	201.7 ± 69.4	1.27 ± 0.65	0.85 ± 0.71
	$p < 0.01$		NS	

TABLE 4.3.

THE PHARMACOKINETICS OF VERAPAMIL AND NORVERAPAMIL FOLLOWING ACUTE ORAL ADMINISTRATION IN 6 HYPERTENSIVE PATIENTS

Patient	A (ng/ml)	α (1/h)	B (ng/ml)	β (1/h)	k_a (1/h)	k_{1m} V_c/V_m (1/h)	k_{mo} (1/h)	tlag (h)
1	251	0.55	36.9	0.116	1.13	0.73	0.86	0.5
2	144	0.97	94.8	0.202	5.43	0.82	0.94	0.9
3	555	1.64	69.9	0.126	2.73	14.6	24.6	0.45
4	258	0.80	55.4	0.155	1.46	1.92	1.48	0.28
5	534	0.43	358	0.368	0.50	6.10	5.91	1.97
6	298	0.39	183	0.176	0.84	1.45	1.76	0.66

A = coefficient
α = hybrid first order rate constant
B = coefficient
β = hybrid first order rate constant
k_a = first order rate constant
k_{1m} = rate constant describing metabolite formation
V_c/V_m = Volume of central and metabolite compartment
k_{mo} = rate constant describing metabolite elimination
tlag = time before drug is detected in the systemic circulation

TABLE 4.4.

THE PHARMACOKINETICS OF VERAPAMIL AND NORVERAPAMIL FOLLOWING CHRONIC ORAL ADMINISTRATION IN 6 HYPERTENSIVE PATIENTS

Patient	A (ng/ml)	α (1/h)	B (ng/ml)	β (1/h)	k_a (1/h)	k_{1m} V_c/V_m (1/h)	k_{mo} (1/h)	tlag (h)
1	215	0.60	118	0.08	21.2	0.17	0.19	0.99
2	597	1.33	45.5	0.031	2.83	0.40	0.37	0.50
3	276	0.72	52.6	0.044	24.8	0.88	1.29	0.49
4	256	0.72	101	0.063	14.9	0.55	0.56	2.49
5	284	2.01	121	0.106	1.5	1.97	2.05	1.72
6	329	0.65	39	0.036	2.83	0.73	0.65	0.40

No significant change in the relative clearance of norverapamil, as judged by the ratio of areas under the metabolite and parent drug time curves (AUC_m/AUC_d) was shown (Table 4.5.).

4.3.3. Concentration effect analysis

Concentration effect analysis allows us to characterise the dynamic profile on both acute and chronic therapy. Initially an attempt was made to model the effect using both parent drug and metabolite as described in previous studies with alpha adrenoceptor antagonists (Meredith et al, 1983) but in all subjects on both acute and chronic study days the model attributing effect of parent drug was most appropriate, as assessed by the general linear (F ratio) test (Bauxenbaum et al, 1974), and therefore used and presented here. The effect model parameters for both acute and chronic study days are given in Table 4.6.

The rate constant K_{eq} characterises the time course of the effect following any rapid change in blood concentration of drug and thus reflects the onset and offset of drug effect. There is wide interindividual variation and in some subjects this is large enough to suggest that concentration and effect are coincidentally correlated. However, in other subjects the K_{eq} is less than 3 which suggests that there is a considerable discrepancy between drug concentration and peak effect. Despite some intraindividual variations there were no significant or

TABLE 4.5.

DERIVED PHARMACOKINETIC PARAMETERS FOR ACUTE AND CHRONIC ORAL ADMINISTRATION OF VERAPAMIL

Patient	AUCd (ng.h/ml)		AUCm/AUCd		βt1/2 (h)	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
1	516	1822	0.84	0.91	5.98	8.66
2	575	1682	0.86	1.09	3.43	22.3
3	665	967	0.59	0.66	5.51	15.8
4	464	1941	1.30	0.98	4.47	10.9
5	411	1011	1.03	0.96	1.88	6.56
6	1238	1460	0.82	1.13	3.94	19.3
Mean ± S.D.	645 ± 304	1314 ± 391	0.91 ± 0.24	0.96 ± 0.17	4.20 ± 1.48	10.6 ± 6.2
	$p < 0.001$		NS		$p < 0.005$	

AUC_d = area under the curve for the parent drug
AUC_m = area under the curve for the metabolite
βt1/2 = terminal elimination half-life

TABLE 4.6.

CONCENTRATION-EFFECT ANALYSIS OF ACUTE AND CHRONIC VERAPAMIL - DERIVED PARAMETERS

	Slope (m) Sensitivity (mmHg/ng/ml)		keq (1/h)	
Patient	Acute	Chronic	Acute	Chronic
1	-0.080	-0.053	3.1	2.0
2	-0.164	-0.151	0.53	0.78
3	-0.112	-0.124	0.73	0.47
4	-0.129	-0.119	2.4	0.32
5	-0.090	-0.081	3.4	1.5
6	-0.053	-0.073	4.6	1.7
Mean ± S.D.	-0.105 ± 0.039	-0.100 ± 0.037	2.5 ± 1.6	1.13 ± 0.70
	NS		NS	

systematic differences between K_{eq} values on acute and chronic study days. The effect model parameter m or slope showed similar potency on both study days in terms of blood pressure fall per unit drug concentration in blood (Table 4.6.).

4.4. DISCUSSION

Verapamil showed in this small group of patients acute antihypertensive activity which was maintained and even prolonged throughout the day after 1 month treatment as reported by other authors (Leonetti et al, 1980; Corea et al, 1981). Heart rate did not change significantly either acutely or chronically. A slight reduction of the post-prandial increase in standing heart rate following verapamil administration was observed compared to placebo (Figure 4.1.). A similar attenuation by verapamil of the increase in heart rate is more clearly observed in Chapter 6 following co-administration of prazosin in normal subjects. The placebo corrected effect-time profiles showed a similar fall in blood pressure on both acute and chronic day.

A significant increase in the area under the curve and elimination half-life with chronic dosing was observed as reported by other groups (Freedman et al, 1981; Shand et al, 1981) and also in this thesis after repeated dosing in normal volunteers (Chapter 7).

Simple approaches to analyse the dynamic profiles seemed inappropriate due to these changes in kinetics.

Concentration effect analysis using an effect compartment has shown that the responsiveness on acute and chronic therapy is similar suggesting that any differences in the effect profile are likely to be due to differences in pharmacokinetics. The change in K_{eq} although not significant indicates an earlier and longer lasting effect with chronic dosing of verapamil. In conclusion the mathematical model applied has shown that a relationship between plasma concentration of verapamil and blood pressure fall exists and is also maintained with chronic dosing. For a fixed increase in blood concentration of drug in the effect compartment a similar change in blood pressure is expected on both acute and chronic treatment. Plasma concentration is, indeed, another determinant of verapamil antihypertensive activity.

CHAPTER 5

EFFECT OF CALCIUM ANTAGONISTS ON ADRENERGIC AND NON ADRENERGIC VASCULAR RESPONSES AND PLATELET AGGREGATION

VASCULAR PRESSOR RESPONSE AFTER VERAPAMIL AND NISOLDIPINE

5.1. INTRODUCTION

Animal studies have shown that at least two types of alpha adrenoceptor contribute to peripheral vasoconstrictor tone (Bentley et al, 1977; Drew and Whiting, 1979; Docherty & McGrath, 1980; Timmermans & van Zwieten, 1980; Yamaguchi & Kopin, 1980) and recent evidence from human studies supports this view (Kiowski et al, 1983; van Brummelen et al, 1983; Elliott & Reid, 1983; Murphy et al, 1984a). The experimental evidence is that the responses mediated via alpha adrenoceptors are dependent upon modifications in the transport of Ca^{++} across cell membranes and also by mobilization of intracellular calcium (Bohr, 1963; Fain & Garcia-Sainz, 1980). It has been suggested that stimulation of the different post-synaptic alpha-adrenoceptor subtypes on vascular smooth muscle may modify transmembrane calcium flux by different mechanisms (Van Breemen et al, 1982): in particular, that activation of the $alpha_1$ receptor is associated with an augmented intracellular Ca^{++} mobilization which is not directly inhibited by calcium entry blockers (Langer & Shepperson, 1982) and that activation of the $alpha_2$ receptors is associated with an increased entry of extracellular Ca^{++} which is inhibited by calcium entry blockers (Van Meel et al, 1981). It has been claimed that calcium antagonists exert their peripheral vasodilator actions by preferential

antagonism of alpha$_2$ receptors (Van Zwieten et al, 1982).

Calcium antagonists drugs have been widely used for the treatment of ischaemic heart disease and hypertension. The papaverine derivative, verapamil, has shown not only peripheral vasodilator activity but also cardiac effects with decreased myocardial contractility and atrioventricular conduction. In relative contrast dihydropyridine derivatives like nisoldipine and nifedipine, exert their greatest effects on vascular smooth muscle with little direct action on the heart.

These two subgroups of calcium antagonists also have different affinities for different tissues and vascular beds and furthermore there is evidence that they have different actions on calcium channels (Karliner et al, 1982) and different effects on alpha adrenoceptor function (Motulsky et al, 1983; Saeed et al, 1983).

The first of the studies described in this chapter investigates in normotensive males the relationship between calcium channels and alpha adrenoceptors and also possible differences in the peripheral vascular actions of verapamil and the new dihydropyridine, nisoldipine, which has been claimed to have a more selective action on the peripheral vasculature (Kazda et al, 1980; Knorr, 1982) than its analogue, nifedipine.

The second part of this chapter deals with the effects of the two drugs on platelet function by investigating platelet aggregation and activation which is dependent upon

both calcium ions and alpha$_2$ adrenoceptors.

5.2. METHODS

The study was performed on nine healthy normotensive males aged 20-40 years, body weight within 10% of ideal values (67.6 ± 7 kgs).

In a single blind randomised order study the subjects received either placebo or verapamil 160 mg orally or nisoldipine 20 mg orally for four days. Subjects were studied on the first and the fourth day of each treatment period. Treatment periods were separated by at least two weeks. Studies were undertaken in a quiet temperature controlled (20 ± 2^{o}C) Clinical Pharmacology Research Unit, after an overnight fast, and subjects remained supine throughout each study day. Blood pressure and heart rate were measured by automatic blood pressure recorder (SENTRON) at intervals up to 8 hours after dosing.

Between 1 and 4 hours after drug administration vascular responsiveness was assessed by a series of intravenous infusions of increasing doses of three pressor agents: phenylephrine, a selective alpha$_1$ agonist; alpha-methylnoradrenaline, a relatively selective alpha$_2$ agonist; and angiotensin II, a non-adrenergic "direct" vasoconstrictor. The sequence of the infusions was randomised but kept constant for each individual throughout the whole study. Each agonist was administered by incremental infusion, with 5 minutes at each of not less than 3 dose levels, until mean

arterial pressure increased by about 30 mmHg, with limits of 45 mmHg systolic and 30 mmHg diastolic pressure. The doses ranged from 0.5-10 μg/kg/min for phenylephrine, 0.01-5.0 μg/kg/min for alpha-methylnoradrenaline and 5.0-150 ng/kg/min for angiotensin II.

After each infusion subjects rested for 30 minutes to allow blood pressure and heart rate to return to within ± 5 mmHg of basal values of mean arterial pressure before the next infusion was started.

5.2.1. Verapamil Assay

Whole blood concentrations of verapamil and its metabolite, norverapamil, were determined by HPLC with fluorescence detection as described in Chapter 2.

5.2.2. Data analysis.

The change in blood pressure was plotted against the log dose of each agonist and fitted to a quadratic function (to include all data points) to derive dose-response curves as described by Sumner et al (1982). The dose of agonist required to raise mean arterial pressure by 20 mmHg (PD_{20}) was obtained by interpolation from each individual curve and the dose ratios calculated for each treatment when compared to placebo. Data were submitted to log transformation (which was shown to satisfy statistically the assumptions of constant variance and normality of distribution) and treatments were compared by paired t-test with Bonferoni

correction.

5.3. RESULTS

The baseline (pre-treatment) blood pressures and the control (pre-infusion) blood pressures are shown in Table 5.1. There were no significant differences between the different study days.

The results of the pressor response studies are shown as the computer fitted mean curves in Figures 5.1. - 5.3. and are summarised in Table 5.2. with statistical evaluation based on comparisons of individual curves.

5.3.1. Phenylephrine

The pressor responsiveness to phenylephrine was significantly altered, with progressive shifts to the right of the log dose pressor response curves after both acute (1st day) and multiple dosing (4th day) of treatment with both drugs (Figure 5.1.). The mean PD_{20} following placebo was 2.5 μg/kg/min and this was significantly increased to 4.6 following the first dose of verapamil and to 6.4 μg/kg/min following 4 days of verapamil ($p < 0.01$). Following nisoldipine, both acute treatment with a mean PD_{20} of 6.4 and 4 days of treatment with a mean PD_{20} of 9.9 μg/kg/min were significantly different ($p < 0.02$) from placebo. There were no significant differences between the two drugs.

TABLE 5.1.

SUPINE BLOOD PRESSURE FOLLOWING VERAPAMIL AND NISOLDIPINE ADMINISTRATION IN 9 SUBJECTS (MEAN ± SD)

	PLACEBO	VERAPAMIL		NISOLDIPINE	
		Acute	"Chronic"	Acute	"Chronic"
Baseline 0 hours	120/67 ± 9/9	122/67 ± 9/6	120/68 ± 5/7	120/65 ± 8/7	118/65 ± 7/9
Pre-Angiotensin	116/67 ± 7/6	113/66 ± 8/7	114/62 ± 7/5	115/61 ± 8/6	115/63 ± 9/8
Pre-Phenylephrine	114/64 ± 6/6	115/61 ± 7/6	114/65 ± 7/5	117/63 ± 6/5	116/60 ± 8/7
Pre-Alphamethyl-noradrenaline	119/67 ± 5/7	116/64 ± 7/7	116/61 ± 6/5	114/62 ± 8/7	114/61 ± 6/8

TABLE 5.2.

PRESSOR RESPONSES TO PHENYLEPHRINE, ALPHAMETHYLNORADRENALINE AND ANGIOTENSIN II INFUSIONS IN 9 SUBJECTS AFTER VERAPAMIL AND NISOLDIPINE ADMINISTRATION
MEAN ± SD

		Phenylephrine	Alpha Methylnor-adrenaline	Angiotensin II
PLACEBO	PD_{20}	2.45 ± 1.54	0.54 ± 0.37	30 ±24.6
VERAPAMIL Acute	PD_{20}	4.59 ± 2.78	0.54 ± 0.4	54.3 ±32.5
	D.R	* 2.1 ± 1.2	0.9 ± 0.3	* 2.4 ± 1.7
Chronic	PD_{20}	5.41 ± 3.39	0.74 ± 0.7	109.5 ±70.1
	D.R	* 3.0 ± 1.6	1.1 ± 0.6	* 4.6 ± 3.5
NISOLDIPINE Acute	PD_{20}	6.36 ± 3.39	0.46 ± 0.29	67.6 ±44.6
	D.R	* 3.1 ± 2.1	0.9 ± 0.3	* 2.4 ± 1.2
Chronic	PD_{20}	9.94 ±13.4	1.08 ± 1.08	91 ±97.7
	D.R	* 3.6 ± 1.9	* 1.7 ± 0.6	* 3.1 ± 1.6

* = $p < 0.05$ compared to placebo.

PD_{20} = the dose required to increase blood pressure by 20 mm Hg.
D.R = Dose ratio = the ratio between the PD_{20} for each active treatment and the PD_{20} for placebo.

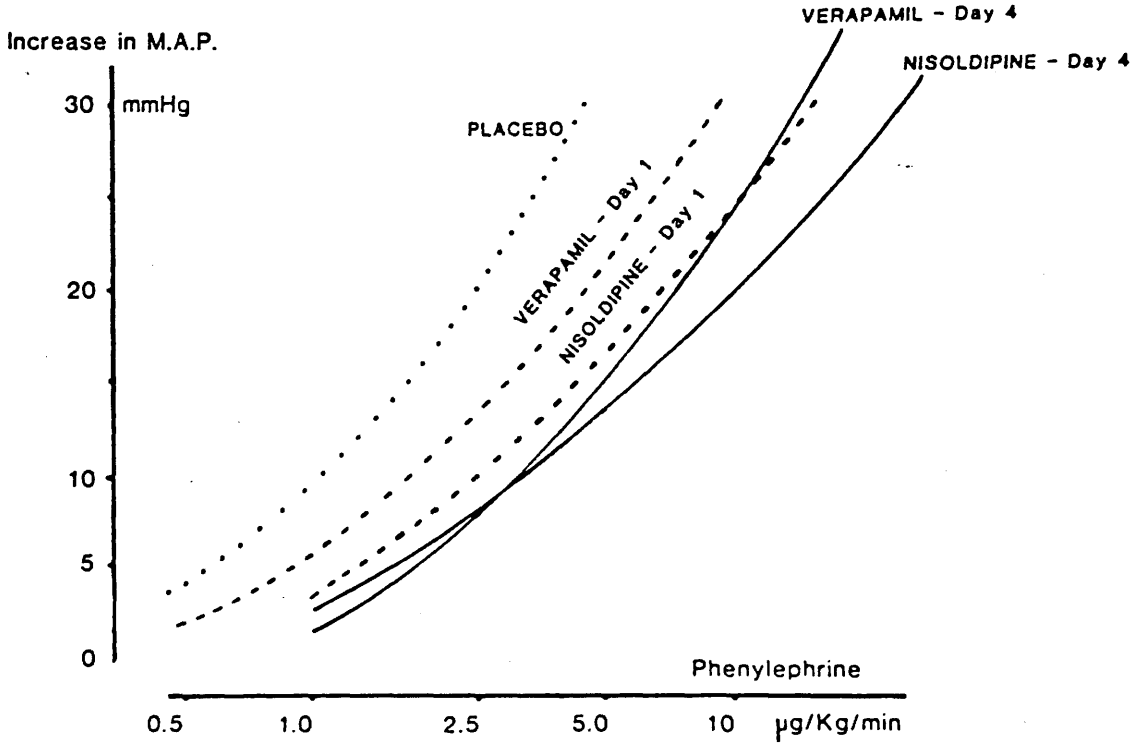


Fig.5.1. Mean pressor dose-response curves for phenylephrine (9 subjects). Responses for all treatments.

5.3.2. Alpha-methylnoradrenaline.

The pressor responsiveness to alpha-methylnoradrenaline showed a similar pattern but the shifts to the right of the log dose pressor response curve particularly after the first dose administration of the two drugs were modest (Figure 5.2.). Only after 4 days of nisoldipine was a significant shift obtained: PD_{20} of 1.1, compared to 0.5 μg/kg/min after placebo, and this shift was also significantly greater than that observed with the first dose of nisoldipine ($p<0.05$).

5.3.3. Angiotensin II

Both active treatments modified the pressor responsiveness to angiotensin II shifting the dose-response curves to the right (Figure 5.3.). These shifts were both marked and statistically significant following continued therapy with both drugs: the mean PD_{20} was 110 after verapamil and 91 ng/kg/min following nisoldipine, compared to 30 ng/kg/min after placebo.

5.3.4. Drug concentration-effect relationships

There were significant relationships between the whole blood verapamil concentrations and the dose ratios for phenylephrine ($r = 0.63$; $p < 0.01$) and for angiotensin II ($r = 0.61$; $p < 0.05$). There was no such correlation for the more modest shifts after alpha-methylnoradrenaline. The inclusion of norverapamil concentrations in these analysis

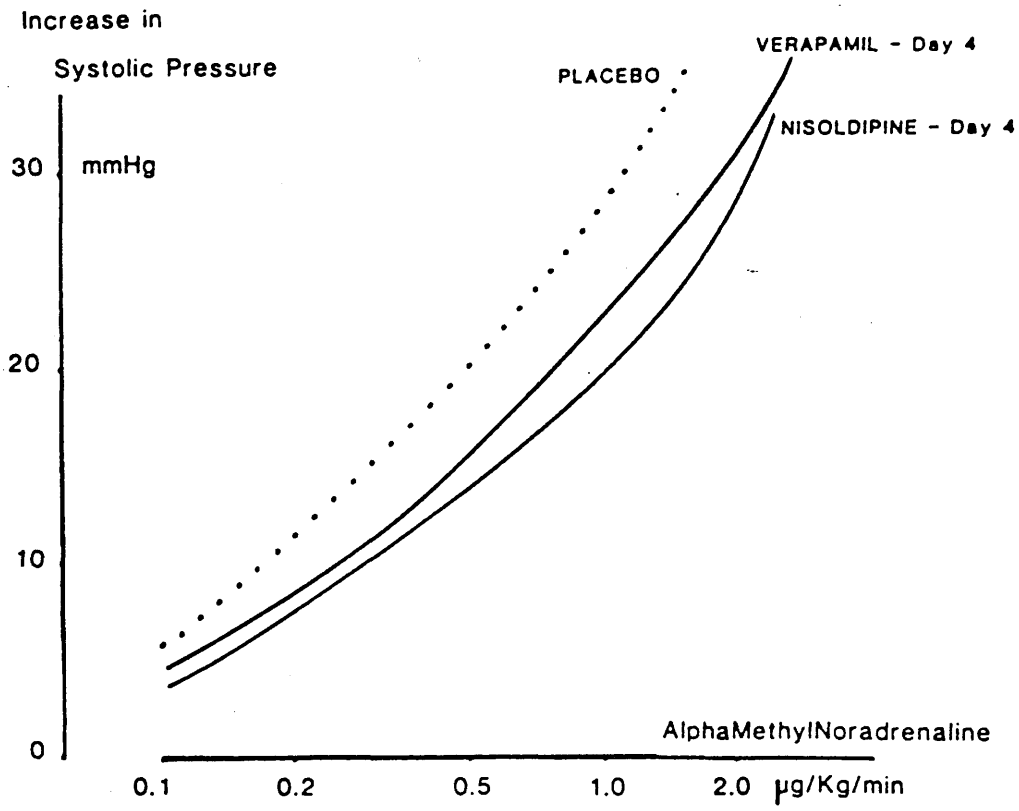


Fig.5.2. Mean pressor dose-response curves for alphamethylnoradrenaline (9 subjects). Response for continued administration with verapamil and nisoldipine compared with placebo.

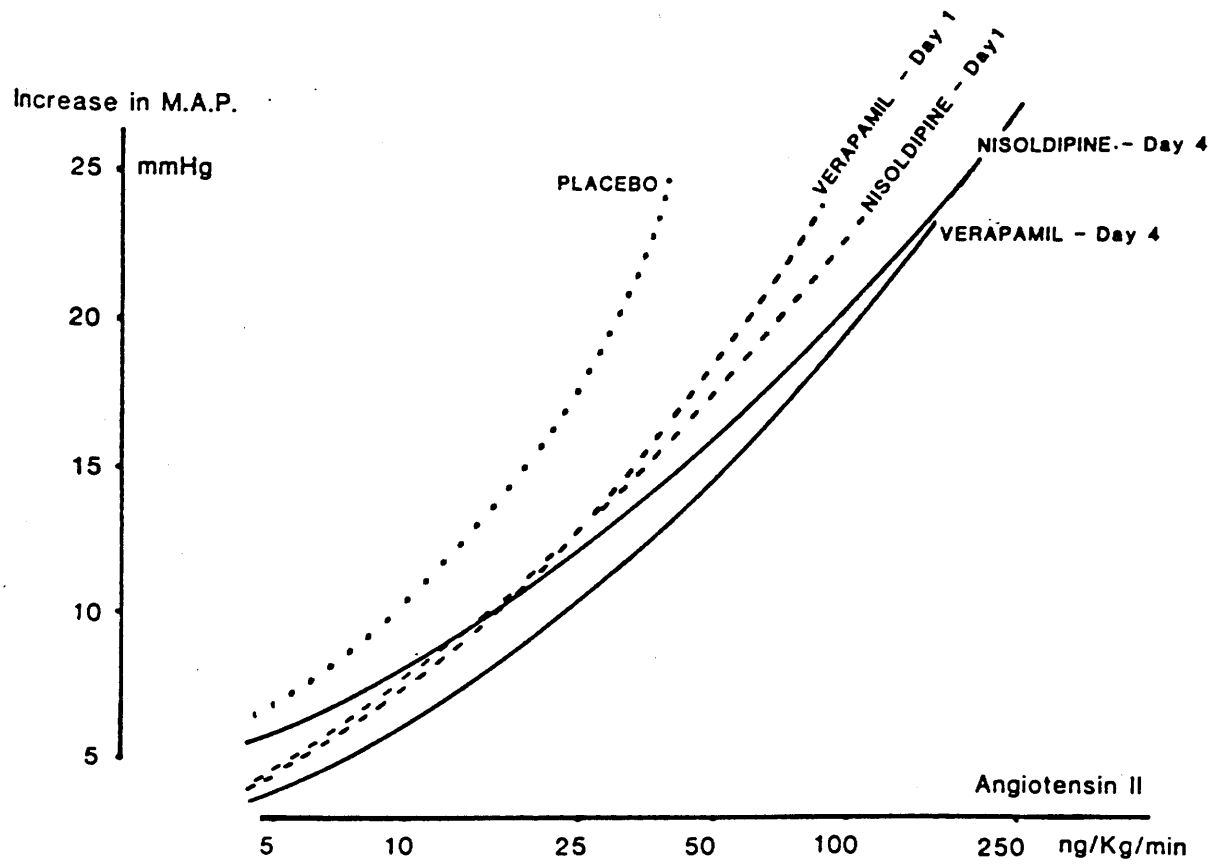


Fig.5.3. Mean pressor dose-response curves for angiotensin II (9 subjects). Responses for all treatments.

did not significantly improve these correlations.

5.4. DISCUSSION

Previous studies in man have shown that calcium channel blocking drugs impair the pressor response to the "direct" vasoconstrictor angiotensin II (Vierhapper et al, 1982; Millar et al, 1983) and the results of this study confirm such a peripheral effect for both verapamil and nisoldipine, particularly with continued administration. After the first dose of each drug, however, interference with angiotensin-induced vasoconstriction tended to be more marked with nisoldipine and this may be an indication of its greater selectivity for peripheral vascular sites.

The relationship between alpha adrenoceptors and the activation of calcium channels has not been well defined in man. Impairment of adrenergic responsiveness to noradrenaline has been previously ascribed to a "non-specific" effect of calcium channel blockade rather than a direct effect on alpha adrenoceptors. In this study, both verapamil and nisoldipine caused significant shifts in the pressor responses to alpha agonists. This was most obvious with the $alpha_1$ agonist phenylephrine where there were 3-fold shifts in the PD_{20} for both drugs. Because of its relatively lesser selectivity for peripheral vascular smooth muscle the $alpha_1$ antagonist activity of verapamil (Motulsky et al, 1983) has been previously attributed to a less specific calcium antagonist action for this particular drug.

The results in this study, however, indicate that a comparable alpha$_1$ antagonist effect was obtained with nisoldipine for which there is no other direct evidence of alpha$_1$ antagonist properties. Similar conclusions were reached in a recently published study on alpha adrenoceptor antagonist activity of nifedipine (Murphy et al, 1984b).

It has been suggested that activation of peripheral alpha$_2$ adrenoceptors is specifically linked with transmembrane calcium fluxes and the actions of calcium antagonists (van Meel et al, 1981; van Zwieten et al, 1982; Timmermans et al, 1983) and so the pressor responses to an alpha$_2$ agonist might have been expected to show the most marked changes. Alpha-methylnoradrenaline has relatively selective alpha$_2$ agonist properties (Starke et al, 1975; Starke, 1977) and pressor responses to alphamethylnoradrenaline have been shown to be markedly attenuated in man by the selective alpha$_2$ antagonist idazoxan (Elliott and Reid, 1983). However, only after 4 days treatment with nisoldipine was a significant shift in the dose-response curve for alpha-methylnoradrenaline observed. The overall effects of both calcium antagonists were more marked on the alpha$_1$-mediated responses and the non-adrenergic response to angiotensin II, compared to their effects on the alpha$_2$-mediated responses. These results are consistent with those reported with nifedipine which has also been shown to have an adrenergic antagonist action without demonstrable selectivity for alpha$_2$ adrenoceptors in

both animals (Alabaster and Solca, 1985) and man (Murphy et al, 1984b).

Although apparent selectivity of alpha$_2$ receptor mediated pressor responses has been demonstrated in several species (van Zwieten et al, 1982) there is now considerable doubt as to the interpretation of these findings. It has been shown in animals that alpha$_1$ mediated responses become sensitive to the action of calcium antagonists following pre-treatment with phenoxybenzamine (Ruffolo & Yaden, 1984) and this would be consistent with other observations that "spare" receptors of the alpha$_1$ sub-type may be present in vascular muscle whereas the reserve capacity of alpha$_2$ receptors is limited so that there is a closer link between response and alpha$_2$ receptor number (Hamilton et al, 1983). Alternative explanations include differences in sensitivity between full agonists, like alpha-methylnoradrenaline, and partial agonists or even differences between phenylethylamines and imidazolines in their binding to receptors or post-receptor mechanisms and associations with calcium channels.

In conclusion, this study demonstrates in man that adrenergic responses mediated via both alpha$_1$ and alpha$_2$ receptors are affected by calcium antagonist drugs. There was no evidence with either verapamil or nisoldipine that this effect was specifically linked to the alpha$_2$ adrenoceptor.

VERAPAMIL, NISOLDIPINE AND PLATELET AGGREGATION

5.5. INTRODUCTION

Calcium ions are involved in several stages of platelet activation including platelet adhesion to endothelium, platelet shape change, the excitation contraction coupling in the release of vasoactive substances and the synthesis of the metabolites of arachidonic acid (Ardlie, 1982).

Similarities exist between the role of calcium in platelet activation and in contraction of vascular smooth muscle where it has been shown that calcium antagonist drugs will reduce both the entry of calcium associated with agonist activation (Rosenberg et al, 1979; Vanhoutte, 1982a) and the mobilisation of intracellular calcium from sites of storage (Wang et al, 1984).

Since platelet abnormalities have been found in both hypertension (Mehta and Mehta, 1981) and ischaemic heart disease (Burns and Frishman, 1983) the antiplatelet actions of the calcium antagonists may expand the therapeutic role of these agents (Barnathan et al, 1982).

5.6. SUBJECTS AND METHODS

From the subjects participating in the study described in the previous section of this chapter a blood sample was withdrawn 1 hour after either drug or placebo administration to assess platelet aggregation.

5.6.1. Platelet preparation and aggregation

Venous blood samples were anticoagulated with 0.1% W/V 3.28% sodium citrate and centrifuged at 180 g for 15 minutes at 20°C to prepare platelet rich plasma (PRP). Platelet poor plasma was prepared by further centrifugation of the remaining blood at 1700 g for 15 minutes. Platelet aggregation was quantified by the turbidometric method of Born (1962). The change in optical density through the samples was measured in a Payton dual channel aggregometer. Aggregation studies were performed at platelet counts of 300 x 10^9/L adjusted by platelet poor plasma after counting in a Coulter counter at a wavelength of 880 nM.

In vitro additions were made of adenosine diphosphate (ADP)(Sigma Chemical Company) or (l)-adrenaline bitartrate (Sigma Chemical Company) dissolved in 0.9% saline with 1mM ascorbic acid and diluted from stock solution stored at -70°C. A dose response curve to adrenaline was produced by plotting the concentration of adrenaline (11-12 concentrations) against the maximum rate of aggregation and the results fitted by an iterative technique to a generalised model of the Hill equation to obtain parameter estimates for maximum aggregation (R_{max}) and the concentration of adrenaline required to produce 50% maximum aggregation (C_{50} μM). For determination of inhibitory responses the response was plotted against the concentration of antagonist required to cause 50% inhibition, at agonist concentrations of 1 μM for adenosine diphosphate and 5 μM

for adrenaline. Verapamil powder was dissolved in 0.9% saline and nisoldipine powder in 1% ethanol in platelet poor plasma. All experiments with nisoldipine were performed under sodium light as this dihydropyridine is photolabile.

5.6.2. Alpha$_2$-adrenoceptor binding assay

Platelet rich plasma was spun at 1700 g for 15 minutes at 4^{o}C to produce a platelet pellet. The pellet was suspended in 0.1% EDTA 150 m M NaCl pH 7.4 to give a platelet concentration of 100 x 10^9 platelets/ litre. Whole platelet suspensions (0.8 ml) were incubated for 20 minutes at 25^{o}C with 6.5 nM ^{3}H yohimbine in triplicate with varying concentrations of nisoldipine and verapamil. Non specific binding was defined by 1 μM phentolamine; incubations were terminated with 20 ml of ice cold Tris (50mM pH7.4) through a Millipore multiport filtration apparatus on to Whatman GFC filters and bound radioactivity determined by liquid scintillation counting. The K_i was calculated from the IC_{50} values for inhibition of binding of the alpha$_2$ adrenoceptor ligand ^{3}H yohimbine which were found from dose response curves for verapamil inhibition of ^{3}H yohimbine binding and converted into K_i values according to the equation of Cheng and Prussof (1973):

$$K_i = \frac{IC_{50}}{S/K_D + 1}$$

IC_{50} is the concentration of the competing agent which

inhibits specific ^{3}H yohimbine binding by 50%. S is the concentration of ^{3}H yohimbine in the assay (6.25 nM) and K_D is the equilibrium dissociation constant for ^{3}H yohimbine binding determined from saturation experiments from the six subjects whose blood was used in the displacement K_DnM (2.42 ± 1.02, n = 6).

5.6.3. Statistical Analysis

Statistical analysis was by paired Student's 't' test with $p < 0.0125$ taken as significant to allow for multiple comparisons (Ingelfinger et al, 1983). All results are expressed as mean ± standard deviation.

5.7. RESULTS

5.7.1. In Vitro Studies

Platelet Aggregation

Verapamil inhibited the aggregatory response to adrenaline. The IC_{50} was 16.8 ± 2.6 μM. The aggregatory response to adenosine diphosphate was also inhibited but the concentration to inhibit the response by 50% was over 40-fold higher at 723 ± 102 uM (Figure 5.4.). Nisoldipine at a concentration of up to 100 uM had no effect on the primary aggregatory response to adrenaline concentration range but caused a 67% ± 13%inhibition of the secondary aggregation response to 5 μM adrenaline (Figure 5.5.) when compared with the control response in the presence of vehicle. There was no alteration of aggregatory response to 1 μM adenosine

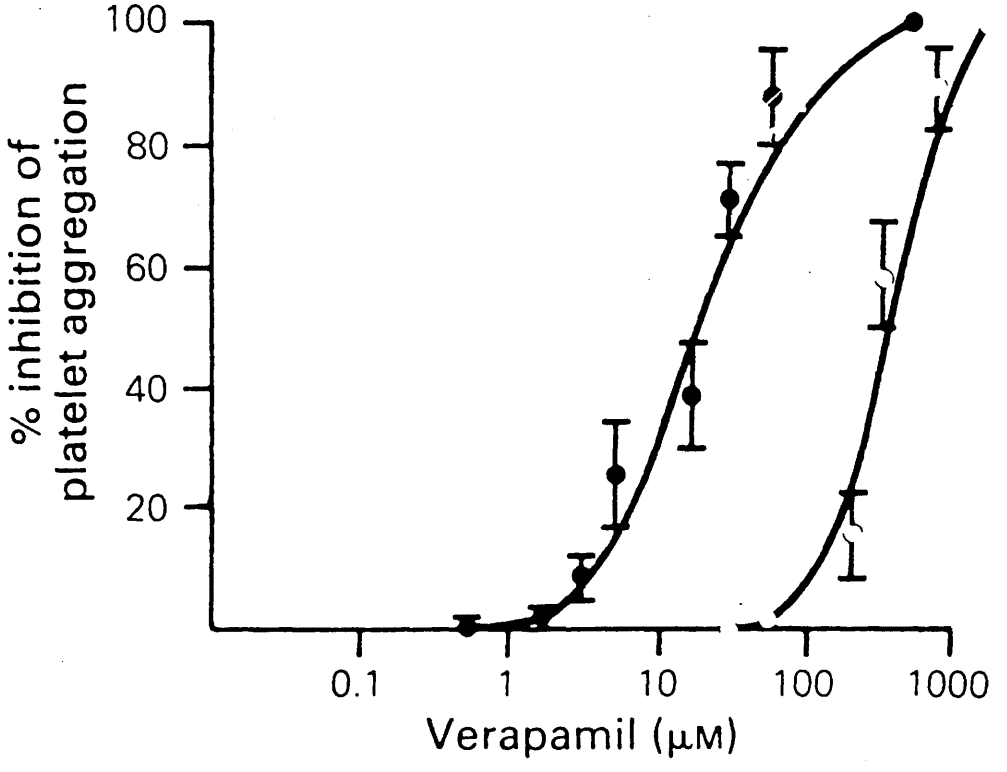


Fig. 5.4. The percentage inhibition by verapamil of the primary aggregation response to adrenaline (5 μM) (●) and the threshold response to adenosine diphosphate (1 μM) (○) in 6 subjects.

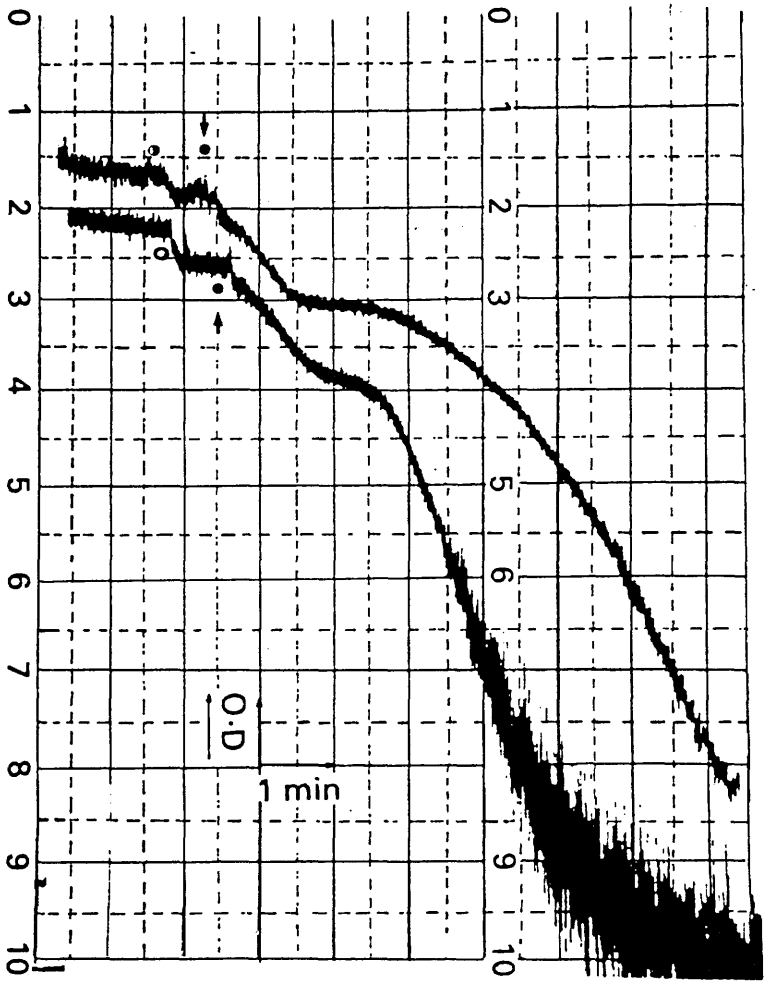


Fig. 5.5. A representative platelet aggregation tracing for the inhibition of secondary platelet aggregation by nisoldipine; the ordinate represents optical density (O.D.) and the time scale of 1 min. marked on the abscissa. Top tracing adrenaline (5 μM arrow) in the presence of nisoldipine (100 μM) (●). Bottom tracing adrenaline (5 uM arrow) in the presence of vehicle (o).

diphosphate.

Radioligand Binding

Verapamil inhibited the binding of [^{3}H] yohimbine to platelets with an IC_{50} of 2.73 ± 0.26 uM (K_i = 0.75 uM) whereas nisoldipine did not affect ^{3}H yohimbine binding (Figure 5.6.).

5.7.2. In Vivo Studies

Platelet Aggregation

Neither nisoldipine nor verapamil had any significant effect on the aggregatory responses to adenosine diphosphate either after acute dosing or after 4 days treatment (Table 5.3.). Verapamil for 4 days altered the aggregatory dose response curve to adrenaline with significant reductions in both the maximal rate of aggregation from 47 ± 18 to 28 ± 16 OD/min ($p < 0.002$) and increases in the C_{50} for adrenaline induced aggregation from 0.77 ± 0.25 to 1.14 ± 0.54 M ($p < 0.003$). Nisoldipine after 4 days caused an increase in the C_{50} value but no change in the maximal rate of aggregation (Figure 5.7.).

Plasma Levels of Verapamil

There were no significant correlations with plasma levels of verapamil or its metabolite, norverapamil, and the changes in platelet aggregation (C_{50} or E_{max}).

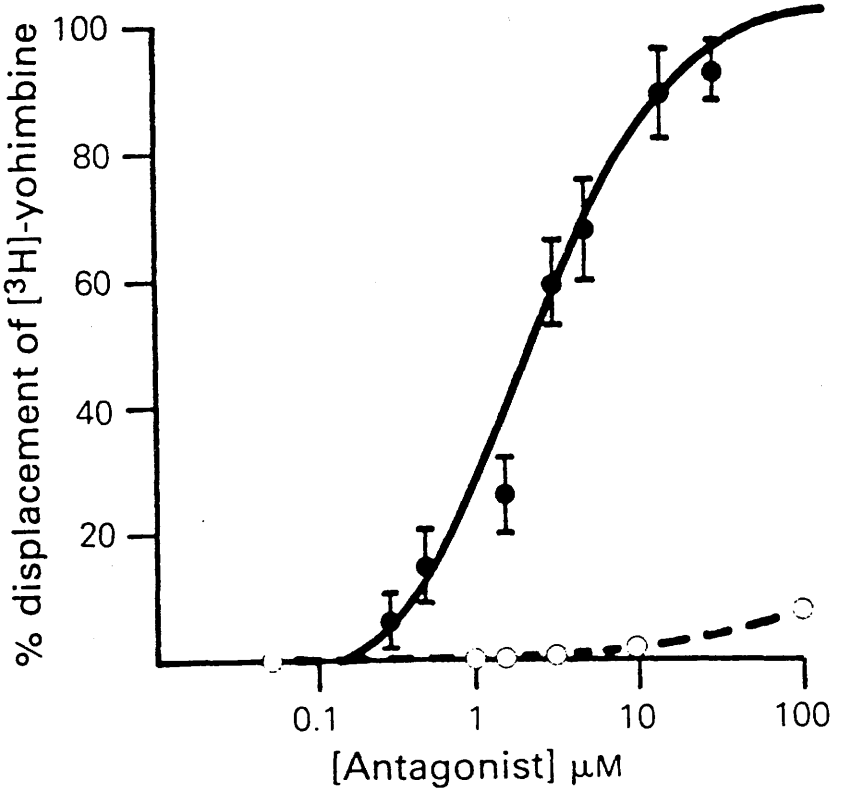


Fig. 5.6. Verapamil and nisoldipine displacement of specifically bound tritiated-yohimbine to whole platelets: verapamil (●) and nisoldipine (o).

TABLE 5.3.

PLATELET AGGREGATION RESPONSES TO 1 uM ADENOSINE DIPHOSPHATE AFTER ADMINISTRATION OF VERAPAMIL AND NISOLDIPINE IN 6 SUBJECTS
(Δ O.D. MEAN ± SD)

	ACUTE	CHRONIC
PLACEBO	32 ± 12	33 ± 13
VERAPAMIL	28 ± 13	26 ± 14
NISOLDIPINE	24 ± 13	23 ± 13

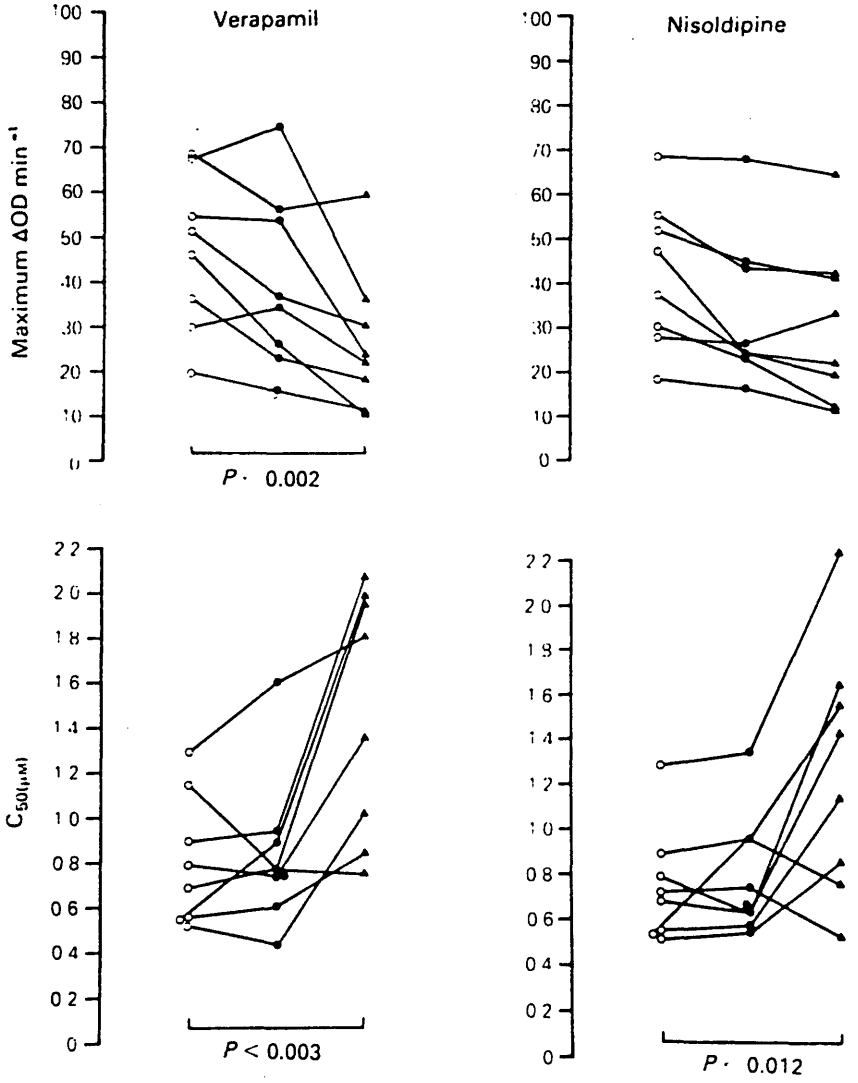


Fig.5.7. The effects of placebo (o), the first dose (•) and 4 days treatment (▲) with verapamil or nisoldipine on platelet aggregation in vitro induced by adrenaline expressed as the maximum rate of primary aggregation upper (Emax Δ OD min^{-1}), and the concentration of adrenaline to achieve 50% of maximal aggregation C_{50} (μM) lower. p values refer to significance levels obtained by comparing placebo with 4 day treatment values by paired t test. No significant difference was found between the Emax values with nisoldipine treatment.

5.8. DISCUSSION

The effects on platelet aggregation of verapamil and the dihydropyridine nifedipine have been examined in vitro in both animals and man (Johnsson, 1981; Kiyomoto et al, 1983). It has been shown that platelet aggregation induced by adenosine diphosphate is relatively resistant to inhibition by both verapamil and nifedipine. The results obtained in this study confirm these findings in vitro and in vivo. This suggests that adenosine diphosphate triggers platelet aggregation through pathways which are not sensitive to blockade by calcium channel blockers. The role of calcium in adrenaline-induced platelet aggregation is under debate. Some authors using ^{45}Ca and chlortetracycline (Owen et al, 1980), the calcium fluorescent probes Quin II (Erne et al (1983) and aequorin (Johnson et al, 1983) have shown that adrenaline induced platelet aggregation is associated with calcium influx. Other authors have shown no change in platelet calcium during adrenaline activation in calcium free media with Quin II (Bryden et al, 1984). The present study shows that caution must be exercised when using verapamil to examine whether or not pharmacological responses are calcium dependent. The inhibition of the adrenaline response by verapamil cannot be used to resolve this question since verapamil has been shown to have other properties in addition to its calcium channel blocking effects, with activity as an $alpha_1$ and muscarinic antagonist in rat

myocardium (Karliner et al, 1982). Other authors using a different ligand have also found an interaction between verapamil and human platelet alpha$_2$ adrenoceptors using [^{3}H] RX781094 to measure platelet alpha$_2$ adrenoceptor number (Maisel et al, 1984). Verapamil has also been reported to act as an antagonist to platelet activating factor (PAF) induced calcium changes in platelets (MacIntyre and Shaw, 1982). Similarly caution must be used when interpreting the effects of nisoldipine in inhibiting the secondary phase of platelet aggregation. The related dihydropyridine nifedipine has been shown to be a thromboxane A_2 antagonist (Addonizio et al, 1982). Platelets possess adrenoceptors of the alpha$_2$ subtype as detected by ^{3}H yohimbine binding (Motulsky et al, 1980). The demonstration of the inhibition of specific ^{3}H yohimbine binding could be due to a direct interaction with alpha$_2$ adrenoceptors or to steric hindrance due to the proximity of receptor operated calcium channels. The potency of verapamil as an alpha$_2$ blocker at platelet alpha receptors is similar to that observed with phenoxybenzamine (Brodde et al, 1982). The results of the clinical study in which oral dosing was continued for 4 days show that a significant inhibition of the aggregatory response to adrenaline, but not to adenosine diphosphate, may occur after both verapamil and nisoldipine in vivo in man. The peak plasma concentration measured during verapamil therapy was 10-fold less than the concentration required to inhibit specific yohimbine binding in vitro by

50%. Although there was some accumulation of the metabolite, norverapamil, during continued dosing this is less active at inhibiting platelet aggregation. The discrepancy between the effects during multiple dosing and the effects in vitro might also be due to accumulation of drug within the platelet. Alternatively, chronic ingestion of these agents may deplete intracellular calcium as has been reported with other antihypertensive agents (Erne et al, 1984).

In summary, the antiplatelet actions of both verapamil and nisoldipine may have implications for the primary prevention of atherosclerosis and the prevention of platelet mediated thrombosis in the treatment of hypertension and ischaemic heart disease. However, although adrenaline-induced platelet aggregation can be inhibited in vitro by both verapamil and nisoldipine, the evidence of this study indicates that the antiplatelet effects in vivo are mediated by a different mechanism.

CHAPTER 6

STUDIES ON THE PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTIONS BETWEEN VERAPAMIL AND PRAZOSIN

6.1. INTRODUCTION

The peripheral vasodilator action of the calcium channel blocker, verapamil, is not associated with a reflex increase in cardiac output, even after acute dosing. This is in part due to its also having a depressant effect on myocardial contractility and atrioventricular conduction (Rowland et al, 1979).

In contrast, the antihypertensive drug prazosin, a selective antagonist of peripheral vascular $alpha_1$ adrenoceptors (Graham et al, 1977) has an acute hypotensive effect which is associated with reflex increases in heart rate and cardiac output (Lund Johansen, 1980). Thus, the combined use of verapamil and prazosin may have therapeutic advantages with an additive peripheral vasodilator action and a counterbalancing of verapamil's cardiac depressant effect with the reflex cardiostimulant effect of prazosin. However, these drugs may also interact in other ways. There is evidence that some types of Ca^{2+} channels are closely linked to $alpha_2$ adrenoceptors (Van Meel et al, 1981) and also that Ca^{2+} fluxes are also mediators of the vasoconstrictor response to $alpha_1$ adrenoceptor stimulation (Vanhoutte, 1982a). The practical consequences of combined calcium channel blockade and $alpha_1$ adrenoceptor antagonism have not been adequately established. Furthermore, in addition to the potential pharmacodynamic interctions there may also be pharmacokinetic interactions. Both verapamil and prazosin undergo extensive first pass metabolism

following oral administration (Schomerus et al, 1976; Taylor et al, 1977) and there may be competition for hepatic uptake or pathways of metabolism, and there may also be modification of hepatic or splanchnic blood flow.

In this chapter the blood pressure and heart rate responses to oral administration of verapamil and prazosin, alone and in combination have been evaluated. The effect of combined therapy on the pharmacokinetics of the individual drugs has been investigated.

6.2. SUBJECTS AND METHODS

Eight healthy normotensive males, aged 20-40 years, gave written informed consent to take part in a double blind, randomised, crossover study. They reported to the Clinical Pharmacology Research Unit at 8.30 am on each study day, at weekly intervals, to receive the following oral treatments, in random order:

1) Verapamil 160 mg (+ placebo prazosin)
2) Prazosin 1 mg (+ placebo verapamil)
3) Prazosin 1 mg + verapamil 160 mg.
4) Placebo prazosin + placebo verapamil

Blood samples were withdrawn via an indwelling intravenous cannula in a forearm vein for subsequent drug and hormone assays.

6.2.1. Blood Pressure and Heart Rate.

Blood pressure was measured by semi-automated recorder

after a minimum of 10 minutes recumbency prior to each supine reading. On standing, readings were taken after 2 and 5 minutes. The standing period was curtailed if the subject complained of orthostatic symptoms or if the systolic blood pressure fell to under 80 mmHg. The corresponding heart rates were measured by one minute radial pulse count. Blood pressure and heart rate were determined at baseline and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4, 5, 6, 7, and 8 hours after dosing.

6.2.2. Plasma Noradrenaline, Renin and Aldosterone

Venous samples were collected for noradrenaline (supine and 5 minutes erect) and for renin activity and aldosterone (both supine) at 0, 2, 4 and 8 hours.

Hormones were assayed as described in Chapter 2.

6.2.3. Drug Assays.

Prazosin, verapamil and norverapamil were measured by high performance liquid chromatography with fluorescence detection as described in Chapter 2.

6.2.4. Pharmacokinetic analysis

The choice of pharmacokinetic model was determined according to the General Linear (F ratio) test (Boxenbaum et al, 1974) whereby the most appropriate fit is statistically confirmed by comparison of the weighted sum of squares. The pharmacokinetics of prazosin were most appropriately

fitted by a one compartment model described by the following equation:

$$C = A(e^{-\alpha(t-tlag)} - e^{-ka(t-tlag)})$$

The analysis was carried out using computer-assisted least squares fitting with an inverse weighting of drug concentrations.

The pharmacokinetic profiles of verapamil and norverapamil were most appropriately fitted to an integrated three compartment model. The disposition of drug and metabolite was described by the following equations:

$$C_{(d)} = Ae^{-\alpha(t-tlag)} + Be^{-\beta(t-tlag)} - (A+B)e^{-ka(t-tlag)}$$

and

$$C_m = \frac{A \cdot \frac{V_c}{V_m} \cdot k_{1m}}{k_{mo} - \alpha} (e^{-\alpha(t-t_{lag})} - e^{-k_{mo}(t-t_{lag})} +$$

$$\frac{B \cdot \frac{V_c}{V_m} \cdot k_{1m}}{k_{mo} - \beta} (e^{-\beta(t-t_{lag})} - e^{-k_{mo}(t-t_{lag})} -$$

$$\frac{(A + B)\frac{V_c}{V_m} \cdot k_{1m}}{k_a - k_{mo}} (e^{-k_a(t-t_{lag})} - e^{-k_{mo}(t-t_{lag})})$$

The data were fitted simultaneously to these equations using non linear least squares fitting regression analysis. Parameters derived from this approach are the coefficients (A+B); the hybrid first order rate constants for drug disposition ($\alpha + \beta$) and absorption (ka); the first order rate constant describing metabolite elimination (k_{mo}); and the constant, $\frac{V_c}{V_m}$ .k1 m where V_c and V_m are the volumes of the central and metabolite compartments respectively.

6.2.5. Statistical Analysis:

Student paired t test with Bonferoni correction was used in the comparisons of the kinetic parameters. Statistical evaluation of the pharmacodynamic measurements was by repeated measures analysis of variance.

Results are expressed throughout as mean ± S.D.

6.3. RESULTS

6.3.1. Blood Pressure:

Supine blood pressure (systolic and diastolic) following each treatment is shown in Figure 6.1. Compared to placebo, neither verapamil nor prazosin had a significant effect on supine blood pressure whereas the combination of verapamil and prazosin caused a significant reduction ($p<0.05$) which was maximal between 2 and 6 hours with a nadir of 100 ± 9 for systolic blood pressure and 60 ± 7 mmHg for diastolic.

On standing, blood pressure fell with prazosin but not

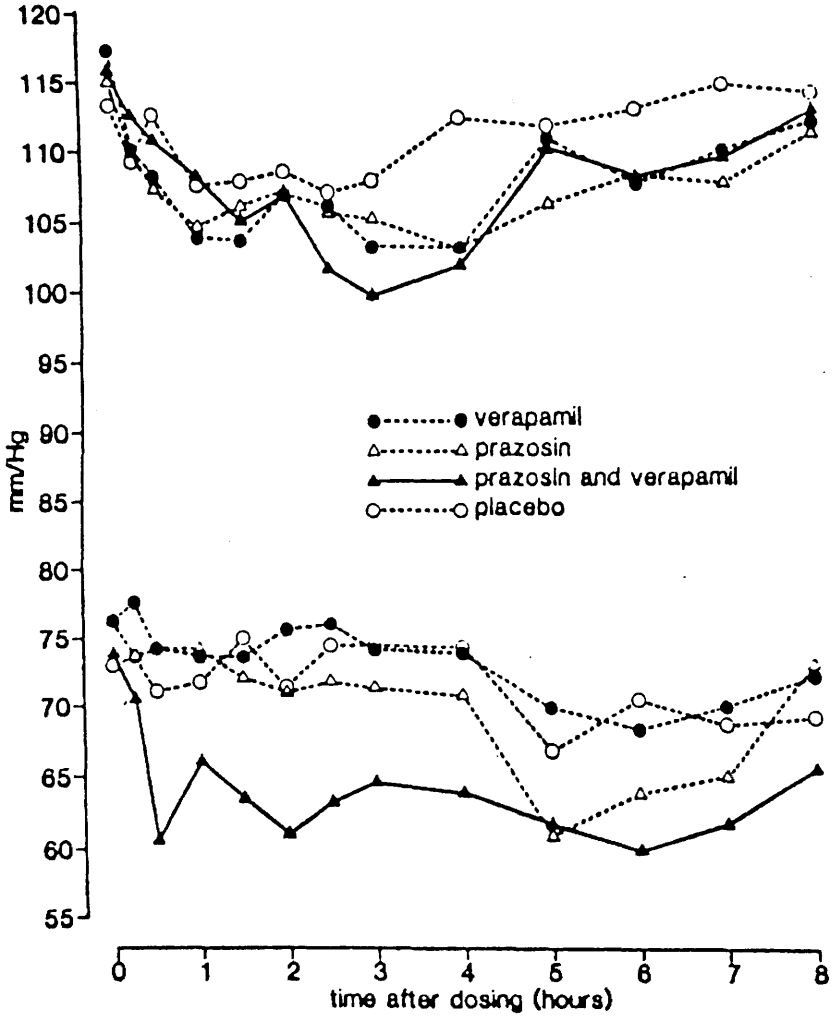


Fig.6.1. Mean supine systolic and diastolic blood pressures in 8 normotensive subjects.

with verapamil alone. The lowest systolic pressure with prazosin was 99 ± 17 at 4 hours compared to 114 ± 9 with placebo and 110 ± 8 mmHg with verapamil (Figure 6.2.). The corresponding pressure with the combination was 89 ± 13 mmHg. The overall hypotensive effect of the combination was greatest ($p < 0.05$) and in addition the reduction in blood pressure occurred earlier (within 0.5 hours) and persisted for longer (up to 7 hours).

The pattern of diastolic response was comparable and is shown in figure 6.2.

6.3.2. Heart Rate

There was a significant increase in supine heart rate (Figure 6.3.) following prazosin alone compared to placebo ($p < 0.05$). With verapamil alone there was no significant change. The greatest increases in supine heart rate occurred within 2 hours of dosing with the combination, maximal at 1 hour at 78 ± 9 bpm, compared to 72 ± 11 with prazosin. Over the 8 hours, however, the combination caused less increase than prazosin alone. On standing (Figure 6.4.), significant increases in heart rate occurred with prazosin alone and with the combination ($p < 0.05$). The tachycardia following prazosin alone reached a maximum of 112 ± 6 bpm at 5 hours after dosing The combination also caused a significant increase in heart rate to a maximum of 102 ± 9 beats/min but it was less than with prazosin alone.

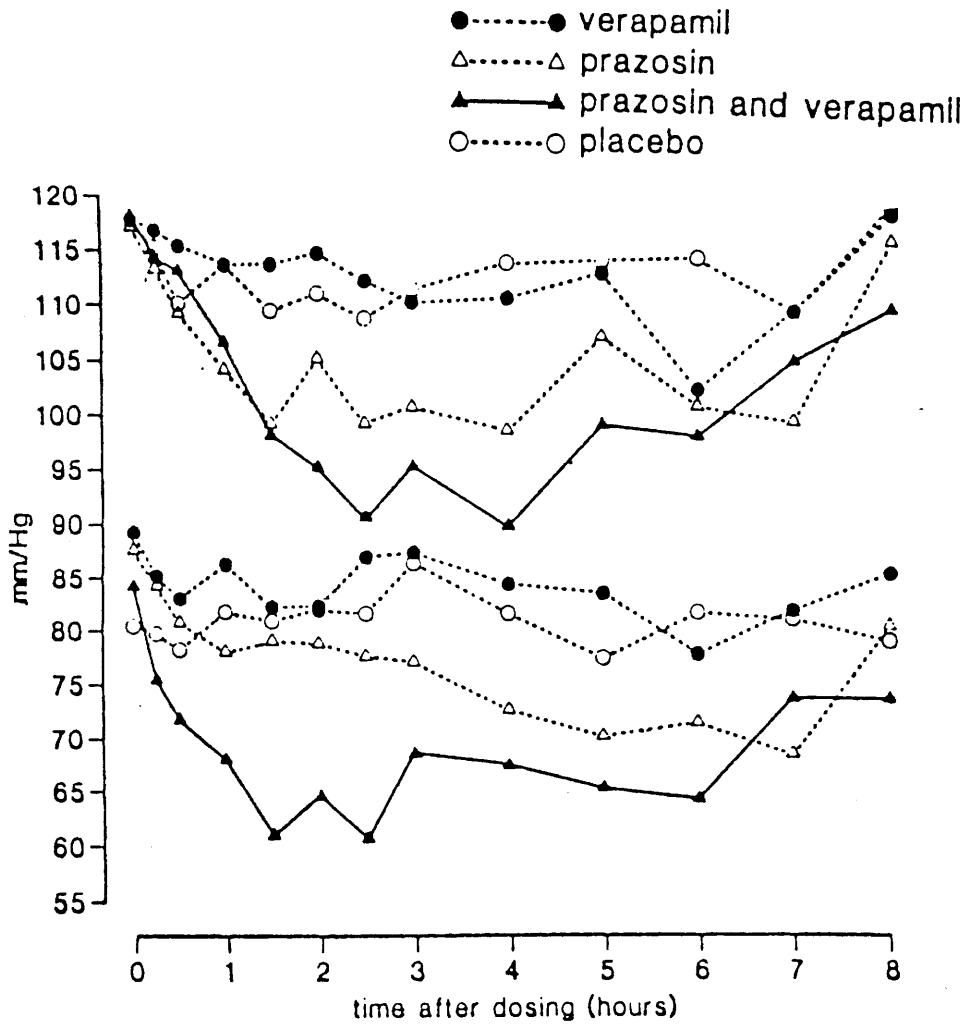


Fig.6.2. Mean standing (5 min) systolic and diastolic blood pressures in 8 normotensive subjects.

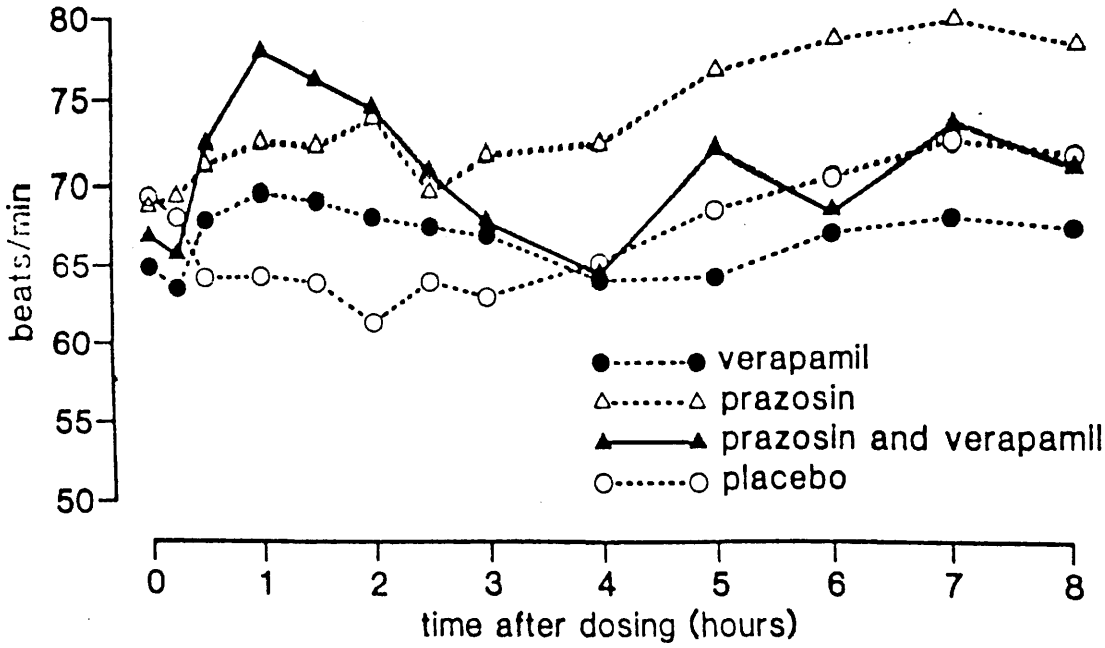


Fig. 6.3. Mean supine heart rate in 8 normotensive subjects.

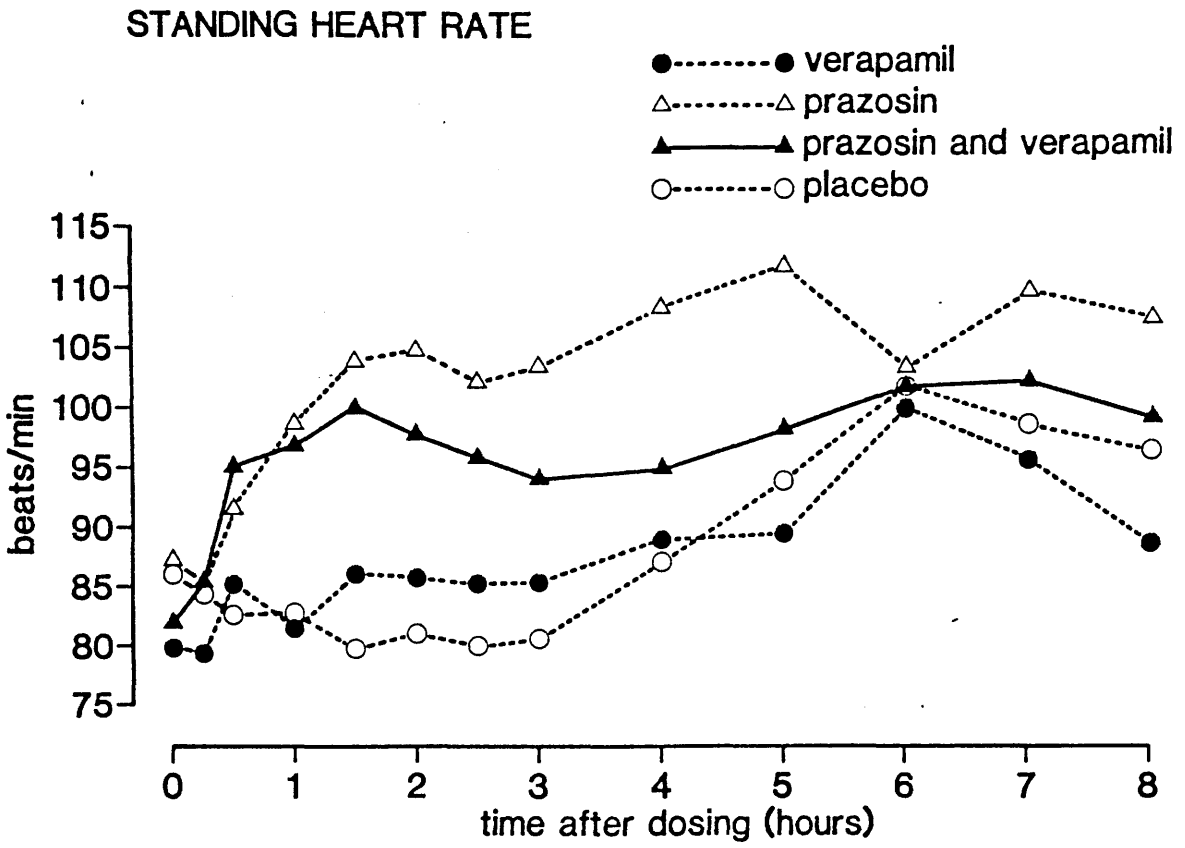


Fig. 6.4. Mean standing heart rate in 8 normotensive subjects.

6.3.3. Side Effects

The frequency of orthostatic symptoms was increased when prazosin and verapamil were given together. Six subjects complained of postural symptoms or had a systolic pressure less than 80 mmHg at 18 times between 1 and 6 hours after the combination. After prazosin alone postural hypotensive episodes occurred on 11 occasions. With verapamil only a single subject had orthostatic symptoms and with placebo there were no hypotensive problems.

6.3.4. Noradrenaline, Renin, Aldosterone

Supine and erect plasma noradrenaline levels were significantly ($p<0.05$) increased after prazosin and the prazosin plus verapamil combination, particularly at 2 hours after dosing (table 6.1.). The greatest increase was observed with the combination. Similarly plasma renin activity was increased by the combined treatment more than by prazosin alone with values still elevated 8 hours after dosing ($p<0.05$)(table 6.2.). Plasma aldosterone showed a slight increase 4 hours after combined treatment only.

6.3.5. Pharmacokinetics

The derived pharmacokinetic parameters obtained by fitting the verapamil and prazosin concentrations to the appropriate models are summarised in tables 6.3. and 6.4. There were no significant differences in the disposition of verapamil or norverapamil when the drug was given alone or

TABLE 6.1

NORADRENALINE PLASMA CONCENTRATIONS AFTER ADMINISTRATION OF VERAPAMIL AND PRAZOSIN IN 8 NORMOTENSIVES

SUPINE PLASMA NORADRENALINE (MEAN ± SD) nmol/l

Treatment	HOURS AFTER DOSING 0	2	4	8
Verapamil	2.05 ± 1.2	2.11 ± 0.8	2.03 ± 0.4	2.08 ± 0.8
Prazosin	2.64 ± 1.2	3.45 ± 1.1	3.4 ± 1.3	3.98 ± 2.0
Verapamil + prazosin	2.01 ± 1.5	5.01 ± 2.8	3.53 ± 2.1	2.46 ± 1.9
Placebo	2.31 ± 0.6	2.66 ± 1.2	2.48 ± 0.7	2.4 ± 0.9

ERECT PLASMA NORADRENALINE (MEAN ± SD) nmol/l

Treatment	HOURS AFTER DOSING 0	2	4	8
Verapamil	3.67 ± 1.6	5.5 ± 2.4	5.3 ± 1.5	5.68 ± 2.7
Prazosin	4.67 ± 3.0	8.04 ± 5.0	7.17 ± 3.5	7.08 ± 3.4
Verapamil + prazosin	4.77 ± 3.2	9.08 ± 4.2	6.6 ± 3.6	7.56 ± 4.6
Placebo	4.85 ± 1.8	5.65 ± 2.1	5.53 ± 2.0	5.18 ± 2.1

TABLE 6.2.

PRA FOLLOWING ADMINISTRATION OF VERAPAMIL AND PRAZOSIN IN 8 NORMOTENSIVES

SUPINE PLASMA RENIN ACTIVITY (MEAN ± SD) ng ANGI/ml/hr

	HOURS AFTER DOSING			
Treatment	0	2	4	8
Verapamil	4.5 ± 3.7	6.8 ± 5.3	4.1 ± 2.6	5.5 ± 5.3
Prazosin	3.7 ± 1.4	5.5 ± 2.3	5.6 ± 2.9	7.4 ± 4.9
Verapamil + prazosin	4.0 ± 1.8	12.3 ± 7.4	10.6 ± 6.5	11.0 ± 6.7
Placebo	5.1 ± 2.5	4.6 ± 3.2	4.83 ± 3.4	6.1 ± 4.2

TABLE 6.3.

PHARMACOKINETIC PARAMETERS OF VERAPAMIL ALONE AND COMBINED WITH PRAZOSIN IN 8 NORMOTENSIVES (MEAN ± SD)

	Verapamil $\beta t_{\frac{1}{2}}$ min	Norverapamil $kmot_{\frac{1}{2}}$ min	Verapamil AUC ng/ml/min	Norverapamil AUC ng/ml/min	Verapamil Peak Concentration ng/ml
ALONE	186 ± 56	228 ± 88	45590 ± 16440	59620 ± 10530	247 ± 147
COMBINED	183 ± 33	199 ± 43	43450 ± 21530	51960 ± 11500	222 ± 144
p <	N.S.	N.S.	N.S.	N.S.	N.S.

$\beta t_{\frac{1}{2}}$ = terminal elimination half-life for verapamil
$kmot_{\frac{1}{2}}$ = terminal elimination half-life for norverapamil
AUC = area under the curve

TABLE 6.4.

PHARMACOKINETIC PARAMETERS OF PRAZOSIN ALONE AND COMBINED WITH VERAPAMIL IN 8 NORMOTENSIVES (MEAN + SD)

	$\beta t_{\frac{1}{2}}$ min	AUC ng/ml/min	Peak Concentration ng/ml
	162	1601	5.17
ALONE	±	±	±
	38	189	0.71
	144	2592	9.64
COMBINED	±	±	±
	19	919	3.58
P <	N.S.	0.02	0.02

when co-administered with prazosin. In contrast the pharmacokinetics of prazosin were significantly altered by the co-administration of verapamil, illustrated by the concentration-time profiles in Figure 6.5. The area under the prazosin concentration time curve was significantly increased ($p<0.02$) by 62%, from 1601 to 2592 ng/ml/min when verapamil was administered concurrently. The peak concentrations of prazosin were also significantly higher with the combination (5.2 ng/ml for prazosin alone compared to 9.6 ng/ml for the combination $p< 0.02$). The absorption rate constant Ka was also larger in seven of the eight subjects when prazosin was given in combination. The elimination half life of prazosin was not changed by co-administration of verapamil.

6.4. DISCUSSION

Only slight reductions in blood pressure were observed in these young normotensive subjects when verapamil was administered alone. This has been noted previously with other calcium antagonists (McAllister and Kirsten, 1982). Prazosin also had little effect on supine blood pressure but a significant orthostatic response was observed, confirming previous reports (Graham et al, 1977). The two drugs in combination produced a significantly greater hypotensive effect than either drug alone. There were greater falls in systolic and diastolic blood pressures, both supine and standing, and an earlier onset and longer duration of

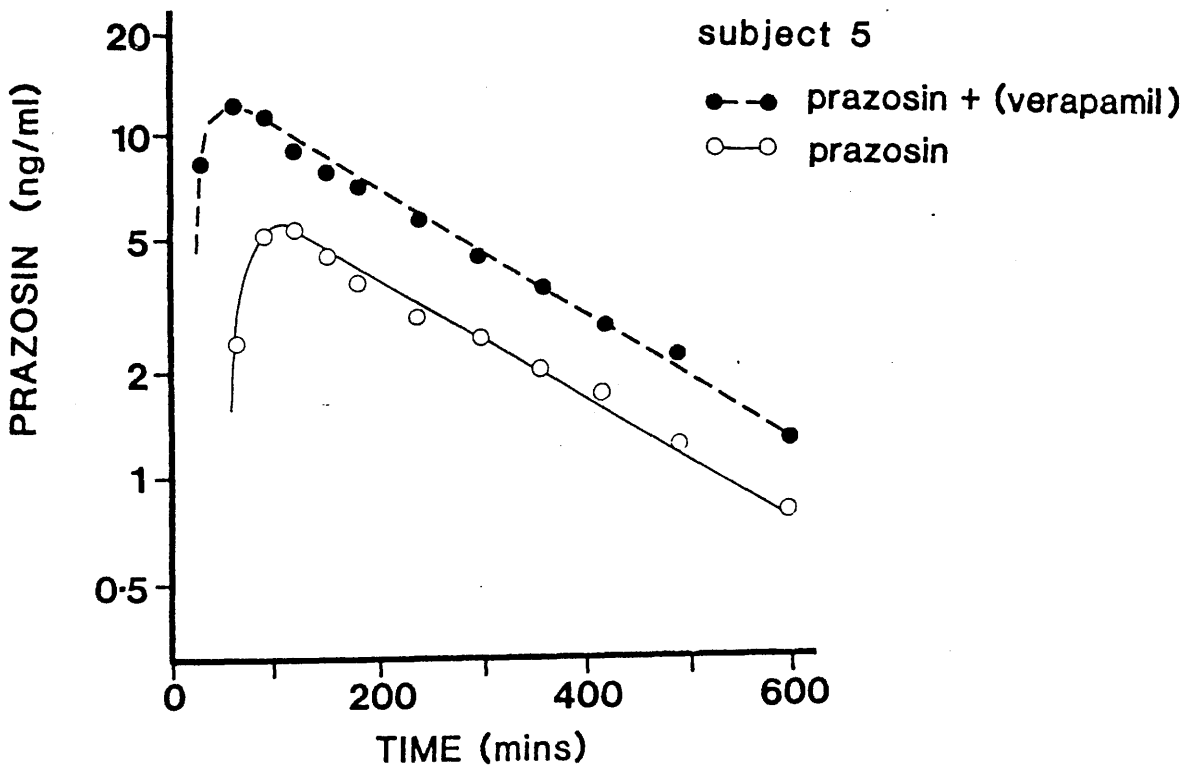


Fig.6.5. Representative blood concentration-time profile for prazosin.

action. This augmented effect of the combination appears to have been more than additive. In particular the reduction in supine diastolic pressure was larger than predictable from the summation of the individual drug effects.

There are a number of mechanisms, pharmacokinetic and pharmacodynamic, by which these drugs might interact to produce an augmented fall in blood pressure. In this study there was evidence of a pharmacokinetic interaction resulting in increased plasma concentrations of prazosin. Both prazosin and verapamil undergo extensive first pass metabolism in the liver and both are highly protein-bound (Hamann et al, 1984; Keefe et al, 1981; Rubin and Blaschke, 1980). If there had been simple competition by each drug for common metabolic pathways or for protein binding sites then changes in the kinetics of both drugs might have been anticipated. Differing affinities and specificities for these processes might explain why only prazosin's pharmacokinetics were affected but there is no evidence to support this proposal.

Plasma concentrations of prazosin have previously been correlated with acute reductions in erect blood pressure (Bateman et al, 1979; Elliott et al, 1981) and it is likely that the increased plasma levels and bioavailability of prazosin contributed to the additional hypotensive effect of the combination. The explanation for the change in prazosin's kinetics, but not those of verapamil, remains unclear but it may be related to acute

changes in liver blood flow.

In Chapter 4 of this thesis, according to other authors (Shand et al, 1981), the elimination of verapamil has been shown to occur more rapidly after acute dosing than after chronic administration. In addition, in this study verapamil, when administered alone, achieved peak plasma levels earlier than prazosin given alone. Verapamil may, therefore, have exerted its vasodilator effect also on the portal and splanchnic vascular bed at an earlier time. If it is hypothesised that liver blood flow is increased by verapamil the pharmacokinetics of verapamil itself might not be changed by the concomitant administration of prazosin, in view of its slightly delayed absorption, whereas the vasodilator effect of verapamil might influence prazosin's kinetics causing a reduction in the extent of prazosin's first pass metabolism as a result of a change in splanchnic blood flow, resulting in higher peak plasma concentrations and increased systemic bioavailability.

If the blood pressure reductions are analysed in terms of the differences in the areas of the blood pressure time curves then increases of 35 and 115% in the hypotensive effect are observed for standing systolic and diastolic pressures respectively, comparing prazosin alone and prazosin with verapamil. These percent changes in the areas of the blood pressure time curves seem to be consistent with the observed mean increase of 60% in AUC for prazosin when verapamil is concurrently administered.

However, it seems disproportionate that this pharmacokinetic effect can entirely account for the 220% increase in hypotensive effect (as determined by the areas of the blood pressure-time curves) obtained for supine diastolic pressure. This suggests that there is an additional pharmacodynamic interaction.

It is possible that verapamil's action to depress cardiac conduction produces an effect analogous to beta-adrenoceptor antagonism in attenuating the reflex increases in heart rate and cardiac output associated with prazosin. Thus, the hypotensive effect of prazosin is enhanced because the reflex cardiac responses are interrupted. In this study the greatest reductions in both supine and erect blood pressures caused by the verapamil-prazosin combination were associated with heart rates which were less than those obtained for prazosin alone. Only within the first 2 hours was there a transiently greater supine heart rate with the combination. This may simply have been due to the higher and more rapidly occurring peak plasma prazosin levels. Alternatively the cardiodepressant effects of verapamil may lag behind the vasodilator effects or may have a particular influence only when the heart rates show a more marked increase.

In conclusion, the combination of these two drugs may have acted at different sites enhancing the vasodilator effect by blocking alpha$_1$ vasoconstrictor and impairing peripheral alpha$_2$ mechanisms exciting a significantly

greater acute hypotensive effect than either drug alone.

CHAPTER 7

STUDIES ON THE EFFECTS OF CALCIUM ANTAGONIST DRUGS ON HEPATIC AND RENAL BLOOD FLOW

7.1. INTRODUCTION

The vasodilator properties of calcium antagonist drugs have been extensively investigated in several animal and in vitro studies particularly for the coronary arteries and the peripheral vasculature (Bou et al, 1983; Kazda et al, 1983). Although it is well established that the selectivity for different tissues and vascular beds varies with individual calcium antagonists, little information is available in man on the effects of this class of drugs on regional blood flow. It is only recently that increases in renal blood flow have been shown to occur after the acute intravenous administration the dihydropyridine calcium antagonists, nicardipine and nifedipine (Yokoyama & Kaburagi, 1981 and 1983). Similarly acute administration of nifedipine has been shown to increase apparent liver blood flow (Feely, 1984).

In the study on the interaction between verapamil and prazosin (Chapter 6), it was suggested that the augmented prazosin bioavailability might result from an acute increase in liver blood flow attributable to verapamil. However, there is evidence that verapamil not only modifies the pharmacokinetics of concurrently administered prazosin but that it also modifies its own pharmacokinetics. During chronic administration there are significant changes in the pharmacokinetics of verapamil, in comparison with the values observed after acute dosing, with prolongation of the elimination half-life and reduction of the clearance of the

drug as observed in chapter 4 of this thesis and by other authors (Freedman et al, 1981; Kates et al, 1981; Shand et al, 1981). These changes have been generally ascribed to a reduction in hepatic clearance as a result of saturation of metabolic processes, with the resultant increase in systemic bioavailability and prolongation of elimination half-life (Kates et al, 1981; Shand et al, 1981) but there are no convincing data to support this hypothesis. While there is no good evidence that verapamil's pharmacokinetics deviate from first order metabolic processes there is evidence in another study (Woodcock et al, 1981) that the systemic clearance of verapamil is well correlated with apparent liver blood flow. Approximately 65% of the blood supply to the liver comes through the portal vein from the splanchnic bed and the gastro-intestinal tract, and since the liver is a crucial site for metabolic transformation of drugs its functional capacity might well be influenced by alterations to its blood supply (Dawson, 1979). Similarly, the kidney has a rich blood supply receiving 25% of total cardiac output and having a blood flow which is higher than any other parenchymatous organs and kept constant by the autoregulation (Shipley and Study, 1951; Bomzon, 1983). Recent studies have demonstrated that blood flow to the kidney may be altered by the acute administration of calcium antagonists (Yokoyama and Kaburagi, 1981 and 1983; Diamond et al, 1984; Ene et al, 1985).

This study was designed to investigate the effects on

apparent liver and kidney blood flow and their possible relationships to pharmacokinetic changes following the acute and continued administration of two different calcium antagonists, verapamil and nisoldipine.

7.2. METHODS

Nine healthy normotensive male volunteers (aged 25 to 40 years; weight 52 to 76 kg) participated in the study. The subjects were studied on five separate days after administration of

(1) placebo
(2) acute verapamil 160 mg and a further dose of
(3) verapamil 160 mg following three days of verapamil treatment (80 mg b.d.)
(4) acute nisoldipine 20 mg and a further dose of
(5) nisoldipine 20 mg following 3 days of treatment (10 mg b.d.).

Active drugs and placebo were taken orally following an overnight fast. Subjects were not fed until 4 hours after dosing and rested supine throughout the study. Placebo and active treatments were randomised and the treatment periods were at least one week apart. From an intravenous cannula inserted in a forearm vein blood samples were withdrawn at the following times: 0 and 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360 and 480 minutes after dosing. Blood pressure and heart rate were measured by an automatic recorder (Sentron) at various times during

the study. Whole blood concentrations of verapamil and its metabolite, norverapamil, were determined by HPLC with fluorescence detection (Cole et al, 1981). The pharmacokinetic profiles of verapamil and norverapamil were most appropriately described by a model incorporating two compartments for drug disposition and a single compartment for the metabolite (see Figure 4.1.).

Parameter estimates were obtained by non linear least squares fitting using an "in house" program based on the Marquhardt algorithm (Bevington, 1969). Verapamil and norverapamil whole blood concentrations were simultaneously fitted to the following equations:-

$$C_{(d)} = Ae^{-\alpha(t_1)} + Be^{-\beta(t_1)} - (A + B)e^{-ka(t_1)}$$

$$C_{(m)} = \frac{Vc.klm}{Vm} \left[\frac{A}{kmo - \alpha} (e^{-\alpha(t_1)} - e^{-kmo(t_1)} + \frac{B}{kmo - \beta} (e^{-\beta(t_1)} - e^{-kmo(t_1)}) - \frac{(A + B)}{kmo - ka} (e^{-ka(t_1)} - e^{-kmo(t_1)} \right]$$

$$(t_1 = t - t_{lag})$$

where A and B are coefficients, alpha and beta are the

hybrid first order rate constants describing drug disposition, ka is the first order rate constant describing drug absorption, Vc.kim/Vm is a constant with Vc and Vm being the volume of central and metabolite compartments respectively, kmo is the first order rate constant describing metabolite elimination, and tlag is the time at which drug is first detected in the circulation.

Hepatic blood flow was assessed one hour after drug administration by the clearance of indocyanine green (ICG) as described in Chapter 2. ICG clearance in a representative subject is shown in Figure 7.1.

Effective renal plasma flow and glomerular filtration rate were estimated by determining the clearance of ^{125}I hippuran and ^{51}Cr EDTA respectively as described in Chapter 2. Effective renal plasma flow and glomerular filtration rate were calculated from the ratio of the amount of urinary excretion over 3 h and the area under the plasma concentration time curve, obtained using the trapezoidal rule, for ^{125}I hippuran and ^{51}Cr EDTA respectively (Harries et al., 1972). Subjects were also asked to collect urine until 24 hours after drug administration on each study day. Three separate collections were therefore taken, one between 0 and 4 hours, another between 4 and 8 hours and from 8 to 24 hours after either drug or placebo administration.

Student's paired 't' test with Bonferroni correction where appropriate, was used for all comparisons. Results are expressed throughout as mean $\pm$ S.D.

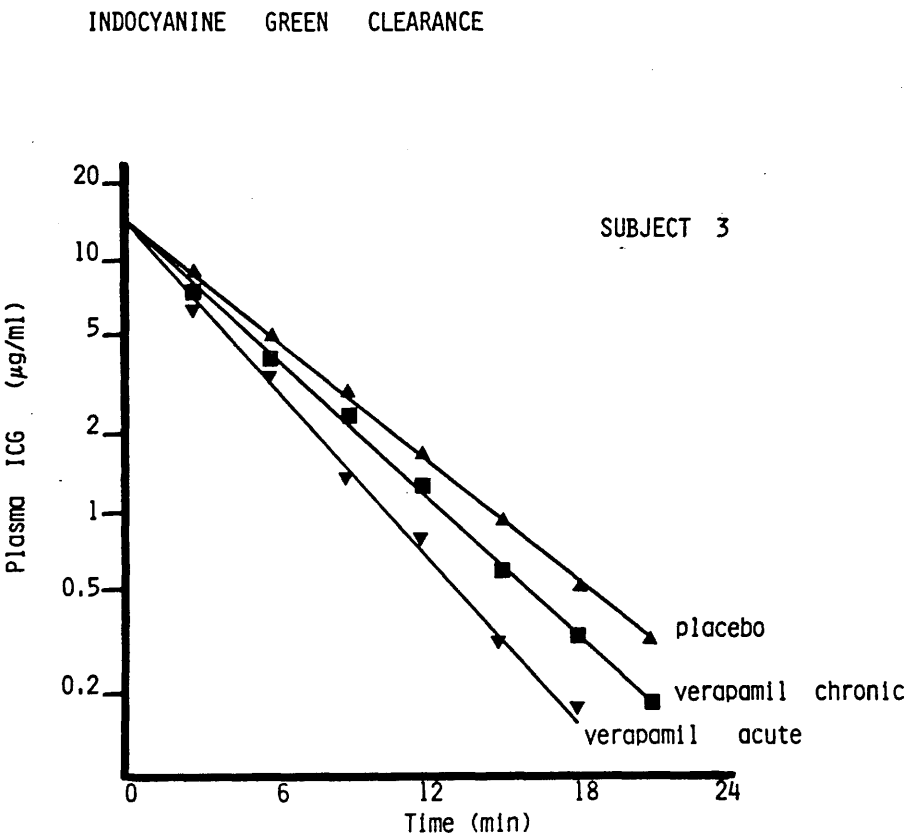


Fig. 7.1. ICG clearance changes in a representative subject.

7.3. RESULTS.

One subject experienced a minor allergic reaction after verapamil administration and therefore data are presented on eight subjects only.

Blood pressure and heart rate did not change significantly after either acute or chronic treatments as compared with placebo. The derived pharmacokinetic parameters following acute and chronic administration of verapamil are summarised in tables 7.1. and 7.2. and a representative pharmacokinetic profile (subject 3) is shown in Figure 7.2. Chronic administration was associated with a significant ($p< 0.001$) increase in area under the concentration time profiles (AUC) for both parent drug and metabolite. The mean drug AUC with acute administration was 800 $\pm$ 353 ng.h/ml compared to 1455 $\pm$ 244 ng.h/ml for single dose administered following chronic treatment. The comparable figures for the norverapamil AUC were 731 $\pm$ 143 ng.h/ml for acute administration and 1374 $\pm$ 365 ng.h/ml for chronic. The ratio of the verapamil and norverapamil AUC, a measure of the relative clearance of drug and metabolite, were not significantly different for acute and chronic administration. Verapamil terminal elimination half life (t1/2), and peak drug concentration (Cp) were both significantly ($p<0.01$) increased when chronic administration was compared with acute. However, absorption rate constant (ka) and time to attain the peak drug concentration (tmax) were not significantly changed by continued drug

TABLE 7.1.

THE EFFECT OF ACUTE AND CHRONIC ADMINISTRATION ON AREA UNDER THE CONCENTRATION TIME CURVES OF VERAPAMIL AND NORVERAPAMIL IN 8 SUBJECTS

Subject	AUC Verapamil (ng.h/ml)		AUC norverapamil (ng.h/ml)		AUC verapamil / AUC norverapamil	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
1	1593	2000	934	1484	0.59	0.74
2	570	1201	664	965	1.16	1.80
3	559	1432	790	1576	1.41	1.10
4	914	1538	487	662	0.53	0.43
5	587	1287	766	1482	1.30	1.15
6	686	1461	706	1482	1.03	1.01
7	907	1387	877	1718	0.97	1.24
8	580	1332	621	1624	1.07	1.22
Mean ± S.D.	800 ± 353	1455 ± 244	731 ± 143	1374 ± 365	1.08 ± 0.31	0.96 ± 0.28
	p<0.001		p<0.005		n.s.	

TABLE 7.2.

THE DERIVED PHARMACOKINETIC PARAMETERS FOR ACUTE AND CHRONIC VERAPAMIL ADMINISTRATION IN 8 SUBJECTS

Subject	Bt.1/2 (h)		ka (1/h)		Cp (ng/ml)		tmax (h)	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
1	3.0	6.8	2.9	5.6	323	360	1.1	1.4
2	5.5	6.6	2.6	4.4	138	240	0.9	0.9
3	6.0	7.0	1.9	3.3	92	268	1.2	0.8
4	2.0	3.1	1.7	2.7	195	335	1.6	1.0
5	8.2	9.7	1.3	1.7	98	248	1.4	1.3
6	3.1	5.9	8.7	3.0	225	329	0.8	0.9
7	8.0	8.6	2.3	2.0	138	196	1.1	1.2
8	5.6	5.9	1.5	3.7	104	262	1.3	0.9
Mean ± S.D.	5.2 ± 2.3	6.7 ± 2.0	2.9 ± 2.4	3.3 ± 1.3	164 ± 80	280 ± 56	1.2 ± 0.3	1.1 ± 0.2
	$p<0.01$		n.s.		$p<0.001$		n.s.	

(Bt.1/2 = terminal elimination half life; ka = absorption first order rate constant; Cp = peak concentration; tmax = time to peak concentration)

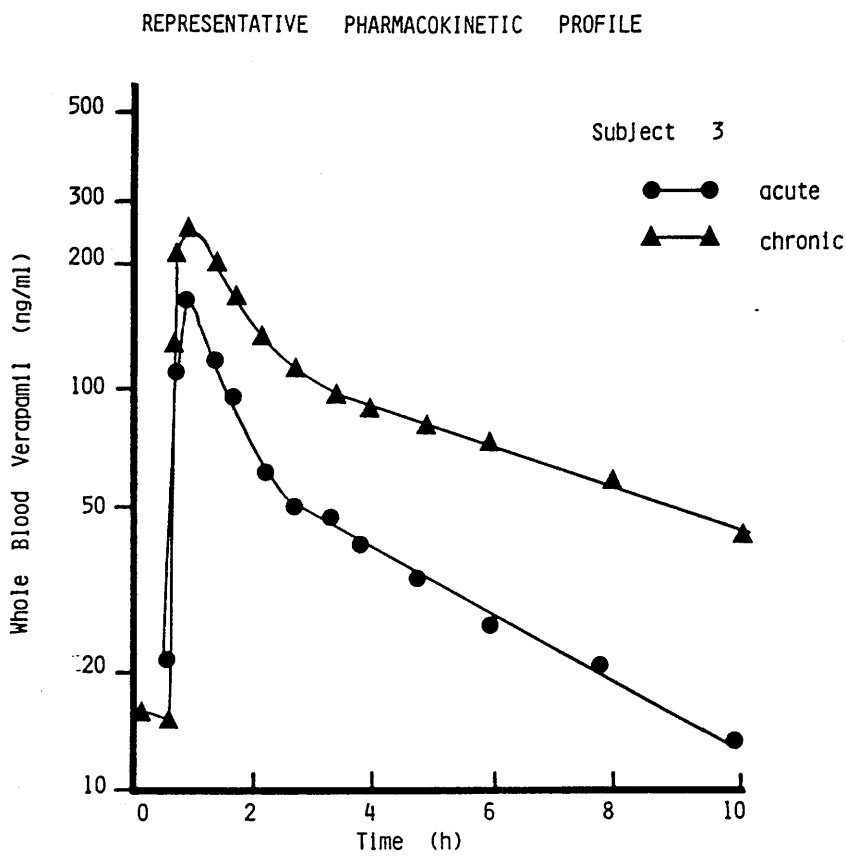


Fig. 7.2. Representative pharmacokinetic profile of verapamil following acute and chronic administration in a normotensive subject.

administration.

7.3.1. Apparent Liver Blood Flow

Acute administration of verapamil was associated with significant ($p < 0.005$) increase to 1501 ± 363 ml/min compared to 945 ± 219 ml/min for placebo. With continued administration apparent liver blood flow was reduced to 1167 ± 375 ml/min (Figure 7.3.). Acute administration of nisoldipine was associated with a significant ($p < 0.01$) increase in apparent liver blood flow to 1310 ± 244 ml/min compared to placebo. Again, with continued administration apparent liver blood flow returned towards placebo values at 1154 ± 216 ml/min (Figure 7.4.). There were no significant changes in the volume of distribution of ICG with acute and continued administration of both drugs.

7.3.2. Renal blood flow

Effective renal plasma flow showed a similar pattern to apparent liver blood flow with a significant ($p < 0.001$) increase following acute administration of the two drugs (to 628 ± 140 ml/min for verapamil and to 624 ± 138 for nisoldipine compared to 507 ± 116 ml/min with placebo). Continued administration of the two drugs was associated with a reduction in renal plasma flow towards placebo values (Figures 7.5. and 7.6.). There were no significant differences in GFR after verapamil compared with placebo. Nisoldipine, however, increased GFR to 123 ± 14 ml/min after

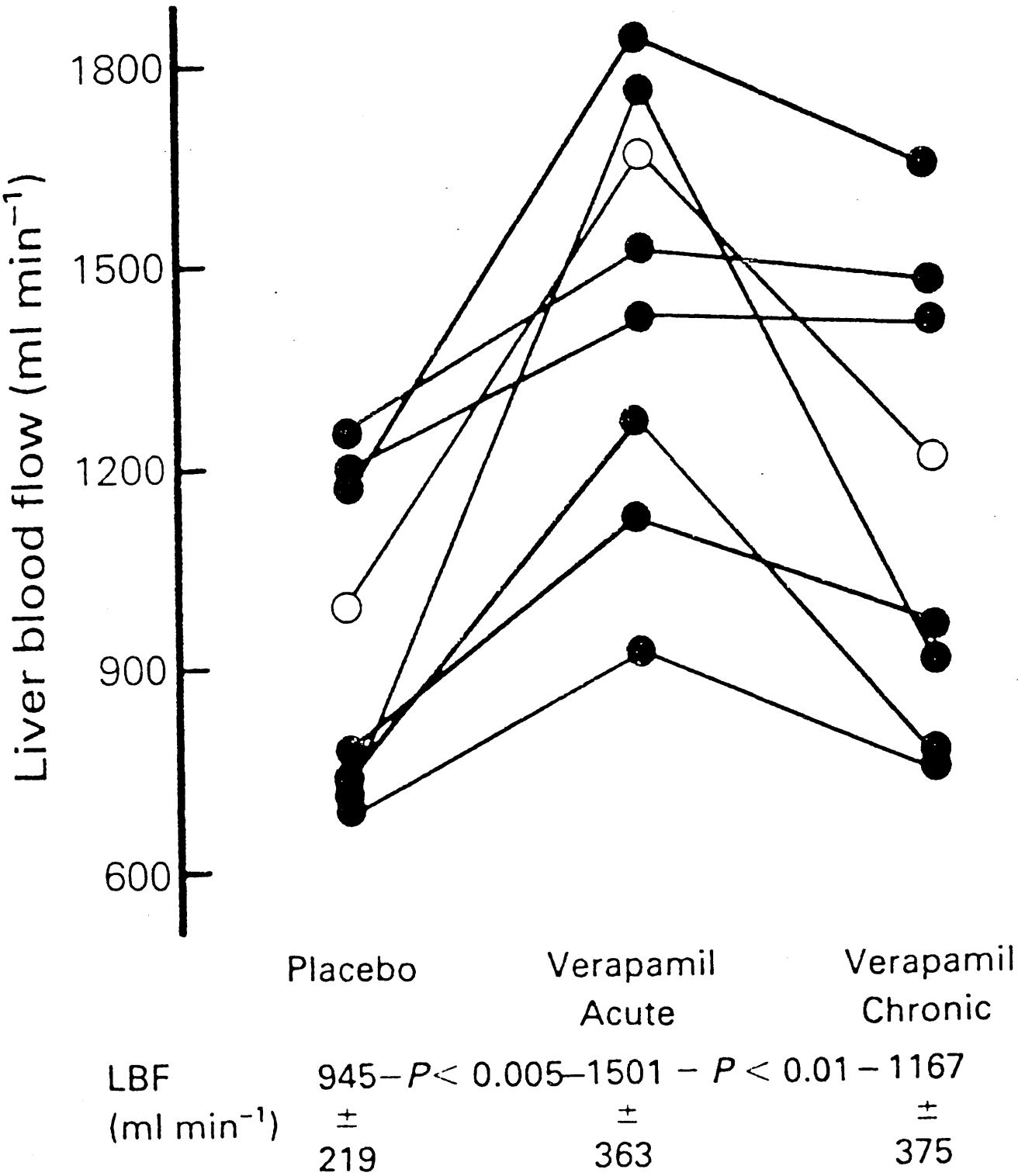


Fig.7.3. Apparent liver blood flow following verapamil administration in 8 subjects. Subject 3 is shown as O .

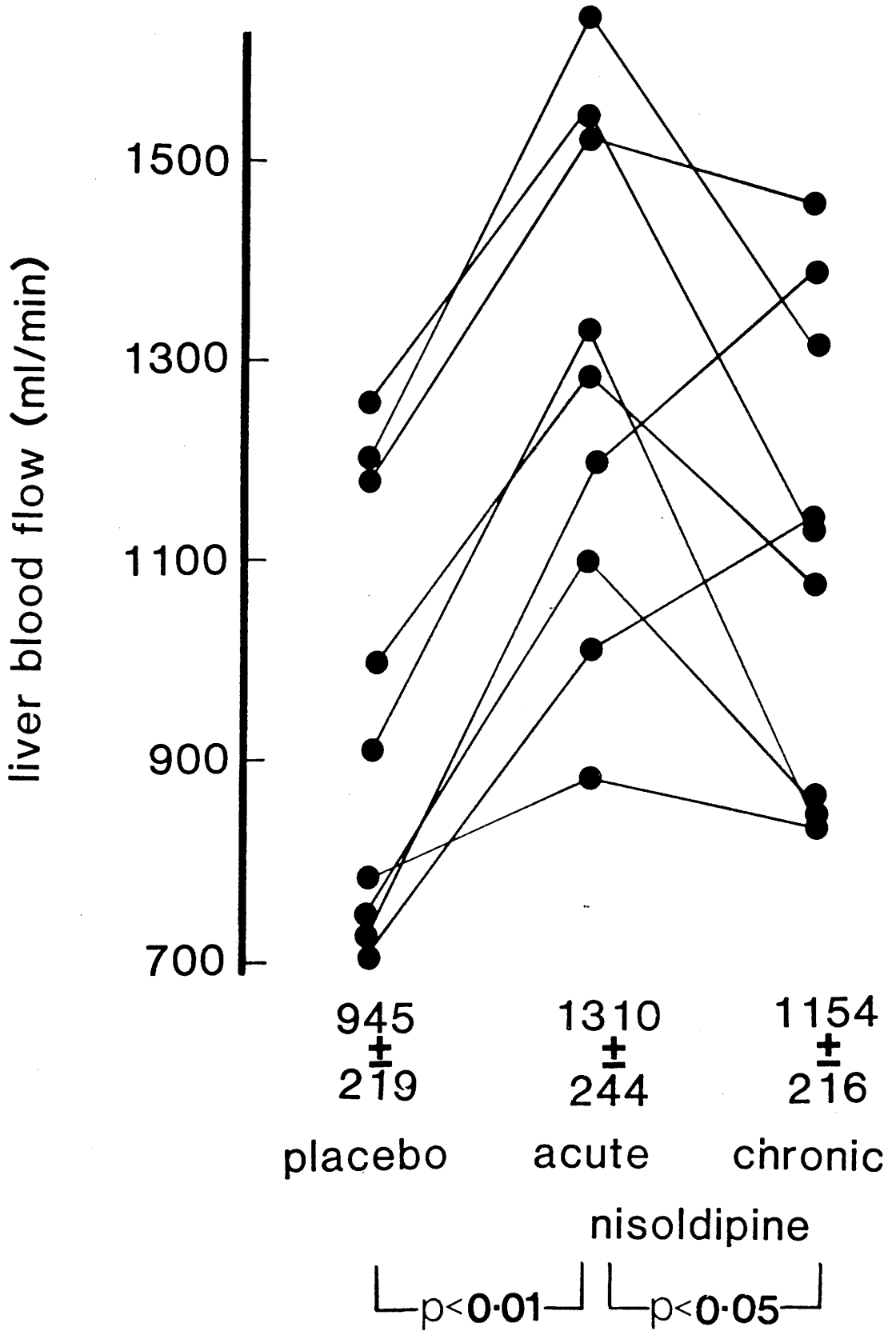


Fig. 7.4. Apparent liver blood flow after acute nisoldipine (20 mg) and after 4 days of administration. (9 subjects).

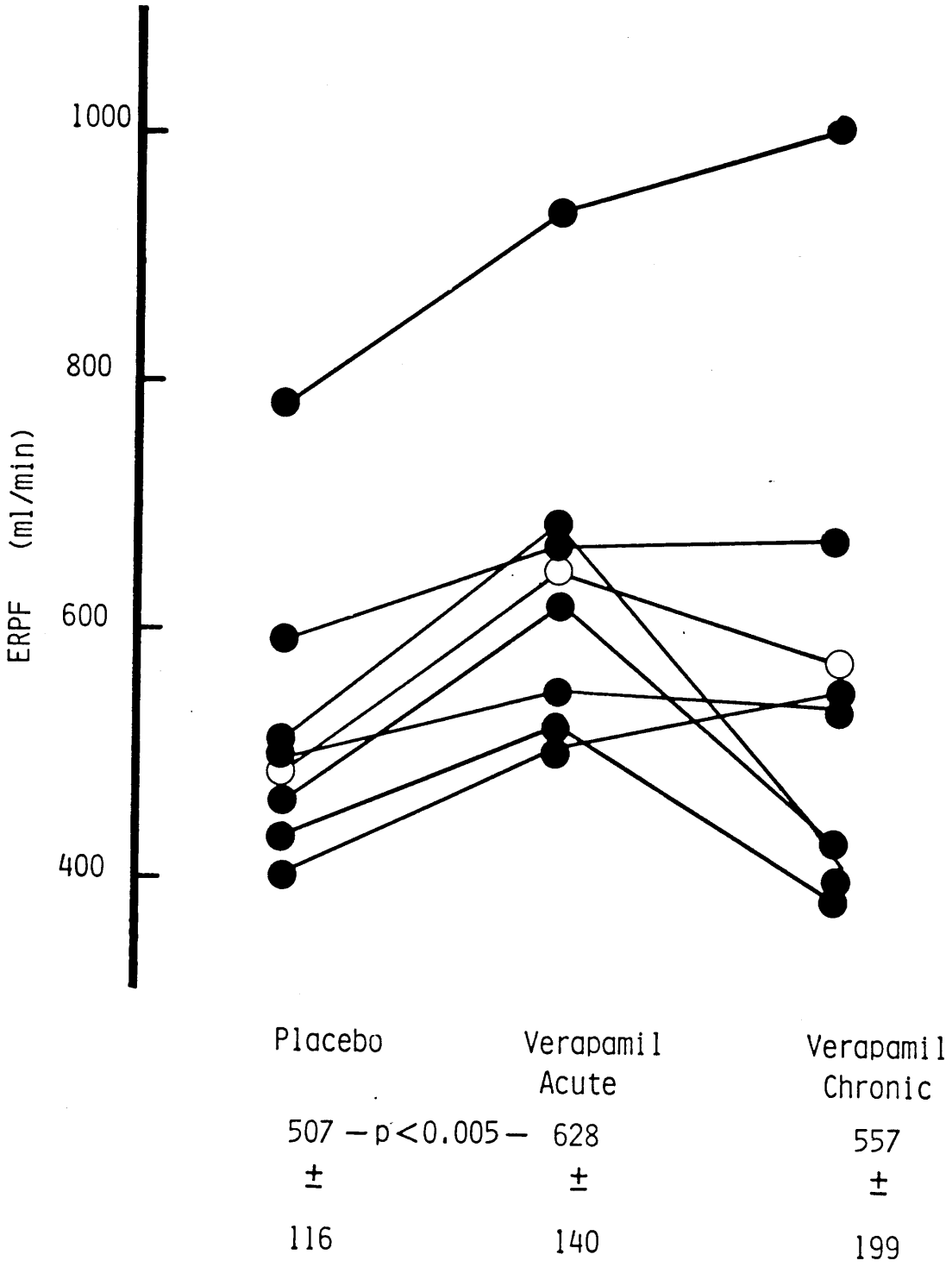


Fig.7.5. Effective renal plasma flow following verapamil administration in 8 subjects. Subject 3 is shown as O.

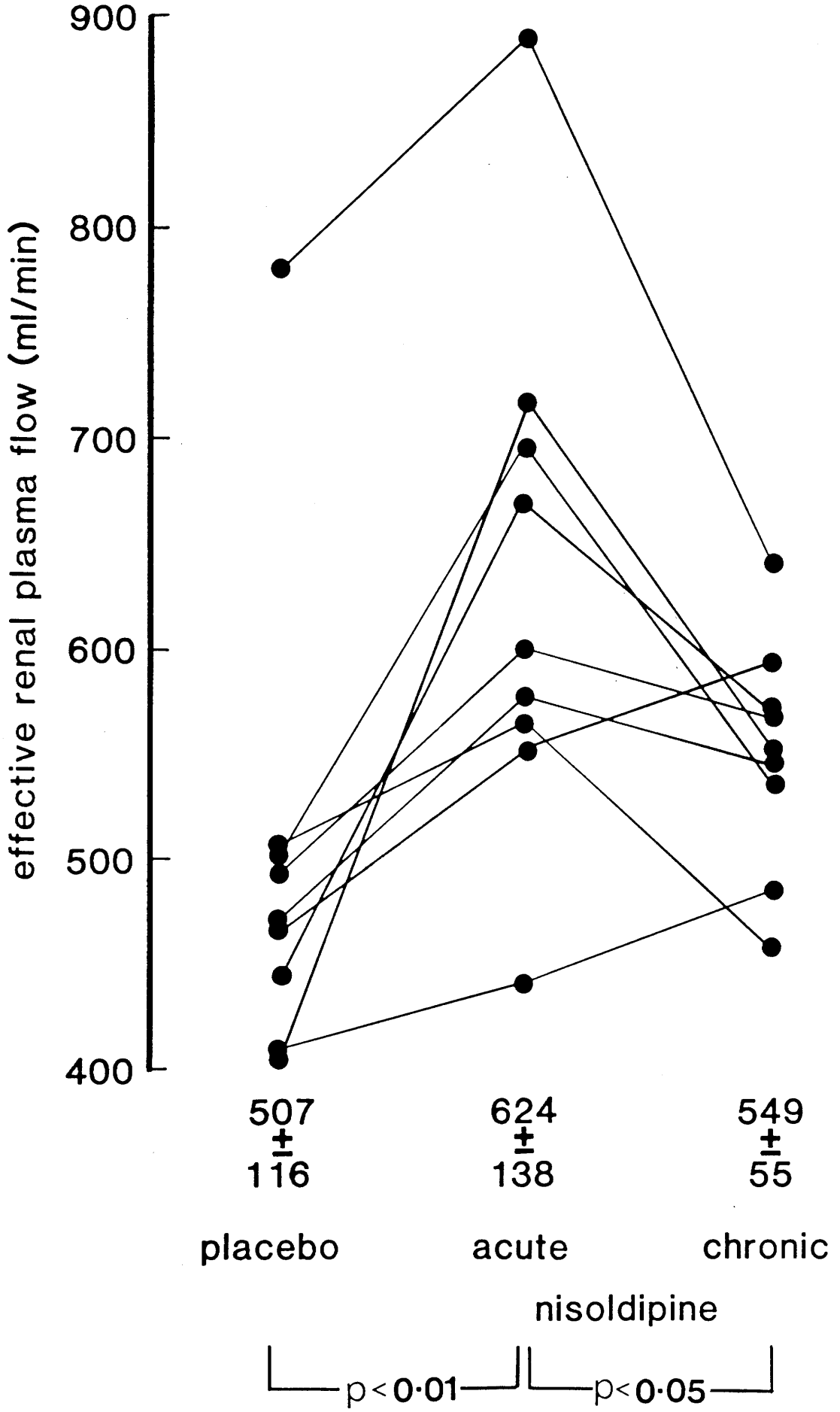


Fig. 7.6. Effective renal plasma flow after acute nisoldipine (20 mg) and after 4 days administration. (9 subjects).

acute administration ($p < 0.01$ compared to placebo); with continued administration the GFR was back to placebo values.

The increase in GFR with acute administration of nisoldipine was paralleled by a modest increase in 24 hour urinary sodium excretion (218 ± 39 mmol compared to 201 ± 54 mmol for placebo). This difference was significant when the Na^+ excretion within 4 hours of drug administration was considered (89 ± 41 mmol compared to 44 ± 27 mmol on placebo ($p < 0.05$) (Figure 7.7.). Total 24 hour urine volume was 1700 ± 500 ml after nisoldipine acute compared to 1380 ± 570 ml on placebo, the difference due to the significant increase in urine output obtaianed during the first 4 hours (653 ± 361 ml after nisoldipine compared to 337 ± 204 ml after placebo).

7.4. DISCUSSION

It has previously been reported (Kates et al, 1981; Shand et al, 1981; Schwartz et al, 1982) that continued oral administration of verapamil is associated with a significant increase in area under the drug concentration time curve (AUC) and thus a decrease in clearance/F. These authors also reported increases in verapamil half-life following multiple dosing. Similar findings were observed in Chapter 4 but other studies, using deuterated verapamil, have failed to demonstrate this (Eichelbaum & Somogyi, 1984). The findings of the present study in normotensive males supports the observations that the decrease in drug

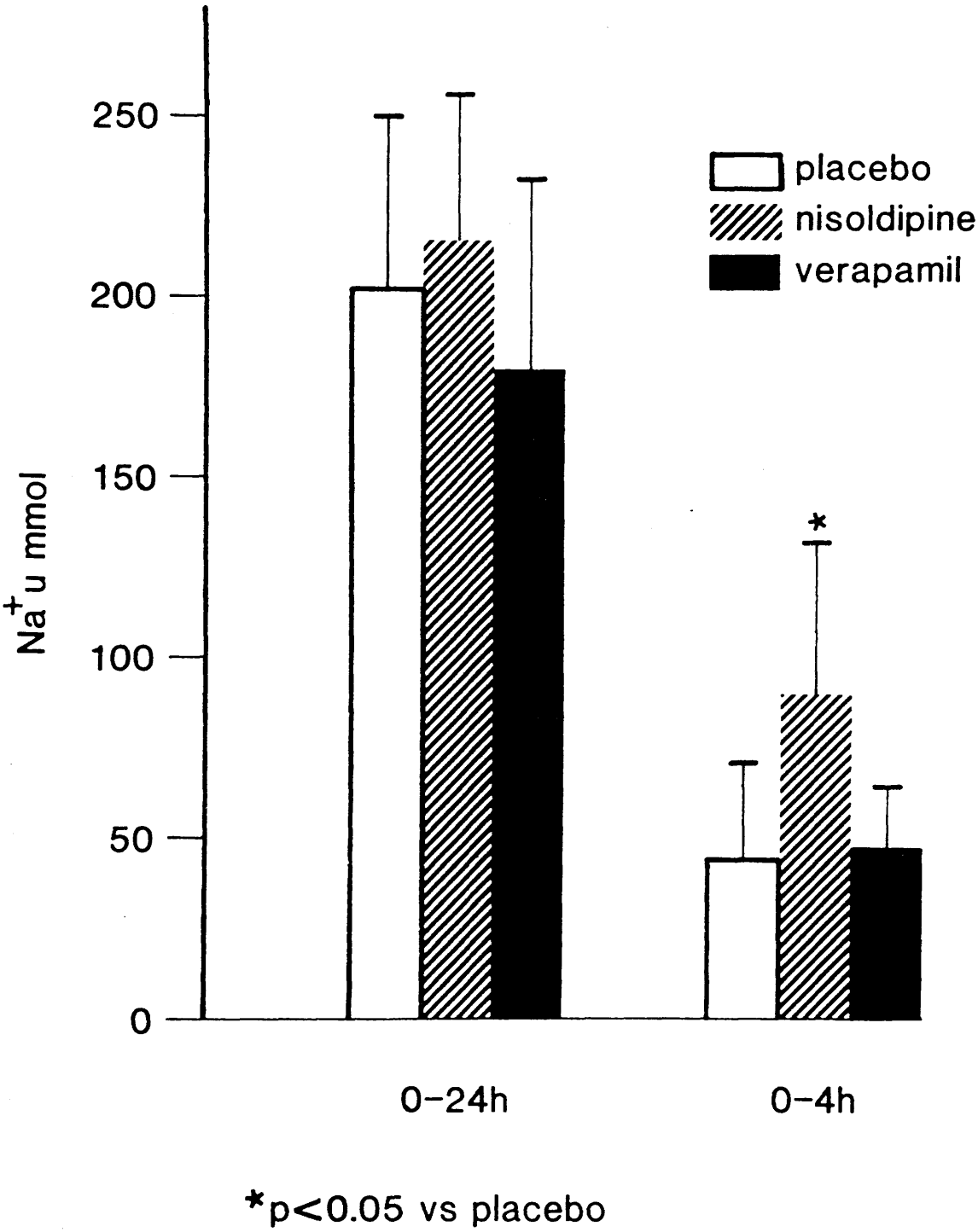


Fig. 7.7. Urinary sodium excretion after acute administration of nisoldipine 20 mg, verapamil 160 mg or placebo in normotensives. (n=9).

clearance/F is also associated with significant but modest increases in elimination half life. A number of possible explanations of this phenomenon have been suggested. Of these the most widely held is the suggestion that the disposition of the drug following continued oral administration exhibits non-linear Michaelis-Menten kinetics associated with saturation of the first pass metabolic processes (Wagner, 1984). Verapamil is metabolised by several pathways to a number of identifiable metabolites including norverapamil (Eichelbaum et al, 1979) and in this present study, as well as in Chapter 4, no significant changes in formation or clearance of this particular metabolite (as reflected by similar values in the ratio of AUC drug to AUC metabolite for acute and chronic dosing) were demonstrated. Whilst this only provides evidence that this particular metabolic pathway is not saturated, it does suggest that other possible mechanisms should be considered. The changes in apparent liver blood flow observed in this study provide such a possible explanation, particularly as Woodcock et al (1981), have shown a good correlation between verapamil clearance and apparent hepatic blood flow. The differences in first dose and steady state pharmacokinetics may be accounted for by the observed pattern of the changes in liver blood flow particularly the profound increase in liver blood flow with acute administration and the subsequent return to baseline during continued drug administration. Indeed the kinetic

changes are not inconsistent with what would be anticipated by theoretical considerations of the changes in liver and splachnic blood flow (Wilkinson and Shand, 1975). However, it must be appreciated that blood flow determinations were made only on one occasion between 1 and 2 hours after dosing, whereas the kinetics were calculated over 8 hours. Thus it is not possible with these data to demonstrate a direct correlation and the hypothesis remains to be conclusively proved. A recently published study in patients (Schwartz et al, 1985) showed that ICG clearance was unchanged after repeated doses of verapamil. This was interpreted as evidence against the role of liver blood flow but unfortunately the acute effects of verapamil had not been assessed.

The changes in apparent liver blood flow with acute drug administration may also be relevant to the pharmacokinetic interaction between prazosin and verapamil described in a previous chapter. The co-administration of verapamil with prazosin gave rise to significantly higher peak plasma levels and increased systemic bioavailability for prazosin, whereas the pharmacokinetics of verapamil were not changed. It was noted that the peak plasma levels of verapamil were consistently achieved earlier than the peak prazosin levels. In the present study the profound changes in LBF with acute verapamil administration were measured almost coincidentally with the attainment of peak verapamil levels. Thus it is plausible to suggest that the increased

hepatic and splanchnic blood flow associated with acute verapamil administration was the main factor underlying the changes in prazosin disposition.

A number of factors including age, posture, exercise and food are known to alter apparent liver blood flow and thereby to affect the systemic clearance of drugs undergoing first pass metabolism (George, 1979). In these studies the subjects were young, healthy and investigated under controlled conditions so that changes in liver blood flow can reasonably be attributed to the administered drugs. The renal circulation, however, is less susceptible to change and the glomerular filtration rate, in particular, is affected only by major haemodynamic disturbances. The efficiency of the renal autoregulation guarantees a steady blood supply which is relatively independent of the pressure within a range of 80-180 mmHg in the renal artery (Shipley and Study, 1951).

In the present study, in the absence of significant changes in blood pressure and heart rate, there were significant increases in effective renal plasma flow and glomerular filtration rate after acute administration of both drugs and a transient increase in urine output and Na^+ excretion after nisoldipine. Similar changes have been reported with other dihydropyridines (Leonetti et al, 1982; Ene et al, 1985). The mechanism by which calcium antagonist drugs cause a transitory alteration of renal autoregulation are not clearly understood. Similar

findings have been reported in animals (Ono et al, 1974) and have been attributed to interference with prostaglandin secretion (Herbaczynska et al, 1973).

Alternatively alpha adrenoceptors mediating vasoconstriction have been identified on rat renal membranes (Pettinger et al, 1976; Schmitz et al, 1981) and the local vasodilator effect may result from interference by calcium antagonists on the adrenoceptor-mediated responses.

It is recognised that alterations in liver blood flow may significantly affect drug bioavailability and clearance (George, 1979; Daneshmend et al, 1981). It is thus important to consider potential pharmacokinetic interactions if calcium antagonist drugs are administered acutely with other drugs. The altered pharmacokinetics of prazosin demonstrate this type of interaction and the explanation may be the transient increased hepatic and splanchnic blood flow attributable to the calcium antagonist.

Whilst increased liver blood flow is an important determinant of the clearance of lipophilic drugs, it remains to be seen whether the corresponding increases in renal plasma flow and glomerular filtration rate will significantly affect the renal elimination of co-administered polar drugs.

CHAPTER 8

STUDIES ON THE EFFECTS OF CALCIUM ANTAGONISTS ON RELEASE OF HORMONES

NICARDIPINE AND ANGIOTENSIN II MEDIATED ALDOSTERONE RELEASE

8.1. INTRODUCTION

Calcium ions were first proposed to be involved in the release of hormones, including catecholamines in 1963 (Douglas & Rubin, 1963; Douglas and Posiner, 1964). Several studies have since demonstrated that adrenal steroidogenesis is also calcium-dependent (Farese and Prudente, 1978) and that the aldosterone response to angiotensin II is associated with the intracellular accumulation of calcium ions (Shima et al, 1978; Fakunding & Catt, 1980; Foster et al, 1981). It has additionally been shown that the plasma concentrations of several peptide and steroid hormones, in response to physiological and pharmacological stimuli, are decreased by calcium antagonist drugs (Lin et al, 1979; de Marinis and Barbarino, 1980) but it is not yet clearly established in man that calcium antagonists have a clinically significant effect on hormone release in doses used in cardiovascular therapy.

For example, verapamil has been shown to inhibit the release of pituitary hormones (de Marinis and Barbarino, 1980) whereas nifedipine has no effect on pituitary hormone release (Struthers et al, 1983) or on cortisol production (Millar et al, 1982). However, nifedipine has been shown to diminish the aldosterone response to angiotensin II (Millar et al, 1981; Vierhapper & Waldhausl, 1982). This inhibitory effect is not sustained during chronic therapy

(Bianchetti et al, 1982; Millar et al, 1983) although it has been observed that aldosterone levels are reduced, particularly in relation to the level of plasma renin activity (Thiebonnier et al, 1980; Hiramatsu et al, 1982).

Nicardipine hydrochloride is a new calcium antagonist under investigation for treatment of essential hypertension. It is a dihydropyridine analogue of nifedipine with similar activities on the heart and the peripheral vasculature but short duration of action (Taylor et al, 1982; Thuillez et al, 1984; Van Schaik et al, 1984).

This study was designed to investigate the effects of acute and repeated dosing with nicardipine on the pressor, adosterone and other hormone responses to infused angiotensin II in healthy normotensive males.

8.2. MATERIALS AND METHODS

A random order double blind study was undertaken in six healthy normotensive sodium-replete males (23-26 years; 63-80 kg). To maintain the double blind design subjects were studied during three treatment phases, each of 1 week's duration with oral medication (including matching placebo tablets) culminating in a final study day when intravenous therapy was added, including 0.9% sodium chloride solution as placebo. Three treatments were thus compared: (1) placebo (2) acute nicardipine by intravenous infusion (at 5 mg/hour for 2.5 hours) (3) steady state nicardipine following 1 week of oral therapy with 30 mg t.i.d.

On the final day of each treatment phase subjects reported to the Clinical Pharmacology Research Unit where they rested supine for 6 hours. An intravenous cannula was inserted into each forearm and a blood pressure cuff was attached. After not less than 20 minutes supine rest the intravenous infusion of nicardipine, 5 mg/hour, or an equivalent volume of 0.9% sodium chloride solution, was started to run for 2.5 hours. One hour later, via the cannula in the opposite arm, an intravenous infusion of angiotensin II was commenced to run for 1.5 hours with fixed incremental doses of 5, 10 and 20 ng/kg/min, each administered for 30 minutes.

Supine blood pressure and heart rate recordings, by semi-automated sphygmomanometer (Sentron) were made at frequent intervals, in particular at 5, 10, 20 and 30 minutes during each infusion dose. Venous blood samples were withdrawn at times 0, 1 hour (i.e. pre-angiotensin) and at 1.5, 2 and 2.5 hours (corresponding to the end of each of the three angiotensin dose levels) for subsequent measurement of plasma sodium and potassium, plasma renin activity, plasma noradrenaline, plasma angiotensin II, plasma cortisol and ACTH. Plasma nicardipine concentrations were assessed by HPLC with fluorescence detection.

8.2.1. Statistical Analysis

Results throughout are expressed as mean ± SD and

statistical evaluation was by repeated measures analysis of variance.

8.3. RESULTS

8.3.1. Blood pressure and heart rate

The supine and erect blood pressure and heart rate data following the first oral dose of 30 mg nicardipine are shown in Table 8.1. The only significant haemodynamic effect following the introduction of oral nicardipine was a transient increase in erect heart rate at 2 hours, 83 ± 11 compared to 72 ± 12 bpm with placebo.

On the three study days there were no significant changes in supine blood pressure or heart rate associated with either acute intravenous administration or one week's therapy with oral nicardipine.

8.3.2. Pressor responses to angiotensin II

The mean blood pressure responses to the incremental infusion of angiotensin II are shown in Figure 8.1. The average increase in mean arterial pressure was significantly reduced by both intravenous and oral nicardipine ($p < 0.05$). Following 5 ng/kg/min the increase in mean arterial pressure was 9.1 ± 5.5 with placebo, compared to 4.7 ± 4.0 with intravenous and 4.8 ± 5.5 mmHg with oral nicardipine. The corresponding increases were 16.7 ± 7.6, 10.4 ± 6.1 and 10.5 ± 6.0 with 10 ng/kg/min and 23.6 ± 9.5, 13.4 ± 6.7 and 16.5 ± 7.3 mmHg with 20 ng/kg/min (Table 8.2.).

TABLE 8.1.

BLOOD PRESSURE AND HEART RATE FOLLOWING 30 mg ORAL ADMINISTRATION OF NICARDIPINE IN 6 NORMAL SUBJECTS MEAN + SD

		PLACEBO		NICARDIPINE	
		Supine	Erect	Supine	Erect
Time (h)	0	121/67	121/76	116/66	122/73
		± 4/4	± 9/6	± 7/5	± 7/3
		62 ±13	71 ±8	59 ±9	74 ±14
	2	121/66	121/76	116/61	119/68
		± 9/3	± 9/6	± 7/5	± 10/7
		59 ±10	72 ±12	63 ±8	83 ±11
	4	122/61	124/72	117/59	118/73
		± 10/4	± 13/7	± 9/8	± 6/9
		67 ± 8	81 ±10	66 ±10	82 ±13

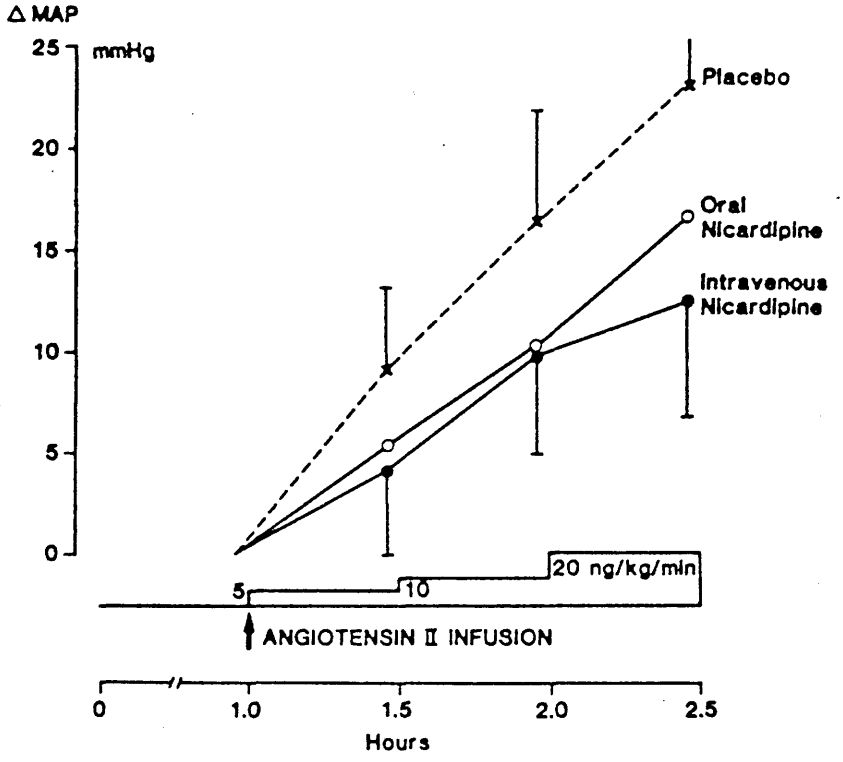


Fig.8.1. Angiotensin pressor responses.
Increase from baseline of mean arterial pressure.
Mean from the measurements in 6 subjects at the given dose level.

TABLE 8.2.

PRESSOR RESPONSES TO AII INFUSIONS AFTER NICARDIPINE ADMINISTRATION IN 6 NORMAL SUBJECTS

	PLACEBO	ORAL	INTRAVENOUS
AII 5 ng/kg/min	8.9 ± 5.5	5.2 ± 5.5	4.2 ± 4.0
10	16.5 ± 7.6	10.4 ± 6.0	10.4 ± 6.1
20	20.9 ± 9.5	16.5 ± 7.3	13.2 ± 6.7

Mean arterial blood pressure changes (mmHg)

Nicardipine (HCl (5 mg/h; 2.5 hrs) i.v. decreased significantly the response to AII infusion compared to placebo (paired t test: $p < 0.05$).

8.3.3. Aldosterone responses to angiotensin II

Plasma aldosterone concentrations are shown in Figure 8.2. and Table 8.3. The baseline values after one week of oral nicardipine were not significantly different at 48 ± 20 compared to 44 ± 13 pg/ml with placebo. In response to infused angiotensin there were wide inter-individual variations but there were no significant differences attributable to nicardipine in the patterns of response, with comparable increases in plasma aldosterone concentrations for all 3 treatments. At the highest dose of angiotensin infusion (20 ng/kg/min) the mean plasma aldosterone levels were 85 on placebo, compared to 73 with intravenous and 101 pg/ml with oral nicardipine.

8.3.4. Plasma Renin Activity

Plasma renin activity is summarised in Table 8.3. There were no significant differences associated with oral or intravenous nicardipine, although with all three treatments plasma renin activity tended to fall during the angiotensin infusion.

8.3.5. Aldosterone: renin ratio

On the placebo day, in response to the infusion of angiotensin II, there was a progressive increase in the aldosterone concentration/plasma renin activity ratio. This increase was significantly attenuated by both nicardipine treatments (Figure 8.3.). At the highest

TABLE 8.3.

PLASMA ALDOSTERONE AND RENIN ACTIVITY DURING AII INFUSION FOLLOWING NICARDIPINE ADMINISTRATION IN 6 SUBJECTS (MEAN ± SD)

	BASELINE	ANGIOTENSIN INFUSION (ng/kg/min)			
		0	5	10	20
ALDOSTERONE					
Normal range:	12-125 pg/ml				
Placebo	43.6 ± 13.3	28.1 ± 10.8	64.3 ± 24.5	61.6 ± 16.6	84.5 ± 15.8
Intravenous Nicardipine	50.6 ± 19.5	41.1 ± 9.2	50.1 ± 20.0	68.3 ± 20.4	73.3 ± 18.3
Oral Nicardipine	47.5 ± 19.6	47 ± 16.4	68.1 ± 18.4	87.8 ± 29.1	101.1 ± 23.9
RENIN ACTIVITY					
Normal range:	4-12 ng AI/ml/hr				
Placebo	1.2 ± 0.8	1.0 ± 0.5	0.5 ± 0.3	0.3 ± 0.1	0.4 ± 0.2
Intravenous Nicardipine	1.5 ± 1.0	2.9 ± 1.8	1.1 ± 0.6	0.7 ± 0.4	0.8 ± 0.3
Oral Nicardipine	2.6 ± 1.5	3.0 ± 1.5	1.7 ± 1.0	1.0 ± 0.5	0.8 ± 0.4

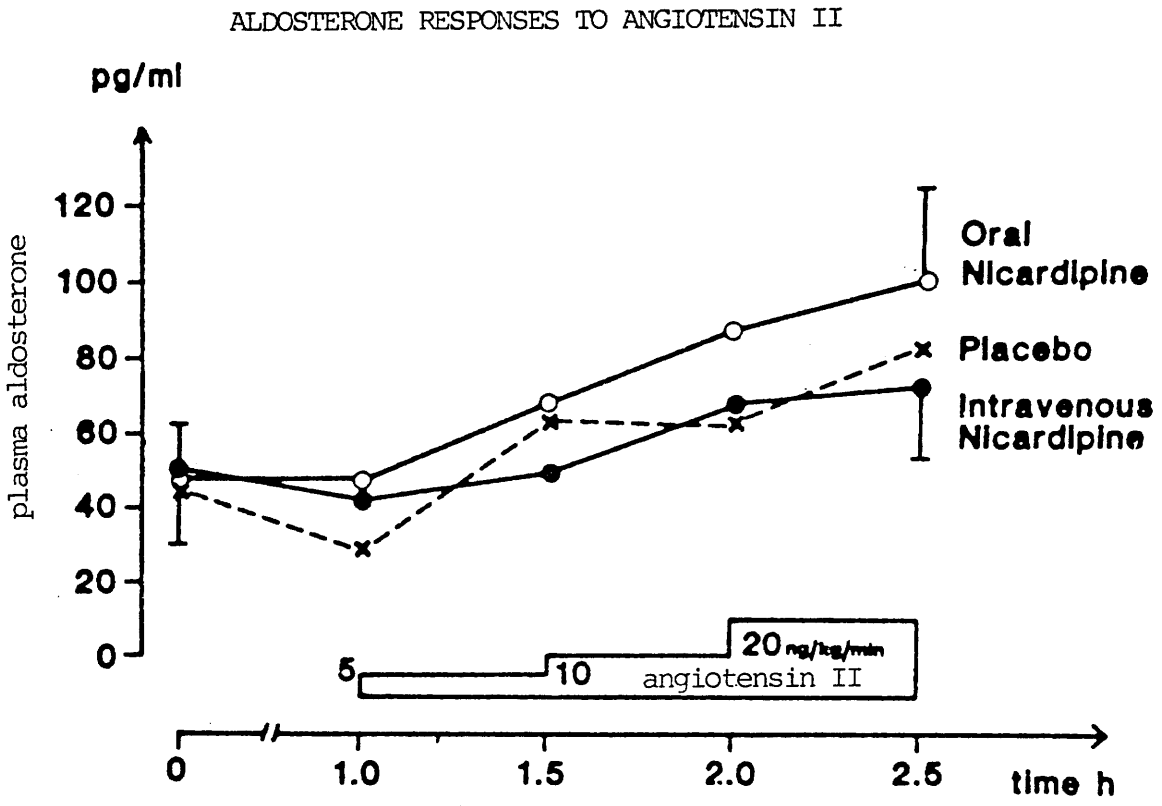


Fig.8.2. Mean plasma aldosterone concentrations before and during the angiotensin infusion at the given dose level in 6 subjects.

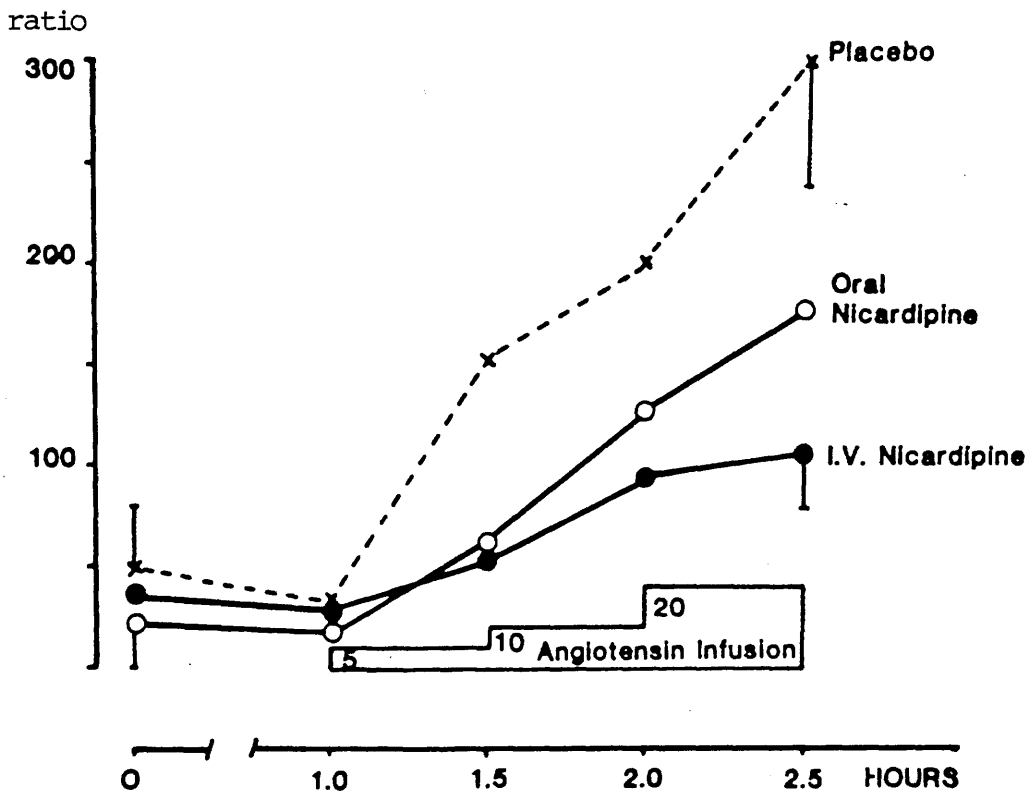


Fig. 8.3 Aldosterone/renin ratios in response to infused angiotensin II at the given dose level in 6 subjects.

level of infused angiotensin the ratios were 298 ± 108, 172 ± 112 and 108 ± 26 on placebo, oral nicardipine and intravenous nicardipine respectively.

8.3.6. Plasma cortisol, ACTH and noradrenaline

These results are summarised in Table 8.4. Only the baseline (0 time) cortisol concentrations after 1 week of treatment with oral nicardipine were significantly different from the corresponding placebo values: 428 compared to 567 nMol/L. The associated ACTH also tended to be lower but this difference was not significant: 12.8 ± 4.9 with oral nicardipine, compared to 16.7 ± 10.9 for the intravenous day and 14.8 ± 4.3 with placebo. Thereafter, during the time of the angiotensin infusion the plasma cortisol concentrations progressively declined but the responses were similar with all treatments. There were no significant differences for noradrenaline.

8.3.7. Plasma sodium and potassium

Plasma concentrations of sodium and potassium are shown in Table 8.5. There were no significant changes associated with administration of nicardipine either oral or intravenous compared to placebo during angiotensin II infusion.

8.3.8. Plasma nicardipine and angiotensin II concentrations

The nicardipine data were not adequate for individual

TABLE 8.4.

PLASMA NORADRENALINE AND CORTISOL CONCENTRATIONS DURING AII INFUSION FOLLOWING NICARDIPINE ADMINISTRATION IN 6 SUBJECTS
MEAN + SD

	BASELINE	ANGIOTENSIN INFUSION ng/kg/min			
		0	5	10	20
NORADRENALINE					
Normal range: 0-7 nMol/L					
Placebo	1.6 ± 0.4	1.9 ± 0.6	1.4 ± 0.5	1.4 ± 0.7	1.7 ± 0.6
Intravenous Nicardipine	2.3 ± 1.3	2.7 ± 1.0	2.7 ± 1.7	2.7 ± 1.2	2.6 ± 1.0
Oral Nicardipine	1.6 ± 0.3	2.2 ± 0.6	2.4 ± 0.5	2.2 ± 0.7	2.3 ± 1.5
CORTISOL					
Normal range (0800): 330-770 nMol/L					
Placebo	567 ± 157	340 ± 98	303 ± 68	257 ± 65	242 ± 52
Intravenous Nicardipine	513 ± 137	343 ± 96	303 ± 99	293 ± 103	332 ± 135
Oral Nicardipine	428 ± 93	345 ± 78	332 ± 92	302 ± 105	308 ± 115

TABLE 8.5.

PLASMA SODIUM AND POTASSIUM DURING AII INFUSION FOLLOWING NICARDIPINE ADMINISTRATION IN 6 SUBJECTS
(MEAN ± SD)

	BASELINE	ANGIOTENSIN II INFUSION (ng/kg/min)			
SODIUM (normal range 135-145 mmol/L)		0	5	10	20
PLACEBO	140.8 ± 2.7	140 ± 2.4	141 ±2.7	140.5 ± 3.2	139.6 ± 1.9
INTRAVENOUS NICARDIPINE	139.6 ± 1.0	140 ± 1.4	139.8 ± 1.7	140.7 ± 1.6	139.3 ± 1.5
ORAL NICARDIPINE	140 ± 1.7	140.6 ± 2.0	140.8 ± 2.7	140.7 ± 1.6	140 ± 1.3
POTASSIUM (normal range 3.5-5.0 mmol/L)					
PLACEBO	4.7 ± 0.1	4.8 ± 0.3	4.8 ± 0.2	4.9 ± 0.5	4.8 ± 0.3
INTRAVENOUS NICARDIPINE	4.6 ± 0.2	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.5 ± 0.3
ORAL NICARDIPINE	4.6 ± 0.4	4.7 ± 0.4	4.9 ± 0.4	4.6 ± 0.3	4.7 ± 0.2

analysis. The mean values following oral administration under steady state conditions and the mean concentrations during the acute intravenous infusion are shown in Figure 8.4.

The angiotensin levels showed wide interindividual variability but with the expected progressive increase during the infusion and with no significant differences during each study day.

8.3.9. General tolerance

Nicardipine was generally well tolerated. Mild headache was frequently reported during the first 24 hours of oral therapy but no subject had to interrupt the study on account of adverse effects.

8.4. DISCUSSION

There was no significant reduction in supine blood pressure, either with acute intravenous administration or during 1 week's treatment with oral nicardipine in this group of young normotensives. A transient, slight increase in standing heart rate observed 2 hours after the first oral dose of 30 mg was the only evidence of a significant haemodynamic effect. These findings are consistent with previous observations in young normotensives (MacGregor et al, 1982; Millar et al, 1983) where only modest haemodynamic effects were seen after treatment with calcium antagonists.

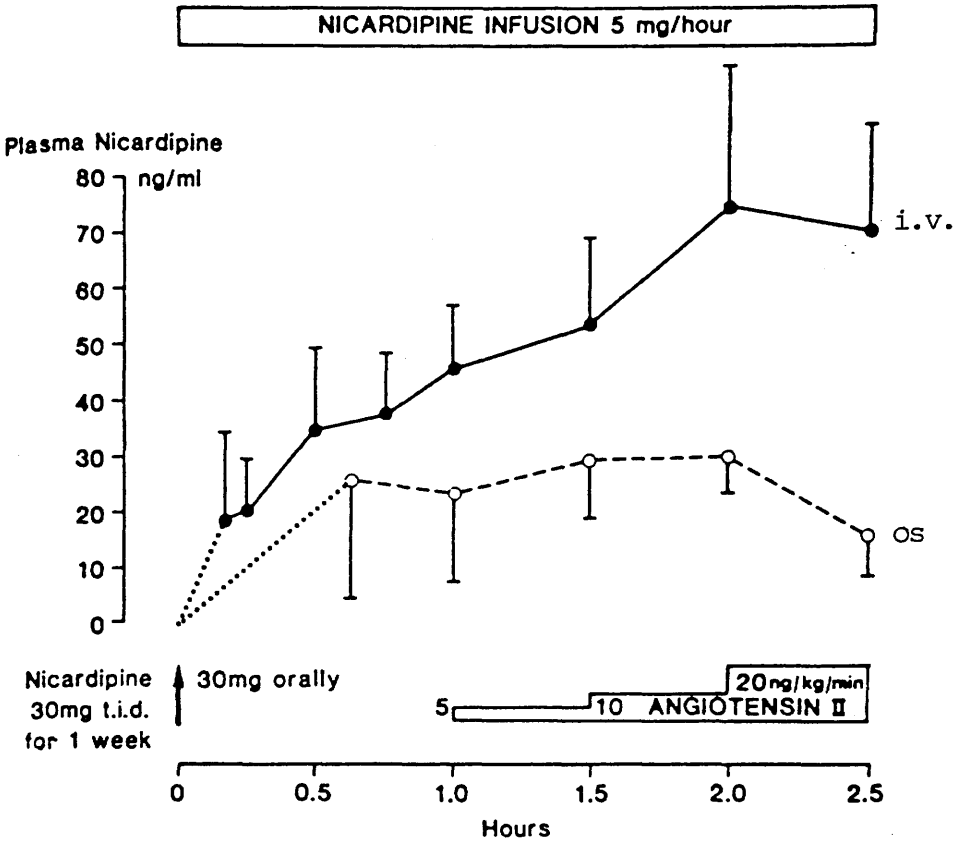


Fig.8.4. Plasma concentration-time profiles for nicardipine following both intravenous and oral administration in 6 subjects.

The intravenous administration of angiotensin II caused the expected dose-related increases in blood pressure (Bean et al, 1979). However, the pressor response to angiotensin was significantly and substantially attenuated by both intravenous and oral nicardipine, and although the expected increase in blood pressure was achieved in all subjects during placebo administration, the increases were very small in some subjects during nicardipine treatment. A similar reduction in pressor responsiveness to angiotensin has been reported for other calcium antagonists, including nifedipine (Millar et al, 1983), nisoldipine and verapamil (Chapter 5). There was no obvious relationship in individual subjects between the degree of antagonism of the pressor effect of angiotensin II and the plasma nicardipine concentrations but, for the whole group, attenuation of the pressor response tended to be greater with intravenous nicardipine and the highest plasma nicardipine concentrations were achieved following intravenous administration.

It has been suggested that inhibition of aldosterone production may be an important component of the antihypertensive effect of calcium antagonists (Millar et al, 1981) and there is evidence that nifedipine acutely inhibits the release of aldosterone (Millar et al, 1981; Vierhapper and Waldhausl, 1982). In this study the acute intravenous infusion of nicardipine did not significantly interfere with the magnitude of the aldosterone response. There is no conclusive evidence that long term therapy with

nifedipine inhibits the aldosterone response (Bianchetti et al, 1982; Millar et al, 1983) and similarly one week's therapy with nicardipine in this study had no significant effect. However, it has been observed that aldosterone levels are low relative to the level of plasma renin activity during chronic therapy with nifedipine (Thibonnier et al, 1980; Hiramatsu et al, 1982). In this study the aldosterone/renin ratio at baseline was not significantly altered by one week's therapy with nicardipine. In response to infused angiotensin, however, there was some evidence of disturbance of the aldosterone/renin ratio, with significant reductions attributable to both nicardipine treatments. The clinical relevance of this remains unclear.

In conclusion this study has shown that nicardipine significantly attenuated the pressor responsiveness to the direct vasoconstrictor angiotensin, both acutely and after one week's therapy. There was no significant effect on the aldosterone response to angiotensin II and no other evidence of clinically significant interference with hormone release.

NIFEDIPINE AND INSULIN SECRETION IN HYPERTENSION

8.5. INTRODUCTION

Several studies using isolated perfused pancreas preparation (Grodsky & Bennett, 1960) as well as islet-cell plasma membranes (Naber et al, 1980) have shown that calcium ions are involved in secretion of insulin (Wollheim and Sharp, 1981). It has been suggested that calcium antagonists might interfere with secretion of this hormone (Malaisse and Sever, 1981; Malaisse and Mathias, 1985). There is also some evidence that the regulation of insulin secretion is under the alpha adrenoceptor mediated adrenergic control (Langer et al, 1983). Studies on peripheral vascular responsiveness (Chapter 5) indicate that effects mediated via alpha adrenoceptors may be affected by the administration of calcium antagonist drugs. Thus, either by a direct action on the secretory process, or by interference with the effector coupling process for adrenergic control mechanisms, calcium antagonists have the potential for reducing insulin release and disturbing glucose homeostasis.

There are a few studies in man which provide supportive evidence of impaired glucose tolerance attributable to calcium antagonists. Following an intravenous glucose load verapamil has been shown to decrease glucose tolerance (De Marinis et al, 1980) and a corresponding impairment of insulin response on oral glucose tolerance test has also

been reported with nifedipine (Giugliano et al, 1980).

This study was designed to investigate the effect of acute and chronic administration of a slow release formulation of nifedipine on insulin secretion in a group of non diabetic patients with essential hypertension.

8.6. PATIENTS AND METHODS

Eight patients (5 M, 3 F; 58 ± 6 years) with moderate essential hypertension gave their informed consent to take part in the study.

Patients were non-obese (Weight 70 ± 6 kg) and non-diabetic and, on no previous treatment; blood pressure on two occasions was confirmed to be greater than 160/100.

Routine biochemical, clinical and ECG screening showed no evidence of concomitant diseases. After a two week period of placebo treatment patients attended the Clinical Research Unit where an intravenous glucose tolerance test (i.v. GTT 0.33 g glucose/kg body weight, as a bolus) was performed after an overnight fast. Blood samples for glucose (measured by standard glucose oxidase method) insulin and C-peptide (by radioimmunoassay) were collected via a cannula inserted in a forearm vein before and 5, 10, 20, 30, 40, 50, 60 and 90 minutes after glucose administration. The i.v. GTT was repeated on the first day (acute) and at the end of a 12 week period (chronic) with 20 mg twice daily nifedipine administered as the slow release formulation.

The test was performed two hours after either placebo

or active treatment administration.

Patients attended the outpatient clinic at monthly intervals for blood pressure recording and review for possible side effects. Blood pressure and heart rate were measured twice in the sitting position by automatic recorder (SENTRON) before i.v. GTT. Throughout the period of trial patients were kept on a standardised well balanced normocaloric diet and were advised not to change their life style. Glucose, insulin and C-peptide responses to i.v. GTT were calculated as incremental areas under the response-time curve. Incremental area (I.A.) was the total area under the curve less the basal area (AUC = AUCt - AUCo). The half time (t1/2) required for blood glucose to fall from a given level was determined and the K constant calculated from the formula K_G = 69.3 / t1/2 and expresses the percentage of blood sugar fall per minute (Seltzer, 1983).

Results are expressed as mean $\pm$ SD and statistical evaluation was by analysis of variance; differences were considered significant when $p < 0.05$.

8.7. RESULTS

The baseline (pre-i.v. GTT) measurements of glucose, insulin and C-peptide were not significantly different during nifedipine treatment. Blood glucose was 5.2 mmol/L on placebo, 5.1 on the acute day and 5.2 on the chronic day. Insulin and C-peptide concentrations were 9.1 and 0.97 respectively on the placebo day, 10.4 and 0.81 on the acute;

10.9 μU/ml and 0.97 nmol/ml after 12 week treatment (Table 8.6.).

Incremental areas under the response-time curve for glucose were 4920 ± 1060 on placebo, 4890 ± 1290 on nifedipine acute and 4240 ± 950 mg/ml min after 12 weeks. Incremental areas for insulin were 2190 ± 490 on placebo, 1910 ± 470 on the first and 2160 ± 620 μU/ml min on the last day of treatment. Incremental areas for C-peptide were 78.5 ± 16 on placebo, 76.3 ± 8 after acute and 72.1 ± 12 nmol/ml min after chronic administration of nifedipine 20 mg as monotherapy (Table 8.7. Figure 8.5.). K_G was 1.78 ± 0.3 (range 1.5 ± 2.2) at baseline, 1.59 ± 0.4 on the acute and 1.69 ± 0.3 on the chronic day.

Both acute and chronic treatment with nifedipine caused significant reduction of blood pressure (BP). BP sitting was 175/104 ± 9/5 on the placebo day and fell to 153/92 ± 9/5 after administration of the first dose of nifedipine; on the chronic day BP was 153/90 ± 8/6 mmHg ($p < 0.01$ compared to placebo). Corresponding heart rates were 74 ± 9 on the placebo day, 79 ± 10 on the acute day and 75 ± 8 b/min after 12 week therapy (Figure 8.6.).

8.8. DISCUSSION

Although there are reports of impaired glucose tolerance with both verapamil and nifedipine there are a number of other studies which have shown no significant effects of calcium antagonists on glucose tolerance or

TABLE 8.6.

BASELINE METABOLIC PARAMETERS FOLLOWING NIFEDIPINE (20mg) ADMINISTRATION IN 8 HYPERTENSIVE PATIENTS (MEAN + SD)

	PLACEBO	NIFEDIPINE ACUTE	NIFEDIPINE CHRONIC	
Glucose (mmol/L)	5.2 ± 0.4	5.1 ± 0.6	5.2 ± 0.5	N.S.
Insulin (μU/ml)	9.1 ± 4	10.4 ± 7	10.6 ± 6	N.S.
C-peptide (nmol/ml)	0.97± 0.4	0.81± 0.4	0.97 ±0.4	N.S.

TABLE 8.7.

INCREMENTAL AREAS FOR GLUCOSE, INSULIN AND C-PEPTIDE FOLLOWING NIFEDIPINE (20mg) ADMINISTRATION IN 8 HYPERTENSIVE PATIENTS (MEAN ± SD)

	PLACEBO	NIFEDIPINE ACUTE	NIFEDIPINE CHRONIC	
Glucose (mg/ml min)	4920 ± 1060	4890 ± 1290	4240 ± 950	N.S.
Insulin (μU/ml min)	2190 ± 490	1910 ± 470	2160 ± 620	N.S.
C-peptide (nmol/ml min)	78.5 ± 16	76.3 ± 8	72.1 ± 12	N.S.

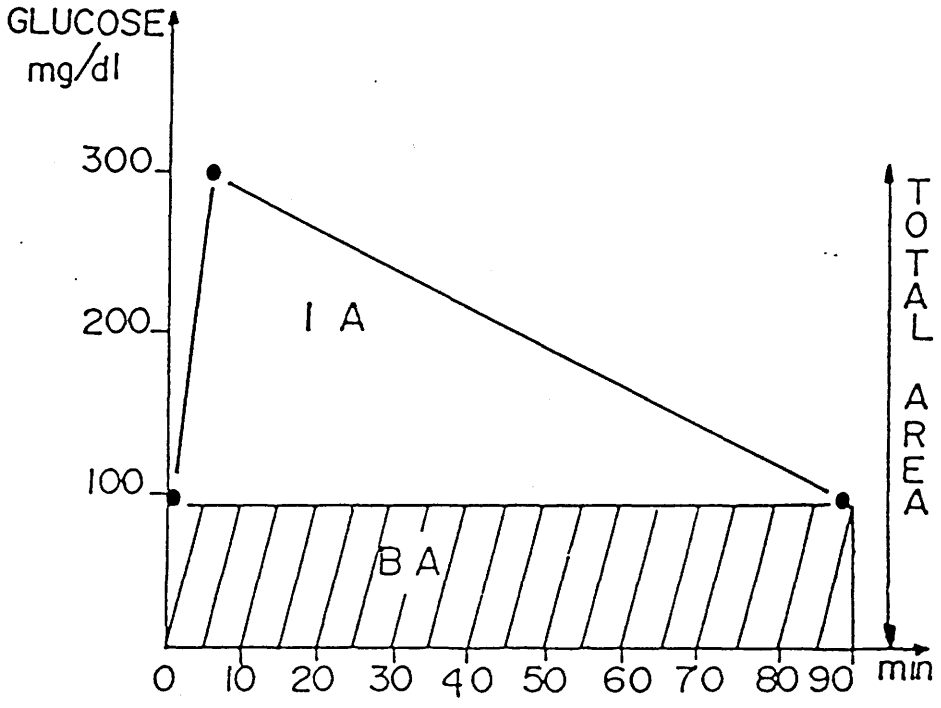


Fig.8.5. A schematic representation of the evaluation of incremental area (IA) = total area - basal area (BA).

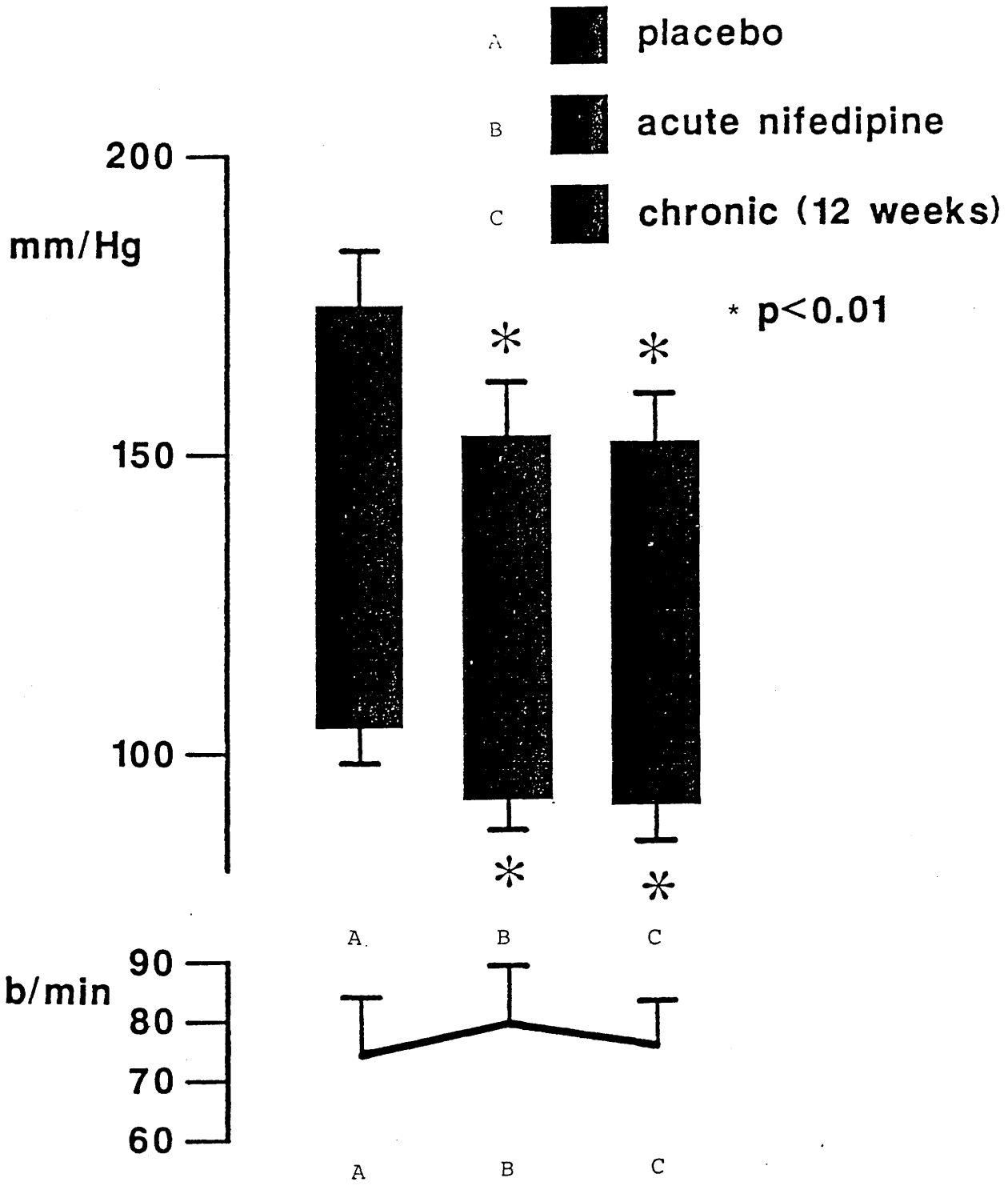


Fig.8.6. Sitting blood pressure and heart rate in 8 hypertensive patients treated with nifedipine 20 mg twice daily. (sig. vs. placebo)

insulin secretion. Whereas verapamil administered intravenously seemed to impair glucose tolerance and to reduce insulin secretion in patients with islet-cell tumor (De Marinis, 1980) chronic oral administration had no significant effects in a group of patients with angina pectoris (Semple et al, 1983).

In contrast, nifedipine 10 mg three times daily for ten days, impaired insulin secretion but improved glucose tolerance in a group of non-diabetics (Giugliano et al, 1980). In addition, nifedipine 20 mg t.i.d. has been associated with hyperglycaemic effect as assessed by oral glucose tolerance testing, in a group of normal subjects (Charles et al, 1981) and with the development and deterioration of diabetes mellitus in hypertensive patients (Bhatnagar et al, 1984). In view of these conflicting reports about the effect of calcium antagonists on glucose/insulin regulation the principal aim of this study was to establish, using i.v. GTT whether or not nifedipine in its most widely used slow release formultion had any significant effect on insulin secretion in a group of hypertensive patients. In this study an i.v. GTT was performed in a group of non-diabetics after acute and chronic administration of nifedipine monotherapy. Calculated incremental areas for glucose, C-peptide and insulin did not show significant differences for nifedipine compared to placebo, indicating that the drug did not impair insulin secretion. Perhaps a balance of subtle inhibition

in secretory process with a subtle inhibition of the $alpha_2$ adrenergic effect, which is itself inhibitory, has occurred leading to unchanged secretion (Nakaki et al, 1980; Langer et al, 1983). Moreover, K_G which is an index of glucose removal rate was not significantly altered by the drug.

These data are consistent with lack of effect on other hormone release after repeated dosing of nifedipine (Millar et al, 1983).

In conclusion this study indicates that nifedipine as standard antihypertensive therapy does not impair insulin release or glucose tolerance in response to an intravenous glucose load.

CHAPTER 9

GENERAL DISCUSSION

GENERAL DISCUSSION

The principal aim of the research described in this thesis was to investigate aspects of the clinical pharmacology of calcium antagonist drugs in both normal subjects and in hypertensive patients. Most of the studies have involved the established calcium antagonist drugs, nifedipine and verapamil, but other newer dihydropyridines, nicardipine and nisoldipine, have also been studied. The inclusion of these newer compounds is a reflection not only of the considerable, recent increase in the number of drugs acting upon slow calcium channels, but also of the increased awareness of the central role of calcium in a variety of intracellular processes.

In contrast with other antihypertensive drugs, the calcium antagonists produce little or no change in the blood pressure of normotensive subjects. This has been reported by a number of other observers (Leonetti et al, 1982; Hulthen et al, 1982; MacGregor et al, 1983; Millar et al, 1983) and was confirmed in the first study described in which the effects of the new dihydropyridine, nisoldipine, were compared with nifedipine in normal volunteers and then assessed in hypertensive subjects (Chapter 3). Based on this difference in responsiveness it has been proposed that increased intracellular ionised calcium in vascular smooth muscle is the final determinant of the increased peripheral vascular resistance observed in essential hypertension (Jones, 1974; Orlov and Postnov, 1982). Therefore, the

hypothesis has been developed that calcium antagonist drugs are particularly appropriate for "correction" of this abnormality (Buhler, 1983). Using the platelet as a model for the vascular endothelial cell, support for this hypothesis has been derived from the observed relationship between an increased Ca^{++} content in the platelets of hypertensive patients and the subsequent "normalisation" of this content with antihypertensive treatment (Erne et al, 1984). However, other groups studying leucocytes have shown that calcium-sodium exchange is not corrected by nifedipine (Haegerty, 1983).

The response to calcium antagonists is not determined solely by an elevated blood pressure and other pathophysiological factors, such as age, sodium intake, plasma renin activity and circulating catecholamines, must be taken into account in the interpretation of the antihypertensive effect. For example, a direct correlation between age and blood pressure response has been demonstrated by some authors (Buhler, 1982) and this has further been related to the renin-angiotensin system whose activity decreases with age (Hulthen et al, 1982). However, the inter-relationship between age, blood pressure response and plasma renin activity has been disputed in both clinical studies (Ferrara et al, 1985) and animal studies (Waeber et al, 1985).

Sodium intake not only affects the activity of the renin-angiotensin system (Brown et al, 1963) but also

affects cardiovascular responses to adrenergic stimulation and circulating catecholamines (Fraser et al, 1981) and plasma noradrenaline tends to rise with age (Ziegler et al, 1976). In addition, calcium antagonist drugs themselves have been shown to acutely increase plasma renin activity (MacGregor, 1982) although this increase is not sustained during chronic treatment (Lederballe et al, 1980; Kiowski et al, 1983a). Similarly an acute increase in circulating catecholamines occurs, particularly with nifedipine (Murphy et al, 1982) as a consequence of a baroreflex-mediated release that also determines a transitory increase in heart rate (Lederballe et al, 1979; Littler, 1983).

Another point of uncertainty was whether or not blood pressure reduction was related to the plasma concentration of calcium antagonist drug, particularly verapamil that has shown changing kinetics with continued administration (Freedman et al, 1981; Shand et al, 1981). Applying concentration effect analysis to the data collected (Chapter 4) the results suggested that the differences in responsiveness observed might be due to change in pharmacokinetics.

In the individual patient, therefore, there are several factors which affect the response to a calcium antagonist drug and the precise mechanism of the antihypertensive action of this type of drug is still subject to discussion. Considering the heterogeneity of the factors involved in the pathophysiology of essential hypertension and the various

factors which can influence the blood pressure response to treatment with calcium antagonists, the interactions of these drugs with other systems, including the adrenergic nervous system were investigated in more detail.

Sympathomimetic amines promote calcium entry and potentiate contraction. Calcium antagonists, on the other hand, affect alpha adrenoceptor function as demonstrated in vitro and in animal studies (Karliner et al, 1982; Pedrinelli and Tarazi, 1984). Several in vitro studies and animal studies have convincing data supporting the hypothesis that either $alpha_1$ or $alpha_2$ adrenoceptors modulate the entry of extracellular calcium in vascular smooth muscle (Vanhoutte, 1982b; Vanhoutte and Rimele, 1982; Van Zwieten et al, 1982). Human studies have also clarified the postjunctional role of $alpha_2$ adrenoceptors in the vasoconstriction of arteries (Elliott et al, 1983; Kiowski et al, 1983; Murphy et al, 1984a).

Some authors had proposed that calcium antagonists selectively inhibit $alpha_2$ adrenoceptor responses (Motulsky, 1982). The data shown in Chapter 5 demonstrate that calcium antagonists impair pressor responses to phenylephrine as well as alpha-methylnoradrenaline showing, therefore, an interference with both $alpha_1$ and $alpha_2$ mediated pressor response. Furthermore, the pressor response to angiotensin II was inhibited suggesting a non-selective interaction with alpha adrenoceptors and non adrenergic mechanisms.

To further investigate the proposed interference of calcium antagonists with alpha$_2$ receptors, a study on platelets was undertaken. Platelets represent a very interesting model to study the alpha$_2$ receptor (Erne et al, 1983, 1985). Several analogies link vascular smooth muscle cell to platelets, and these cells can be used as a model in both cardiovascular research and the study of pathophysiological processes in hypertension. Furthermore, platelets play a key role in thrombosis and in the progression of atherosclerosis. If an antiplatelet action of calcium antagonists could be shown in long term clinical studies, this could be of importance in the prevention of ischaemic diseases. In the platelet study both verapamil and nisoldipine inhibited adrenaline induced platelet aggregation in vitro confirming an interaction with the alpha adrenergic system.

In view of these observations on the relationship between alpha adrenergic mechanisms and calcium channels another study was undertaken on the use of the alpha blocker, prazosin, together with the calcium antagonist, verapamil (Chapter 6). An enhanced hypotensive effect was achieved using the two drugs in normotensives which seemed to result from both a dynamic and a kinetic interaction. The increased bioavailability of prazosin could be responsible for the larger fall in blood pressure observed as, according to other authors, prazosin blood levels are related to response (Bateman et al, 1979; Larochelle et al,

1982). An additional dynamic factor, however, contributed to the interaction causing a significant reduction in supine diastolic blood pressure. Total peripheral resistance may have been significantly reduced by affecting simultaneously alpha$_1$ adrenoceptors and calcium channels in the arterioles. An effect similar to that described for beta blockers (Elliott et al, 1981) was also observed with verapamil that attenuated the reflex tachycardia due to prazosin. It is also conceivable that verapamil slightly depressed atrioventricular conduction. The results of this study open the possibility to further investigate the combination in the treatment of hypertension.

In the study of the interaction between verapamil and prazosin, it was suggested that the observed prazosin pharmacokinetics resulted from a change in the hepatic extraction of prazosin, reflecting an increase in liver or splanchnic blood flow due to the calcium antagonist. To further investigate the effects of calcium antagonist drugs on liver and kidney blood flow a comparative study was undertaken with verapamil and nisoldipine (Chapter 7). The liver has a system of blood flow autoregulation called the hepatic arterial buffer response. Portal venous blood flow is the major regulatory factor within this system whereby total hepatic blood flow is maintained at a constant level through compensatory alterations to hepatic arterial flow (Lautt, 1985). An increase of apparent liver blood flow, as measured by the clearance of indocyanine green, was

demonstrated following the first dose of either nisoldipine or verapamil. This finding was observed within the first 2 hours of drug administration suggesting a transitory alteration of hepatic blood flow autoregulation. A consequence of this alteration to apparent liver blood flow could be changes in the hepatic clearance of drugs co-administered with the calcium antagonist (c.f. prazosin) but this requires further detailed evaluation.

Of additional interest are the findings of acute changes in blood flow and glomerular filtration rate of the kidney which again suggest a transitory impairment of renal autoregulation secondary to the vasodilator influence of the calcium antagonists. With nisoldipine there was also evidence of an effect on the function of the nephron with an increase in sodium excretion within a few hours of administration. To account for this natriuretic effect an interference by the dihydropyridines on the adrenergic receptor affecting sodium reabsorption in the tubule has been postulated (Leonetti et al, 1982; Zanchetti, 1985). The different effect on sodium excretion and urine output may reflect a different affinity of the two drugs for the afferent and the efferent arterioles. An alternative, or additional, factor in increased natriuresis observed with nisoldipine may be inhibition of the effect of aldosterone on the tubule but there is no evidence to support this hypothesis.

Another subject of investigation of this thesis was

the potential interference by calcium antagonists, particularly dihydropyridines, of the calcium-dependent release of hormones (Chapter 8). Previous observations had shown that nifedipine acutely inhibited the aldosterone response to exogenous angiotensin II (Millar et al, 1981) and a corresponding study was undertaken with nicardipine. In contrast to the findings with nifedipine, nicardipine did not affect significantly the magnitude of the aldosterone response to angiotensin II infusion in normal volunteers, although a similar attenuation of the blood pressure response was observed. A possible explanation for this difference is that nicardipine has lesser affinity for the adrenal cortex than nifedipine, but whether or not this mechanism has an important role in the reduction of blood pressure remains to be established.

Hormonal and metabolic aspects were further investigated in a group of hypertensive patients. A number of studies have reported deterioration of blood glucose control in diabetic patients taking nifedipine for treatment of concomitant hypertension (Giugliano et al, 1980; Bhatnagar et al, 1984). In addition to glucose intolerance, antihypertensive drugs, particularly thiazide diuretics, are known to adversely affect other metabolic risk factors, such as increased plasma lipids and augmented uric acid (Ames and Hill, 1976; Weidmann et al, 1985). In the study undertaken with nifedipine no significant impairment of insulin secretion or glucose tolerance after acute and chronic administration was

observed. The lack of side effects of this kind, if confirmed with longer term studies, could be an advantage to consider in the choice of the drug for treatment of hypertension.

Other points must be taken into account. Calcium antagonists are vasodilator drugs but, among them, verapamil does not cause reflex tachycardia nor increase renin secretion (Muiesan et al, 1982). As a class they do not induce fluid or sodium retention and some of them have, at least at the beginning of therapy, a natriuretic and a diuretic effect (Leonetti et al, 1980). These drugs seem to be as effective as beta blockers in the control of high blood pressure and are also devoid of the contraindications that apply to beta blockers, such as asthma or heart failure. Furthermore a possible "cardioprotective" role of these drugs may be suggested considering the favourable use in treatment of angina pectoris. This aspect also requires long term clinical investigations.

These considerations are of important clinical relevance because they may suggest the use of calcium antagonists as a first line drug as well as beta blockers and diuretics. When the blood pressure is inadequately controlled, a rational next step is the addition of a beta blocking agent (Buhler, 1983) since the cardiac and the renin-angiotensin mediated counter-regulatory mechanisms induced particularly by nifedipine can, in principle, be antagonised by beta blockers. In contrast to verapamil, a depressant effect on

cardiac pacemaker cells and prolongation of the AV conduction is not likely to occur with the combination of nifedipine and beta blocker, since nifedipine has fewer direct cardiac effects (Singh et al, 1982). The combination of a calcium antagonist with a diuretic or a converting enzyme inhibitor could theoretically also be effective. However, at present clinical experience with these combinations is limited.

The calcium antagonists certainly represent a fascinating group of drugs whose various properties may prove to be of great value in the treatment of a variety of clinical conditions in the future.

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