STUDIES ON CIRCULATORY EFFECTS OF CLONIDINE AND PAPAVERINE

A Thesis

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SUMMARY

This thesis describes studies on the circulatory pharmacology of clonidine and papaverine, which have in common a hypotensive action but have entirely opposite peripheral vascular effects.

The nature of clonidine's local action on blood vessels and its effect on vascular responsiveness to catecholamines was examined in man and animals. In normotensive man, intra-arterial clonidine caused cutaneous vasoconstriction in the forearm and hand which was mediated by adrenergic alpha receptors. Intravenous clonidine caused a sustained fall in blood pressure and heart rate, and an increase in the vascular resistance of the hand during and briefly after the infusion. Chronic antihypertensive treatment with clonidine also resulted in a fall in blood pressure and heart rate and an increase in hand vascular resistance. The cardiovascular effects of intra-arterial and intravenous noradrenaline were increased by both intravenous and chronic clonidine.

Experiments with animal isolated vascular preparations from normotensive animals confirmed that acute clonidine causes a direct rather than an indirect adrenergic alpha receptor stimulation and that it has the properties of a partial agonist. Chronic administration to rats did not cause arterial or venous smooth muscle supersensitivity to noradrenaline, methoxamine or potassium chloride,

nor did this treatment have any obvious effect on the monoamine fluorescence in these vessels. The doses used in these animals, though insufficient to cause a fall in blood pressure, were more than double those which had previously been shown to decrease vascular responsiveness to vasoconstrictors and vasodilators in the cat. In contrast, chronic reserpine and chronic guanethidine treatment did cause vascular supersensitivity and also catecholamine depletion. Since the animal studies failed to demonstrate vascular supersensitivity after chronic clonidine, it is suggested that the increased responses to noradrenaline observed in the human studies resulted from the additive stimulant effects of clonidine and noradrenaline at the adrenergic receptor, rather than true vascular supersensitivity.

The second drug whose effects on the human were examined was papaverine. It was confirmed that, in contrast to clonidine, intra-arterial papaverine has a vasodilator effect on the human hand and forearm. Papaverine was also administered intravenously and orally to determine whether the doses recommended for oral administration produce blood levels capable of causing vasodilatation. Intravenous infusion resulted in an increase in heart rate, occasional ectopic beats and an increase in forearm blood flow, all of which were of brief duration. Plasma concentrations of the drug reached peak values during or shortly after the infusion and then declined rapidly to about half peak values within thirty minutes. The onset and

duration of cardiovascular effects correlated well with plasma concentrations. In chronic studies papaverine in a sustained release formulation was administered daily. The plasma level at the end of a week with each of the two doses was only about one-sixth of that associated with vasodilator effects after intravenous administration. With the lower dose there was no consistent change in the heart rate or blood flow to the hand or forearm though the reflex vasoconstriction in the hand in response to the Valsalva manoeuvre was potentiated. The higher dose consistently reduced forearm blood flow and reflex responses were not consistently changed. At neither dose level was there any correlation between the plasma concentration and the cardiovascular effect in an individual subject. It was concluded that papaverine lacks significant vasodilator effects when given orally, even in doses five to ten times those which cause vasodilatation and cardiac effects when given intravenously, and the failure to achieve adequate plasma concentrations of the drug is the most probable cause for this finding.

DECLARATION

I declare this thesis to be the record of original work containing no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief it contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

S. M. Robinson

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PREFACE

Clonidine hydrochloride is an imidazoline derivative with antihypertensive properties. In contrast to most other antihypertensive drugs, it probably lowers arterial pressure by an inhibition of sympathetic structures in the central nervous system, rather than by impairment of peripheral autonomic or vascular function. Clonidine also causes adrenergically mediated vasoconstriction, and there is evidence that acute administration causes an increase in the vascular responsiveness to catecholamines. However, there is other evidence that chronic clonidine administration depresses vascular sensitivity to catecholamines. The studies described in Part I of this thesis were therefore undertaken to investigate the effect of acute and chronic clonidine administration on the blood vessels in man and animals, particularly its effect on vascular sensitivity to noradrenaline.

Papaverine hydrochloride is another drug with hypotensive properties. In contrast to clonidine, it causes a dilatation of blood vessels by a direct action on vascular smooth muscle. This effect is potent when the drug is applied locally on the vessel, but vasodilatation after oral administration is weak. However, greater vasodilator effects during oral administration have been claimed for a new sustained release preparation. Part II presents human

studies undertaken to determine the correlation between plasma concentrations of papaverine and its cardiovascular effects during acute (intravenous) and chronic (oral sustained release) administration.

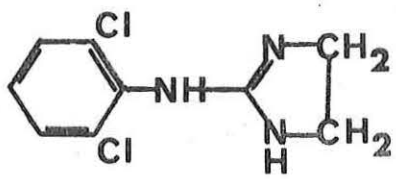
PART I

CLONIDINE

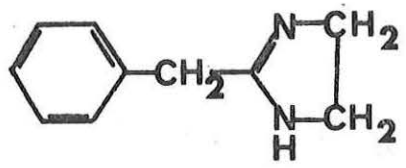
CHAPTER 1

Clonidine (Catpres, Catapresan, ST 155, DCAI) has the chemical formula 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (Fig. 1.1). Its structure resembles that of tolazoline (an adrenergic blocking drug), naphazoline and tetrahydrozoline (sympathomimetics) and antazoline (an antihistaminic). Boehringer Ingelheim (Germany) developed clonidine while searching for new vasoconstrictors among imidazoline derivatives, and its hypotensive effect was observed first in man during trials as a nasal decongestant (Graubner & Wolf, 1966). Thereafter, research on clonidine was directed towards elucidating the mechanism of its hypotensive action and assessing its therapeutic potential as an antihypertensive agent.

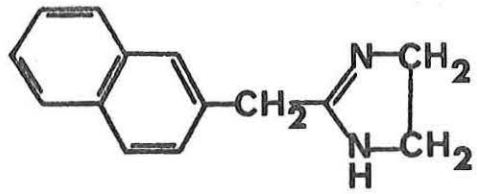
Animal experiments confirmed that a prolonged and dose dependent fall in arterial pressure and heart rate were clonidine's most prominent effects (Hoefke & Kobinger, 1966). A number of other studies verified its antihypertensive effect in patients (Grabner, Michalek, Pokorny & Vormittag, 1966; Kochsiek & Fritsche, 1966; Michel, Zimmerman, Nassehi & Seraphim, 1967). However, clonidine differs from other antihypertensive drugs because blood pressure is probably lowered by an inhibition of central sympathetic structures rather than by ganglionic blockade or adrenergic neurone or receptor blockade (e.g. Hoefke & Kobinger, 1966; Kobinger & Walland, 1967; Sattler & Van Zweiten, 1967; Rand & Wilson, 1968; Bentley & Li, 1968; Schmitt, Schmitt, Boissier, Giudicelli & Fichelle, 1968;



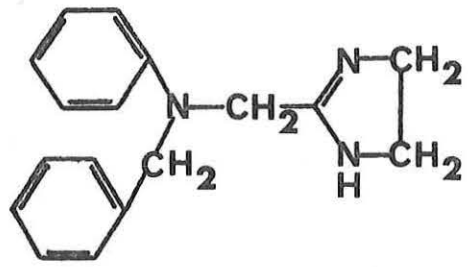
Clonidine



Tolazoline



Naphazoline



Antazoline

Fig. 1.1 The structural formulae of clonidine, tolazoline, naphazoline and antazoline.

Hughes, 1968).

In man, Hökfelt, Hedeland & Dymling (1970) have provided evidence that clonidine (acute and chronic) causes a dose dependent decrease in sympathetic activity in patients with essential and other types of hypertension. A fall in blood pressure and heart rate occurred in all patients except one who had a phaeochromocytoma. These changes were closely paralleled by a reduction in the urinary catecholamine excretion. Plasma renin activity and urinary aldosterone also fell during clonidine treatment, though these changes were delayed in relation to the fall in catecholamines. The authors suggested that a reduction in sympathetic activity was responsible, not only for the antihypertensive effect, but also for the fall in renin activity and aldosterone excretion. Furthermore, interruption of clonidine treatment was accompanied by rebound sympathetic overactivity characterized by hypertension, anxiety, nervousness, restlessness and a marked increase in urinary catecholamines. Although confirmatory studies on the effect of clonidine on urinary or plasma catecholamines have not been undertaken, the withdrawal rebound phenomenon has since been noted by several groups (Toubes, McIntosh, Kirkendall & Wilson, 1971; Hoobler & Sagastume, 1971).

Treatment of hypertension with a number of the antihypertensive drugs which have a sympathetic blocking action, e.g. guanethidine and bethanidine, may be associated with undesirable degrees of postural

hypotension at a time when supine blood pressure is still inadequately controlled. In contrast, this disadvantage is not commonly encountered with antihypertensive doses of clonidine, nor has exercise hypotension been reported (e.g. Kochsiek & Fritsche, 1966; Kellet & Hamilton, 1970). Such evidence suggests that the inhibition of sympathetic activity is only partial. There is experimental evidence that an intravenous dose of 150 mcg, although causing significant hypotension, does not significantly impair the cardiovascular homeostatic reflexes to passive tilting, the Valsalva manoeuvre or supine exercise (Iisalo & Laurila, 1967; Barnett & Cantor, 1968; Muir, Burton & Lawrie, 1969). However, larger single oral doses (e.g. 0.45 - 0.75 mg) are associated with a significant postural hypotension and lack of reflex increase in the total peripheral resistance (Onesti, Schwartz, Kim, Swartz & Brest, 1969). There is evidence from animal studies that sufficiently strong stimuli, such as the diving reflex or an arterial oxygen tension of 30 mm Hg, can still elicit homeostatic cardiovascular reflexes after doses of clonidine of 20 to 200 mcg/kg (Kobinger & Oda, 1969; Shaw, Hunyor & Korner, 1971). The experiments of Shaw *et al.* (1971) also provided evidence which indicated that inhibition of the circulatory reflexes probably occurs at the level of the central autonomic structures.

Briefly then, the animal and human experimental data is compatible with the hypothesis that clonidine's hypotensive effect

results from a dose dependent inhibition of central sympathetic structures. Because such a mode of action is unique among the currently available antihypertensive drugs, clonidine is a useful addition to the therapeutic armamentarium for the treatment of hypertension.

The immediate haemodynamic cause of the hypotensive effect of acute clonidine varies with the dose. Low doses (e.g. 1 mcg/kg) cause a reduction of the total peripheral resistance and little or no change in the cardiac output and heart rate (Kochsiek & Fritsche, 1966; Constantine & McShane, 1968). With larger doses (>2 mcg/kg) the fall in arterial pressure almost invariably results from a decrease in heart rate, cardiac output and stroke volume, while the total peripheral resistance is usually unchanged or only falls slightly (e.g. Grabner *et al.*, 1966; Michel *et al.*, 1966; Onesti *et al.*, 1969; Muir *et al.*, 1969). However, an intravenous injection of doses of 2 mcg/kg or more causes a biphasic blood pressure response in which the hypotension is preceded by a brief rise in pressure resulting from adrenergic alpha receptor stimulation (e.g. Hoefke & Kobinger, 1966; Rand & Wilson, 1968; Merguet, Heimsoth, Murata & Bock, 1968). As Hoefke & Kobinger (1966) showed, the pressor response results from a brief rise in the total peripheral resistance since the cardiac output and heart rate are falling at this time. In man, the vasoconstriction responsible for the rise in peripheral resistance

is probably limited to the skin and cerebral circulations (Bock, Heimsoth, Merguet & Schoenermark, 1966; Deisenhammer & Klausberger, 1966), since pulmonary vascular resistance, renal vascular resistance and hepatic blood flow show little or no change (e.g. Kochsiek & Fritsche, 1966; Grabner *et al.*, 1966). Ehringer (1966) infused clonidine intra-arterially and intravenously to differentiate the local vascular effects from reflex effects of systemic administration on blood vessels. He demonstrated that infusion into the femoral artery caused a dose dependent reduction in blood flow to the calf and foot. Because the effect in the foot (largely composed of skin) was more marked than that in the calf (largely muscle), he concluded that the major site of the vasoconstriction was skin. Unfortunately, Ehringer did not directly measure skin and muscle blood flow, nor did he seek to confirm that the vasoconstriction is adrenergically mediated in man as it is in animals. Thus, in man, there is only indirect evidence that the cutaneous vasoconstriction results from adrenergic alpha receptor stimulation.

The effects of chronic treatment with clonidine have not been adequately documented and the hypotension may result from different haemodynamic mechanisms than apply in the acute situation. Human studies to obtain such evidence are confronted with a number of difficulties, e.g. the variability of cardiac output in an individual at different times even under similar conditions, the probability

that treatment will include other antihypertensive drugs in addition to clonidine, and the frequency with which patients withdraw from intensive follow-up studies. In view of these difficulties, it is not surprising that there has been only one reported attempt to assess the haemodynamic effects of chronic clonidine treatment. Reubi, Vorburger & Bütikofer (1970) studied four patients before and 13 to 31 months after starting treatment with clonidine (0.45-0.9 mg daily) and frusemide (plus guanethidine in two). They found that the fall in blood pressure was accompanied by a fall in total peripheral resistance and a slight increase in the cardiac index. This is the reverse of the haemodynamic response to acute administration of such doses. To explain the discrepancy the authors suggested that the initial reduction in cardiac output and fall in blood pressure caused a compensatory dilatation of blood vessels in order to maintain adequate tissue blood flow. However, there is other evidence that vasospasm, and even Raynaud's phenomenon, occur during prolonged treatment with similar doses (e.g. Iisalo & Laurila, 1967; Kellet & Hamilton, 1970; Winchester & Kennedy, 1971). Most studies have also noted that the bradycardia persists (e.g. Bock *et al.*, 1966; Hökfelt *et al.*, 1970). Therefore, in contrast to the observations of Reubi *et al.* (1970), there is some evidence that two of the haemodynamic consequences of acute administration also occur during clinical use. However, the effect of chronic clonidine on peripheral blood vessels needs to be confirmed by actual measurements of blood flow.

Another aspect of the chronic use of clonidine on which there is conflicting evidence is the development of tolerance to its anti-hypertensive action. An incidence varying from as low as 16% to as high as 60% has been reported by several groups (e.g. Ng, Phelan, McGregor, Laverty, Taylor & Smirk, 1967; Davidov, Kakaviatos & Finnerty, 1967; Smet, Hoobler, Sanbar & Julius, 1969). In these clinical trials the phenomenon developed between one and ten months after starting treatment. Increasing the dose of the drug usually resulted in regaining control of the blood pressure, though three to four times the original dose was occasionally required. In contrast, others (e.g. Sung, Samet & Yeh, 1971; Mroczek, Leibel & Finnerty, 1972) have not observed tolerance occurring with treatment lasting up to two years. There is also divergence of opinion as to the possible mechanism(s) causing tolerance. For example, Davidov *et al.* (1967) considered that tolerance was related to fluid and sodium retention, and they were able to reverse the effect by adding a diuretic to the treatment. But others have not found evidence to support such a mechanism of action (e.g. MacDougall, Addis, MacKay, Dymock, Turpie, Ballingall, McLennan, Whiting & MacArthur, 1970).

There is evidence from animal studies that acute clonidine (5-10 mcg/kg) increases the pressor effect of adrenaline and noradrenaline (e.g. Kundig, Monnier, Levin & Charlton, 1967; Rand & Wilson, 1968; Bentley & Li, 1968). Therefore, the possibility that vascular

supersensitivity to catecholamines may cause tolerance to clonidine should be considered. As studies with other antihypertensive drugs have shown, an increase in general cardiovascular responsiveness to catecholamines does not constitute evidence that the sensitivity of peripheral vessels is also increased. For example, hexamethonium and bretylium do not increase peripheral vascular sensitivity to noradrenaline even though the pressor effect of this catecholamine is increased (Hodge & Whelan, 1962; Cooper, Fewings, Hodge & Whelan, 1963). Furthermore, according to Trendelenberg (1963), an increase in peripheral vascular responsiveness to catecholamines can only be termed supersensitivity if the treatment causes an approximately parallel shift to the left of the complete dose response curve. Without evidence from such curves additive effects may be misinterpreted as supersensitivity. Such additive effects occur when the drug under study causes an effect which is similar to that of noradrenaline, and thus increased responses to subsequent injections of noradrenaline result from simple addition of effects. Dose response curves after pretreatment with these drugs are convergent rather than parallel (Trendelenberg, 1972). The necessary experiments to determine whether or not clonidine causes vascular supersensitivity have not been done. However, Zaimis and Hanington (1969) have shown that chronic clonidine (10-20 mcg/kg/day) decreases rather than increases vasoconstrictor responses of the cat femoral artery to

single injections of noradrenaline, adrenaline and angiotensin. These findings may indicate that chronic treatment actually results in vascular subsensitivity, but such a conclusion needs to be confirmed by determining the effect of clonidine on full dose response curves. Thus the possible role of vascular supersensitivity in the development of tolerance to clonidine remains to be clarified.

The vasodilator effect of isoprenaline is also decreased by chronic clonidine (Zaimis & Hanington, 1969). In view of the evidence that it depresses vascular smooth muscle reactivity to both vasoconstrictors and vasodilators, Zaimis & Hanington (1969) suggested that clonidine might be of value in the prophylaxis of migraine. Clinical trials have confirmed that it is more effective than placebo in preventing migraine attacks (e.g. Sjaastad & Stensrud, 1971; Shafar, Tallet & Knowlson, 1972). However, despite its probable efficacy in this condition, it is not known whether depressed vascular reactivity occurs in man or, if it does, whether such an effect is the correct explanation of the beneficial action it has in migraine.

On the basis of the increased cardiovascular response to exogenous catecholamines after acute clonidine the suggestion has been made that it may influence uptake, storage and release of catecholamines in the body (Dollery & Conolly, 1970). The observation that the pressor response to clonidine is reduced by cocaine (Boissier, Giudicelli,

Fichelle, Schmitt & Schmitt, 1968) supports this possibility. However, in another study, cocaine had no such effect (Rand & Wilson, 1968). In addition, Hoefke & Kobinger (1966) did not find any depletion of the noradrenaline content of the rat heart even after doses of clonidine as large as 5 mg/kg daily given for three days. Thus the possible role that uptake and release mechanisms of sympathetic nerves may play in the action of acute and chronic clonidine remains unclear.

In summary, clonidine probably exerts its antihypertensive effect by inhibition of central sympathetic structures, which, after acute administration, leads to a reduction of cardiac output, heart rate and blood pressure, with the total peripheral resistance being unchanged or only slightly reduced. There is also evidence of a sympathomimetic vasoconstrictor effect which is responsible for the brief initial rise in peripheral resistance and pressor response characteristic of intravenous clonidine administration. During chronic administration of antihypertensive doses there is unconfirmed evidence that the cardiac output is increased and the total peripheral resistance decreased. However, there is also evidence that cutaneous vessels are constricted during chronic use. There is therefore a need for further investigation of the effect of acute and chronic clonidine administration on human peripheral vessels.

Tolerance to clonidine's antihypertensive effect has been noted in a number of studies, although the mechanism by which this occurs

is unknown. Acute administration causes an increased cardiovascular responsiveness to noradrenaline and adrenaline which suggests that vascular supersensitivity to catecholamines may contribute to the development of tolerance. However, there is evidence that vascular responsiveness is depressed during chronic administration. This finding needs confirmation before vascular supersensitivity is excluded as a possible mechanism contributing to the occurrence of tolerance. The greater pressor response to catecholamines seen with acute administration may be an indication that clonidine interferes with the uptake, storage and release of catecholamines by adrenergic neurones although there is other evidence which is not consistent with such an effect. The possibility that clonidine has an effect on peripheral adrenergic neuronal mechanisms needs to be examined by further studies.

CHAPTER 2

INTRODUCTION

The vasoconstrictor effect of clonidine in animals has been shown to result from adrenergic alpha receptor stimulation (e.g. Kobinger & Walland, 1967; Constantine & McShane, 1968), but there has been no direct confirmation of this mechanism of action in man. Another aspect of the human pharmacology of clonidine which has received scant attention is its effect on cardiovascular sensitivity to catecholamines or other vasoactive agents. Merguet *et al.* (1968) studied the effects of acute clonidine on the responses to intravenous catecholamines, but effects on local vascular sensitivity, as distinct from generalized cardiovascular sensitivity, were not determined. Furthermore, the effect of chronic clonidine on sensitivity to catecholamines in man has not been reported.

The studies reported in this chapter were undertaken to:

1. Determine the mechanism and site of acute clonidine's local vascular action in man;
2. Examine, in normotensive and hypertensive subjects, the effects of acute and chronic clonidine on the local vascular and general cardiovascular sensitivity to noradrenaline.

METHODS

Subjects and Laboratory Conditions

The subjects for these experiments were 21 healthy male volunteers, and two patients with essential hypertension, all of

whom gave informed consent to the procedures undertaken on them. The studies were performed in a laboratory at ambient temperatures ranging from 24° to 28° C, though the temperature during any particular study varied less than ± 0.5° C. The subjects lay supine on a couch for at least thirty minutes before recordings began, and during this time the recording apparatus was applied and the appropriate catheters or infusion needles inserted.

Systemic Arterial Pressure

During the studies in which clonidine or noradrenaline were infused intravenously (I.V.) systemic arterial pressure was measured directly through a catheter inserted into the brachial artery. Using local anaesthesia, the catheter was placed in the artery in the antecubital fossa by means of a modified Seldinger technique (Seldinger, 1953) and a 19 or 20 gauge Riley needle. It was then connected to a Statham pressure transducer (P23 DC) by means of a length of saline filled polyethylene tubing and the transducer output was recorded by a Grass polygraph (model 5 D). The frequency response of the whole recording system accurately reproduced the output of a sine-wave generator (Ardill, Fentem & Wellard, 1967) up to 10 Hz which is adequate to accurately record arterial pressure when the heart rate is less than about 100 beats/min (Geddes, 1970). The internal electrical calibration in the polygraph recorder was regularly checked with a mercury manometer. The patency of the intra-arterial

(I.A.) catheter was maintained during the experiment by intermittent flushing with heparinized saline solution. Mean arterial pressure (M.A.P.) was derived using the formula, M.A.P. = 1/3 (S + 2D), where S is the systolic and D the diastolic pressure in mm Hg.

Heart Rate (H.R.)

The subjects who had arterial pressure recorded also had limb electrodes applied, and the standard electrocardiograph lead which was most suitable for counting the H.R. was selected to be recorded by the Grass polygraph. The H.R. was read by counting either the R waves of the E.C.G. or the systolic peaks of the arterial pressure trace.

Hand and Forearm Blood Flow (H.B.F. and F.B.F.)

Two methods which yield information about hand or forearm blood flow were used in the human studies reported in this thesis. Firstly, venous occlusion plethysmography was applied to measure the total hand or forearm flows. Secondly, measurement of oxygen saturation in blood samples from superficial and deep veins in the forearm were used to give a qualitative indication of blood flow through skin and muscle, respectively, in this segment of the limb.

Venous occlusion plethysmography

The basic principle of the technique is that the venous outflow from the limb is occluded for a short interval without directly interfering with arterial inflow. The initial rate of increase in

limb volume is then taken to be the rate of arterial inflow (Brodie & Russell, 1905) (see also Appendix). A water-filled plethysmograph with temperature control (Greenfield, 1954) was used to measure the limb swelling, and temperatures of 32° C and 34° C for the hand and the forearm, respectively (Barcroft & Edholm, 1946), were maintained during the experiments. The plethysmograph was usually coupled by air transmission to a float recorder with a frontal writing pen applied to a smoked drum kymograph (Greenfield, 1960). In some experiments the increase in plethysmograph hydrostatic pressure, rather than volume, was recorded using a Statham transducer (low pressure, P23 BC) and a Rikadenki pen writing recorder. Calibration was by air displacement into the air transmission to the float recorder or by water displacement into the transducer.

During the measurement of F.B.F. distal circulation to the hand was arrested by an arterial occlusion cuff inflated to 200-250 mm Hg at least one minute before flow recording began (Grant & Pearson, 1938; Kerslake, 1949). The duration of continuous occlusion was limited to 15 to 20 minutes which was tolerated by every subject. Inflation and deflation of the venous occlusion cuffs was automated by incorporating a sequence timer (Paton Industries, Adelaide) and electrically operated solenoid valves which released the air from cylinders of compressed air via pressure regulators. Three or four flows were recorded each minute by limiting the venous occlusion to

9 to 15 seconds and deflation time to 4 to 6 seconds.

In the application of the technique and the experimental procedure used the present studies substantially followed the methodology of Greenfield (1960) and Greenfield, Whitney & Mowbray (1963). The interpretation and reading of flow records was as described by these authors, and direct readings of blood flow in ml/100 ml of tissue/min were obtained with the aid of a slide caliper (Greenfield *et al.*, 1963).

Forearm venous oxygen (O_2) saturation measurements

Venous occlusion plethysmography can only indicate changes in the total blood flow through a limb segment and not a change in the pattern of distribution of flow to its various tissues. The detection of changes in flow distribution to two important vascular beds in the forearm, skin and muscle can be obtained in a number of ways, though none of these is completely satisfactory. The method used in the present clonidine studies yields qualitative information only by measuring changes in the O_2 saturation of serial venous blood samples from muscle and skin (Mottram, 1955; Roddie, Shepherd & Whelan, 1956). It is assumed that during the experimental procedure the arterial O_2 saturation and the tissue O_2 extraction remains constant, so that any changes in the venous O_2 saturation sampled from the two circulations accurately reflect changes in the blood flow distribution, i.e. a rise in O_2 saturation in blood from one vascular bed indicates

an increase in flow to that vascular bed and a fall indicates a flow reduction.

Catheters (Intracath No. 17, Bardic) were passed retrogradely into a superficial (skin) and a deep (muscle) vein in the antecubital fossa percutaneously with the aid of local anaesthesia (Mottram, 1955; Roddie *et al.*, 1956). Intermittent flushing with a heparinized saline solution maintained the patency of the catheters. To establish that true representative samples from the skin and muscle circulations were being obtained with minimal mixing of the two, the correct placement of catheters was confirmed by the response to indirect heating, which increased the O_2 saturation of superficial venous samples alone (Roddie *et al.*, 1956), and by raising the legs and lower trunk, which caused the O_2 saturation of deep venous samples to rise and had no effect on superficial venous O_2 saturation (Roddie & Shepherd, 1956; Roddie, Shepherd & Whelan, 1957a). Arterial occlusion (200 mm Hg) at the wrist to exclude the hand circulation was effected at least one minute before sampling started. The venous blood samples (1 ml) were collected anaerobically into heparinized plastic syringes and haemolysed by a saponin sodium carbonate solution. The O_2 saturation of each sample was rapidly determined by measuring the percentage of light transmission through each sample with a Unicam SP 1400 prism absorptiometer using a wavelength of 660 mμ and a self-flushing cuvette. A fully oxygenated and a fully

deoxygenated standard were prepared using the subject's blood so that percentage saturation of each test sample could be derived. Full details of the technique using a SP 400 or SP 350 absorptiometer which were standardized against Van Slyke determination of O_2 saturation were reported by Roddie, Shepherd & Whelan (1957b). The SP 1400 absorptiometer used in the present studies has been calibrated by the Van Slyke method (Skinner, 1961).

Hand Vascular Resistance (H.V.R.)

The resistance (in arbitrary units) to flow in the vascular bed of the hand was calculated from the ratio $\frac{\text{M.A.P. (mm Hg)}}{\text{H.B.F. (ml/100 ml/min)}}$.

Cardiovascular Reflexes

In two subjects the effect of I.A. clonidine on sympathetically mediated reflex responses was tested. The two reflexes chosen were (a) the application of ice for 30 seconds to the neck of the subject which elicits a marked vasoconstriction of hand blood vessels (Pickering, 1932; Cooper *et al.*, 1963); and (b) the performance of mental arithmetic by the subject, the stress of which causes a 2-3 fold increase in forearm muscle blood flow by activation of cholinergic vasodilator fibres to muscle vessels (Blair, Glover, Greenfield & Roddie, 1959).

Intravascular Infusions

Infusions of drugs were given into the brachial artery in the cubital fossa of one arm, either through the catheter which was in

place to record arterial pressure or, where this was not present, through a 22 gauge short-bevel needle introduced percutaneously during local anaesthesia. Intravenous drug administration was made through a catheter placed in an antecubital vein. Needles and catheters were connected through polyethylene tubing to infusion pumps which delivered 1 or 2 ml of solution per min. Saline (0.9% w/v) was infused during control periods and was used as a vehicle for the drugs.

Expression of Results

With both I.A. and I.V. infusions (except I.V. clonidine) changes in all variables were determined from averaged values for the 2 minutes prior to the drug infusion and for the last 2 minutes of the infusion period, by which time the responses to the drugs had become stable. When I.V. clonidine was infused, averaged values for the 2 minutes before infusion were compared with those for the same period at the time when the drug effects were maximum. With I.A. infusion, where the small doses given did not cause systemic effects, the blood flow on the uninfused side was regarded as a control and was taken into account in calculating the change in flows (absolute and per cent.) on the treated side (Duff, 1952) (see also Appendix). The assumption made by Duff, that except for the infused drug the conditions on the experimental side are the same as those on the control side, is not valid when the vessels are pretreated

with an adrenergic alpha receptor blocking drug, and therefore the infused side was used as its own control during these particular experiments.

RESULTS

Acute Administration

Effects of I.A. clonidine

The effect of three doses of I.A. clonidine on hand and forearm blood flows in two subjects is shown in Figs. 2.1 and 2.2, respectively. A similar type of response was seen during I.A. infusions in all subjects. Clonidine caused a fall in blood flow which was rapid in onset; the maximum effect was attained within three to four minutes. In a single experiment with a thirty minute infusion the reduction in H.B.F. was well sustained (Fig. 2.3) and spontaneous fluctuations of flow were diminished in magnitude. Within 2-10 minutes of completing the drug infusion blood flows always returned to resting levels. In all, five subjects received two or three doses of clonidine, with the effects of H.B.F. being measured in three and on F.B.F. in two (Fig. 2.4). In another nine subjects one dose per subject was infused, H.B.F. being measured in six and F.B.F. in three. Fig. 2.5 presents the mean results from all subjects in terms of the effects on absolute blood flow (individual results are in Table A.1). Although 500 ng/min reduced H.B.F. 67% (mean), compared with a 48% reduction during 250 ng/min, the difference in the effects

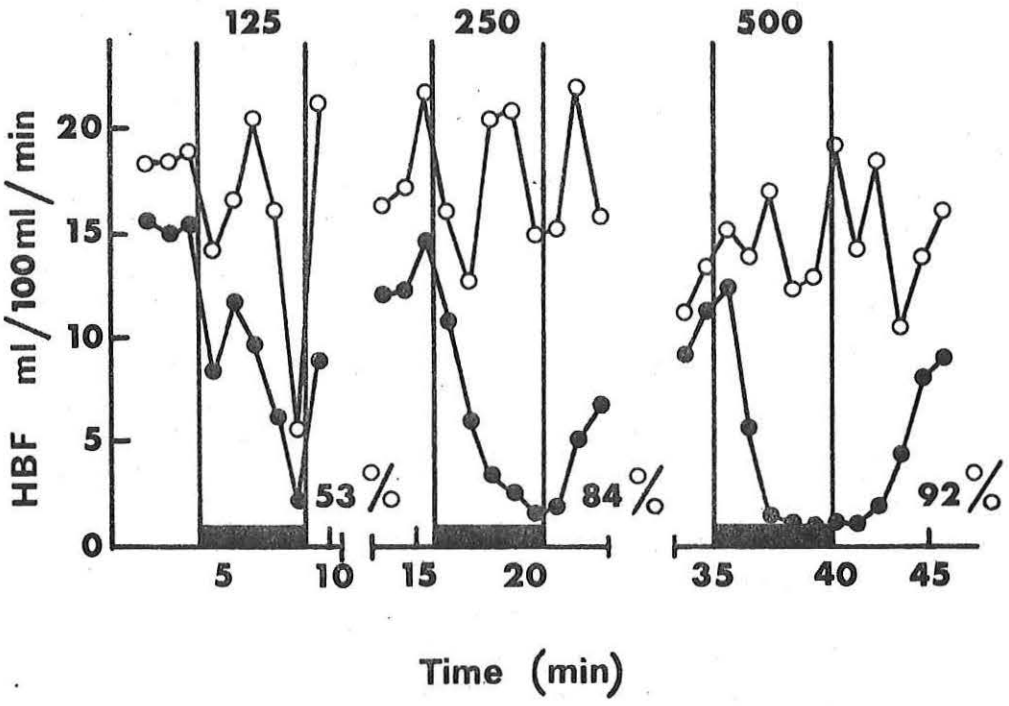


Fig. 2.1 Changes in hand blood flow in one subject (D.K.B.) in response to I.A. clonidine (125, 250 and 500 ng/min). The percentages indicate the falls in flow on the infused side after correction for fluctuations in flow on the control side.

o , uninjected control side; ● , injected side.

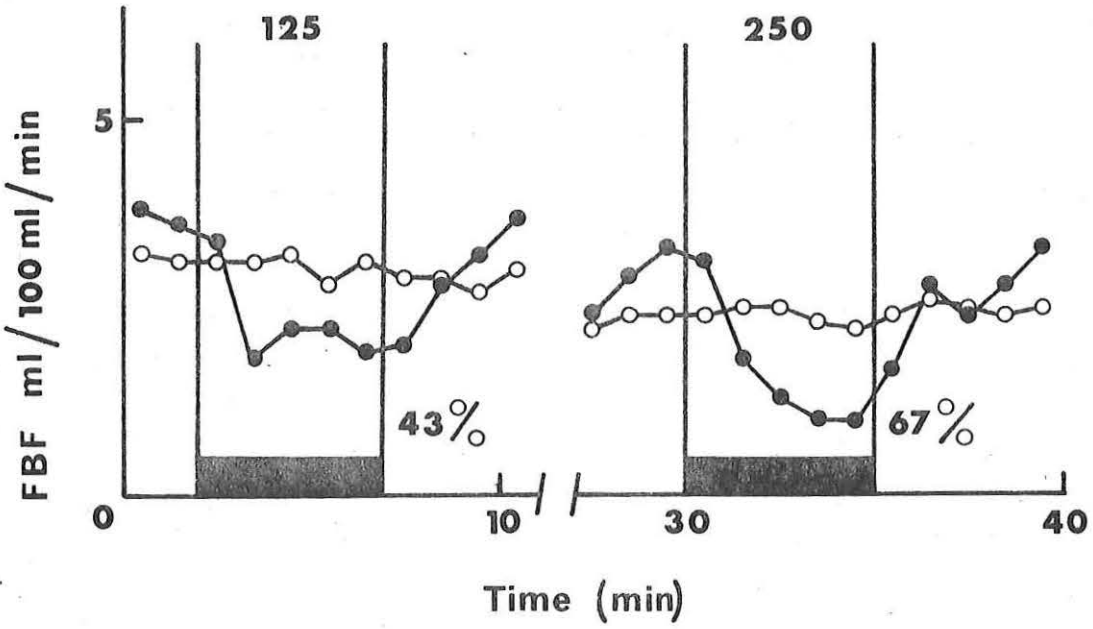


Fig. 2.2 Changes in forearm blood flow in one subject (G.M.) in response to I.A. clonidine (125 and 250 ng/min). The percentages indicate the fall in flow on the infused side after correction for fluctuations in flow on the control side. ○ , uninjected control side; ● , injected side.

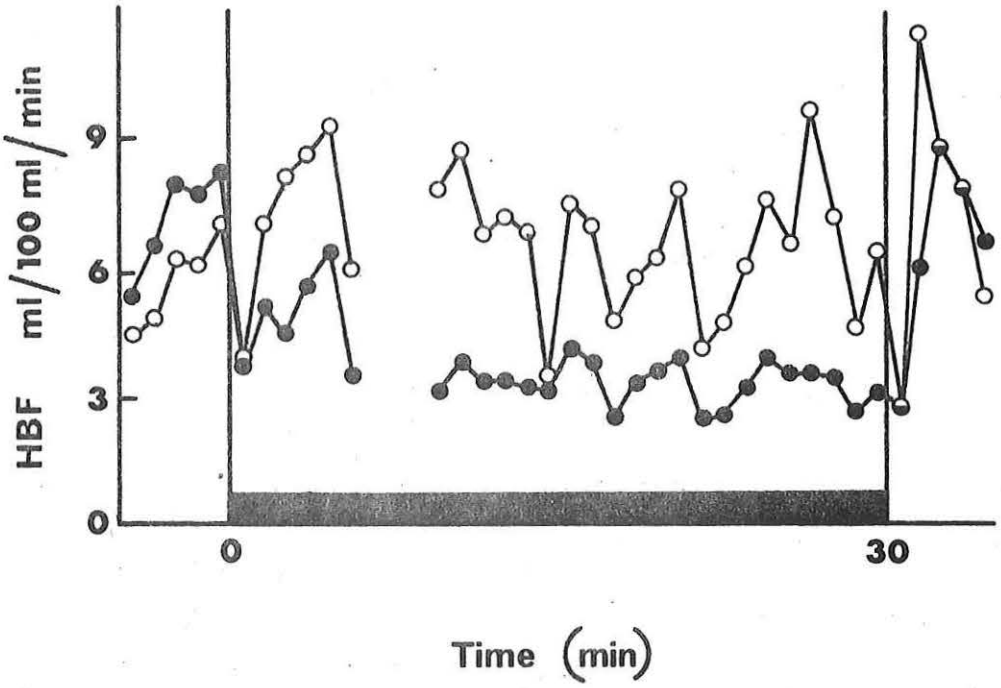


Fig. 2.3 Effect of I.A. clonidine, 250 ng/min, for 30 min (between vertical lines) on hand blood flow of one subject, D.B. o , control, uninjected side; ● injected side.

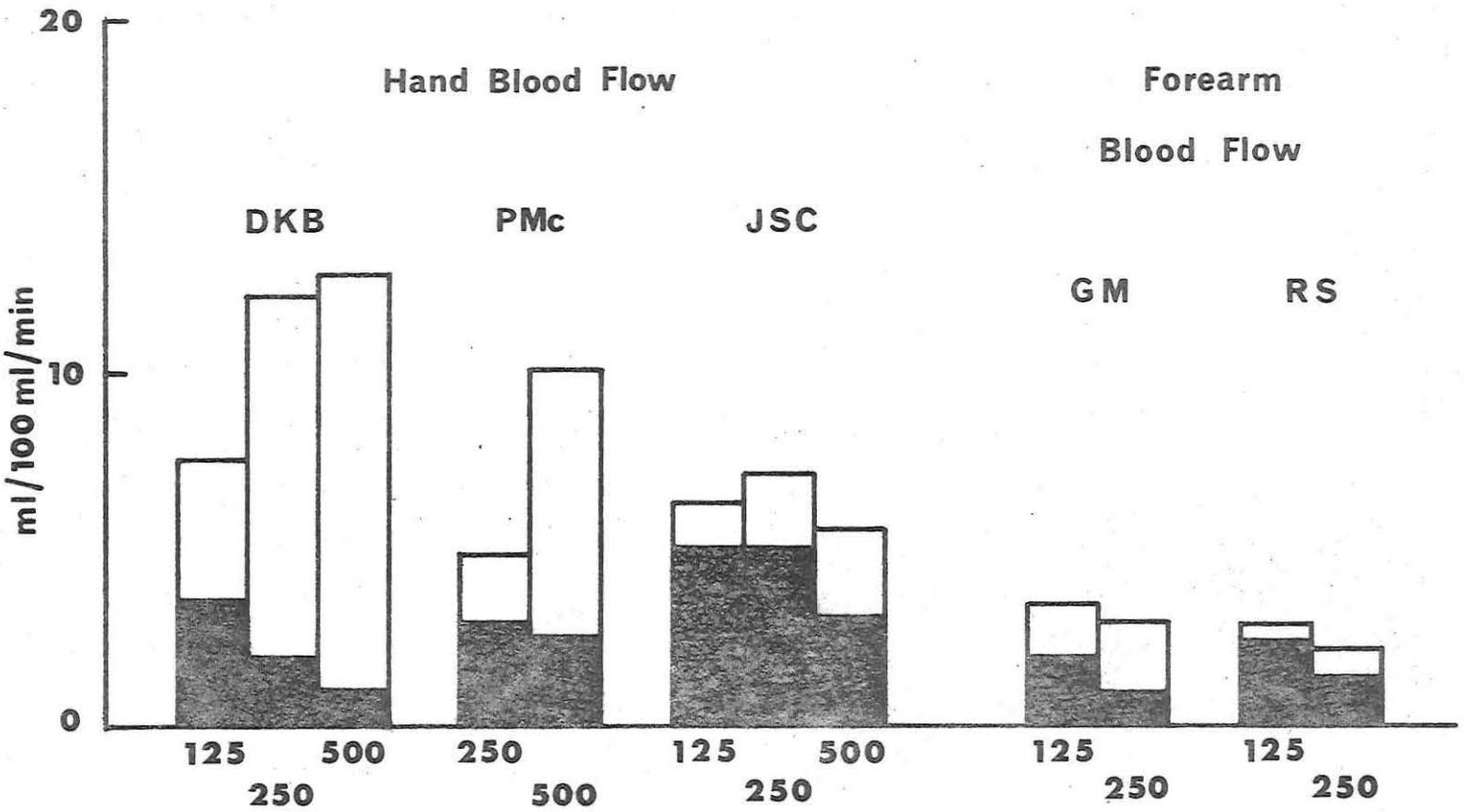


Fig. 2.4 Effect of I.A. clonidine in two or three doses (125, 250 and 500 ng/min) on hand blood flow (3 subjects) and forearm blood flow (2 subjects). Full bar, corrected control flow; shaded bar, flow during clonidine (final 2 min).

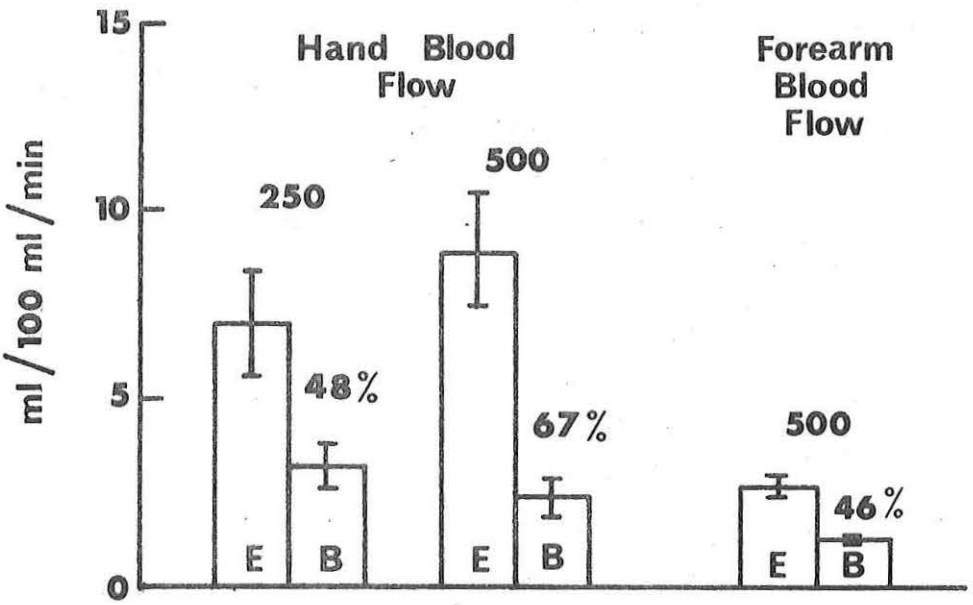


Fig. 2.5 The effect of I.A. clonidine (250 and 500 ng/min) on hand blood flow (6 subjects) and forearm blood flow (6 subjects). Bar E, mean (± S.E.) of corrected control flows; bar B, mean (± S.E.) of flows during clonidine. Percentages, mean of percentage falls in flow.

of these doses is not significant whether compared as percentage or absolute values.

Adrenergic blockade: The effects of this blockade on the action of clonidine were tested in 11 of the subjects given I.A. infusion. I.A. infusions of phenoxybenzamine (0.5 mg/min) in two subjects and phentolamine (50 or 75 mcg/min) in nine subjects blocked the alpha receptors in the test limb only. The effectiveness of blockade was confirmed by the absence of a constrictor effect of I.A. noradrenaline (40 to 80 ng/min for hands and 200 to 400 ng/min for forearms) in ten of the eleven subjects. During blockade H.B.F. was increased approximately 1.6 times and F.B.F. increased approximately 2.6 times. The constrictor response of hand blood vessels was abolished in all but one subject; often there was a slight dilator response. Despite the latter response, the mean flows during clonidine were not significantly different from the pre-clonidine flows (Fig. 2.6, Table A.2). In the case of forearms, clonidine still caused either a slight constriction (2 subjects) or slight dilatation (one subject) after alpha blockade.

Site of vasoconstriction: The effect of I.A. clonidine on the skin and muscle circulations of the forearm was investigated in three subjects. In all, the O_2 saturation of samples from the superficial (skin) vein fell markedly during the infusion of clonidine 500 ng/min, but there was no consistent change in the O_2

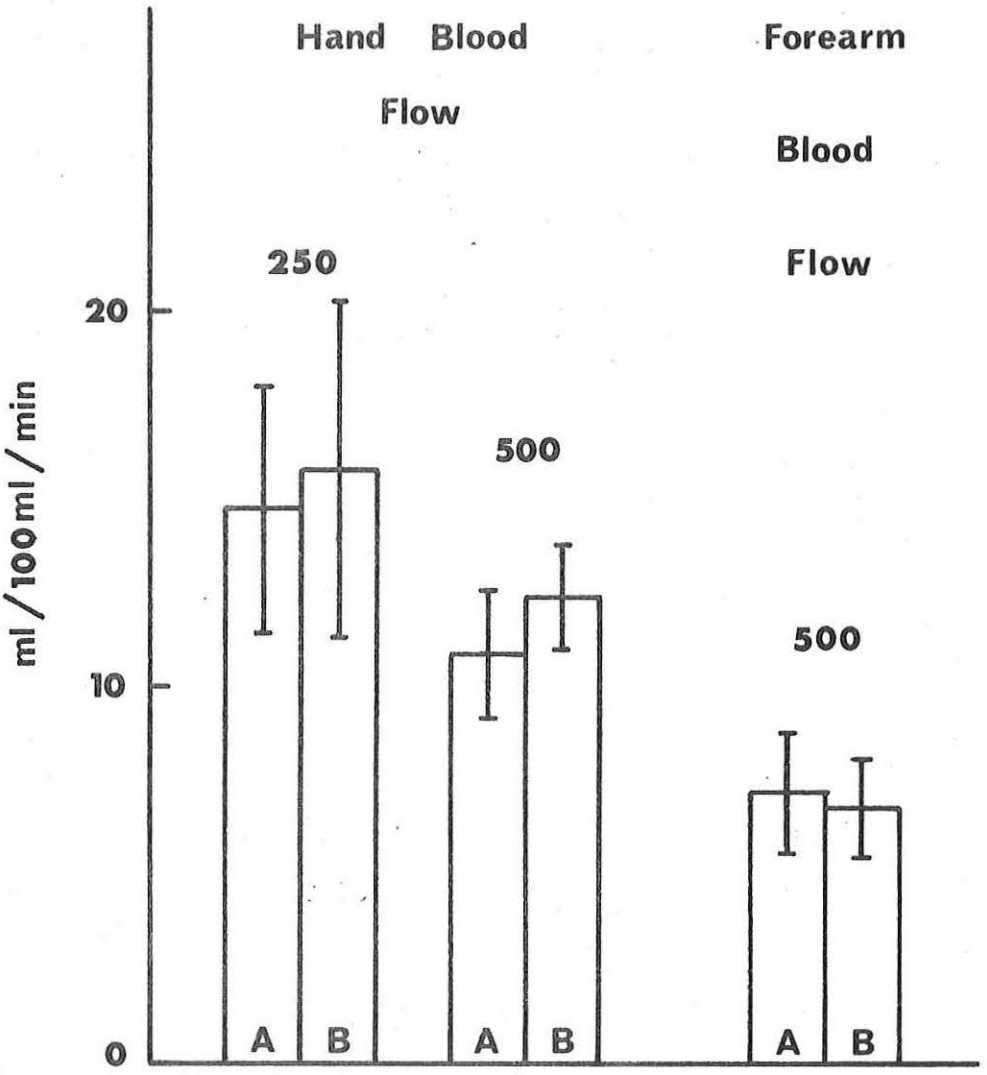


Fig. 2.6 The effect of I.A. clonidine (250 and 500 ng/min) on hand blood flow (3 subjects) and forearm blood flow (3 subjects) during adrenergic alpha receptor blockade. Bar A, mean (± S.E.) of uncorrected pre-clonidine flows; bar B, mean (± S.E.) of flows during clonidine.

saturation of samples from the deep (muscle) vein. Within ten minutes of the end of the infusion the superficial vein O_2 saturation had returned to resting values. The absence of a change in the deep venous O_2 saturation was observed also in a fourth subject who had deep vein sampling only. These changes are illustrated in Fig. 2.7 and are summarised in Table 2.1.

Responses to ice and mental arithmetic: The effect of clonidine (125 and 250 ng/min) on H.B.F. response to a 30 second ice application to the neck was recorded in one subject and the results are shown in Fig. 2.8. There was no detectable impairment of the vasoconstrictor response during the two infusions of clonidine which had reduced flow by 23 and 63%.

In another subject the vasodilator effect of mental arithmetic on F.B.F. was tested during 17% and 34% reductions in flow caused by clonidine (125 and 250 ng/min) and the response was unaffected (Fig. 2.9).

Effects of I.V. clonidine

Clonidine (150 mcg) was infused I.V. into four subjects over five or six minutes. There was usually little or no pressor effect. Instead, a fall in systolic, diastolic and mean arterial pressures began during the infusion (e.g. Fig. 2.10), and the maximum fall in M.A.P. of 10 to 16 mm Hg (12-22%) was significant. Calculated H.V.R. initially rose to a variable degree (49 to 240%), reaching a maximum

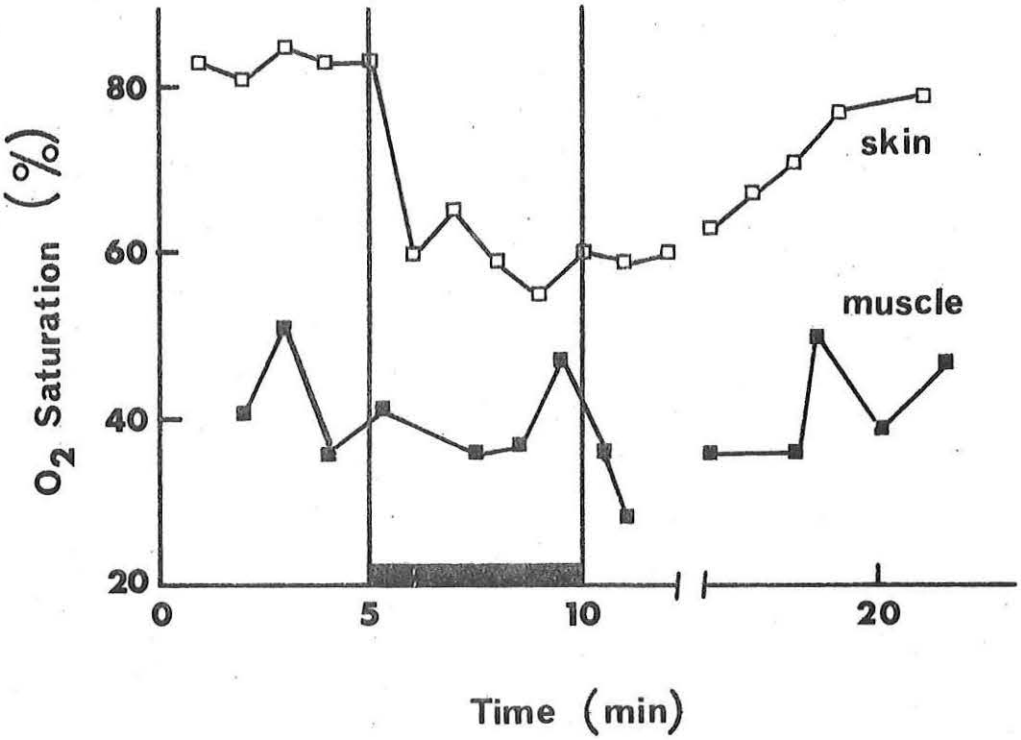


Fig. 2.7 Effect of I.A. clonidine, 500 ng/min (between vertical lines), on the oxygen saturation of separate blood samples aspirated from skin and muscle veins in the forearm of the same side in one subject (M.R.).

Subject	Superficial Vein			Deep Vein		
	Run-in	Final	Difference	Run-in	Final	Difference
M.R.	84	57	-27	44	42	-2
J.A.D.	80	60	-20	40	45	+5
P.G.	71	58	-13	60	60	0
J.C.	-	-	-	36	37	+1
Mean	78.3	58.3	-20	45	46	1
± S.E.	3.8	0.9	4.0	5.3	5.0	1.5
p			<0.05			>0.5

Table 2.1 Effect of I.A. clonidine (500 ng/min) on the oxygen saturation (%) of blood samples from a superficial and from a deep vein in the forearm. Run-in, average oxygen saturation during the two minutes before clonidine; final, average oxygen saturation during last two minutes of clonidine. (Student's paired t-test.)

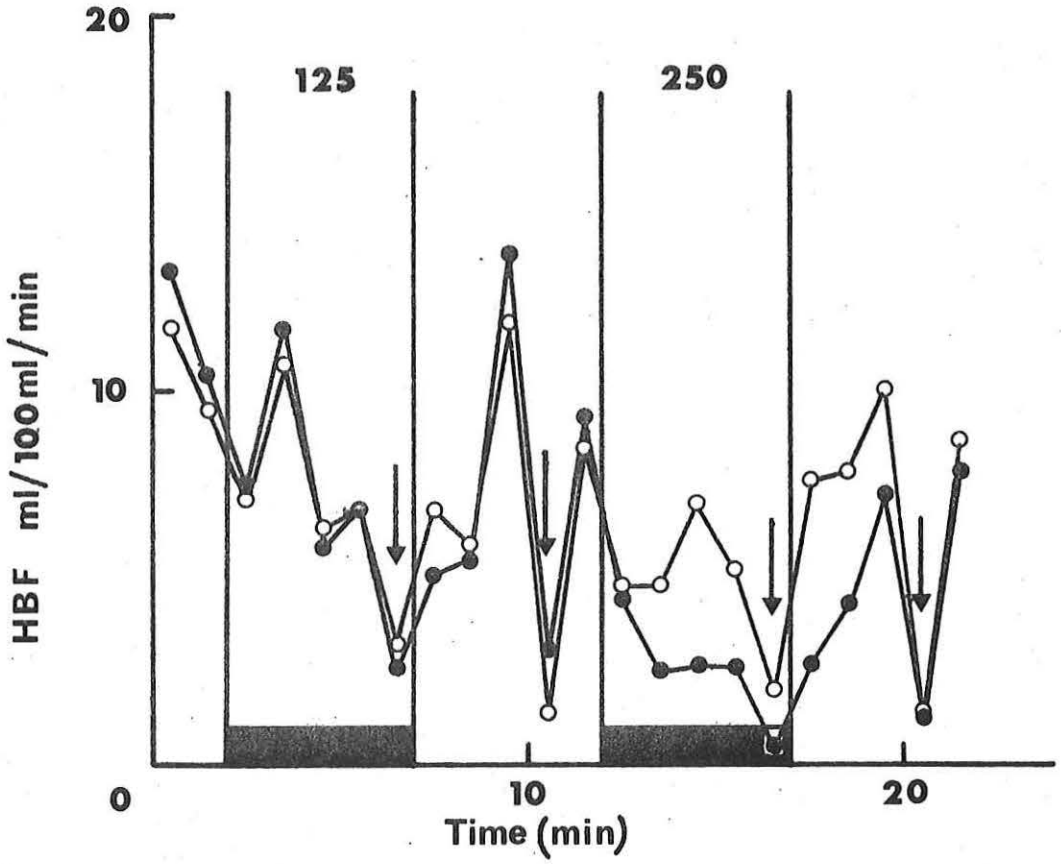


Fig. 2.8 The effect of I.A. clonidine (125 and 250 ng/min) on the response of hand blood flow to the application of ice to the neck (arrows) in one subject (P.L.). o uninjected control side; ● injected side.

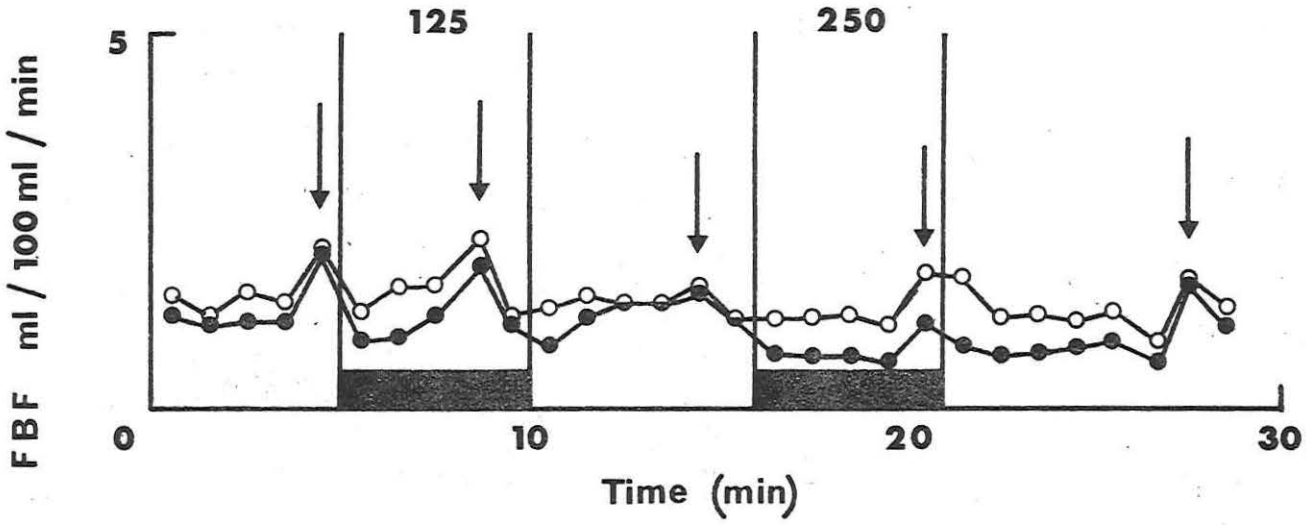


Fig. 2.9 The effect of I.A. clonidine (125 and 250 ng/min) on the response of forearm blood flow to the performance of mental arithmetic (arrows) in one subject (R.S.).

o control uninjected side; ● injected side.

within 2½ to 8 minutes. Thereafter resistance fell, and by 25 to 41 minutes (when hypotension was at or near maximum) was below control values in two subjects but above them in the other two. All subjects had a fall in H.R., beginning during the infusion, which was statistically significant at the time of the maximum fall in M.A.P. The results are presented in Table 2.2 and Fig. 2.11.

Noradrenaline sensitivity: The vascular sensitivity of the hand to two infusions of I.A. noradrenaline (50-200 ng/min) was tested in three of the four subjects before and after I.V. clonidine. The post-clonidine noradrenaline was given at a time when the blood pressure and H.R. changes indicated that clonidine was still acting (53 to 73 min), and resulted in an approximate 1.5 times greater constrictor response during five of the six infusions (Fig. 2.12).

All four subjects received two five minute I.V. infusions of noradrenaline, at two dose levels between 2.5 and 10 mcg/min, before and 25 to 44 minutes after the clonidine infusion. The rise in M.A.P. and in calculated H.V.R., and the fall in H.R. produced by noradrenaline were greater after clonidine during seven of the eight infusions (Tables 2.3 and A.4, and Fig. 2.13).

Chronic Administration

Management

The two patients in the study attended the Medical Outpatients Department of the Royal Adelaide Hospital for management of their

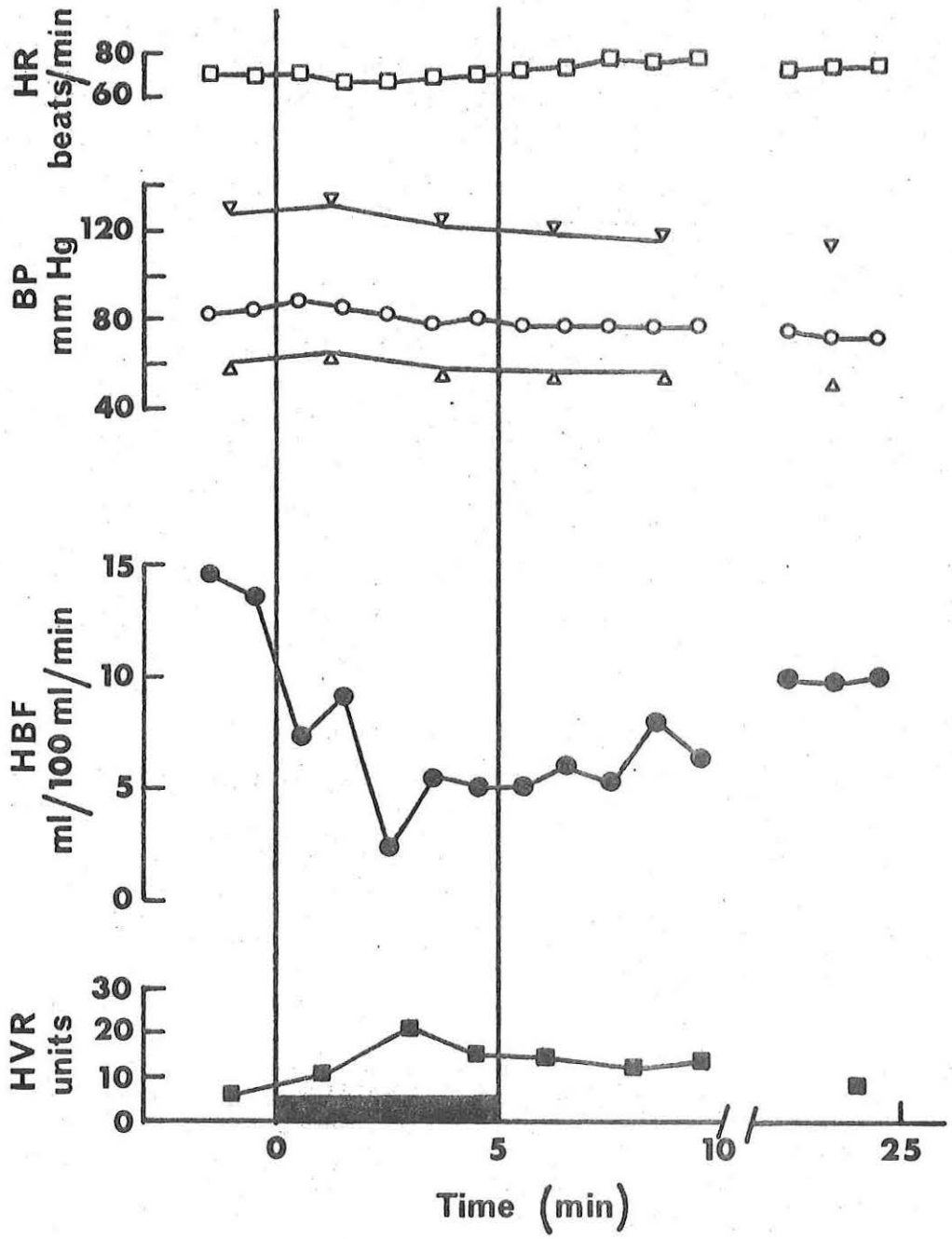


Fig. 2.10 The effect of I.V. clonidine, 30 mcg/min (between vertical lines), on the heart rate, blood pressure, hand blood flow and calculated hand vascular resistance in one subject (G.G.). ▽ systolic blood pressure; △ diastolic blood pressure; ○ mean blood pressure.

Subject	Mean Arterial Pressure (mm Hg)	Fall in M.A.P. Absolute	%	Heart Rate (beats/min)	Fall in H.R. Absolute	%	Time after Infusion min
S.M.R.	B. 78 A. 63	15	19	B. 69 A. 63	6	8.7	40
J.A.D.	B. 82 A. 72	10	12	B. 74 A. 72	2	2.7	41
N.C.	B. 78 A. 62	16	21	B. 66 A. 61	5	7.6	11
G.G.	B. 83 A. 72	11	13	B. 70 A. 68	2	2.9	36
Mean ± S.E.		13 1.5	16		3.75 1.0	5.5	
p		<0.01			<0.05		

Table 2.2 Effect of I.V. clonidine, 150 mcg, on mean arterial pressure (M.A.P.) and heart rate in 4 subjects. The changes were measured when the fall of M.A.P. was maximum; the time after the infusion when this occurred is shown. B, before clonidine; A, after clonidine. (Student's paired t-test.)

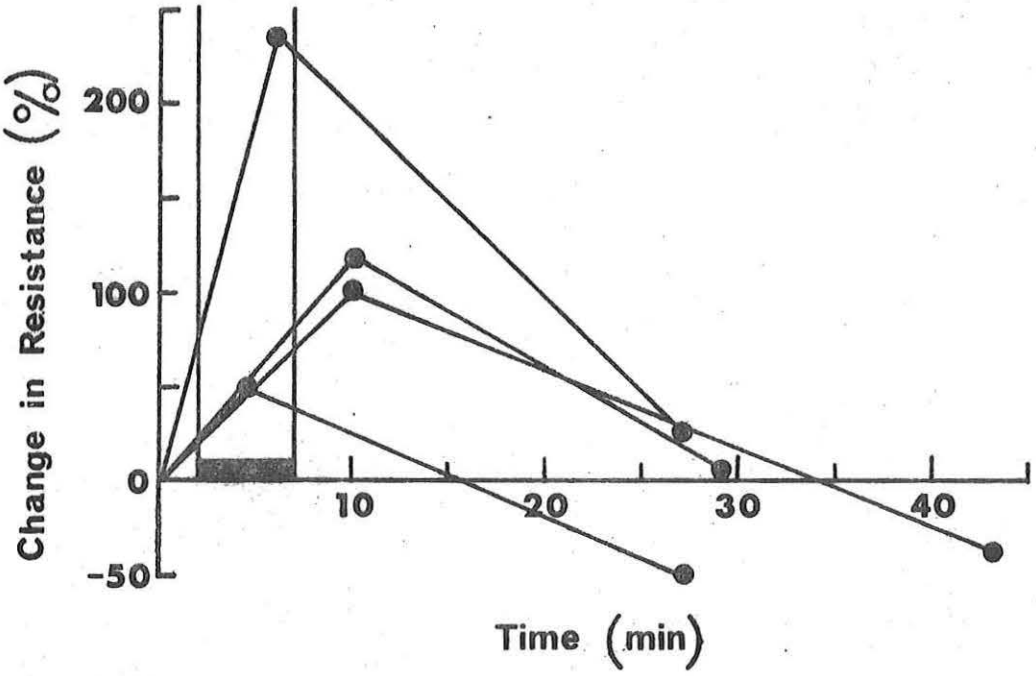


Fig. 2.11 Changes in calculated hand vascular resistance of 4 subjects, expressed as a percentage change from the control value following I.V. clonidine 150 mcg (between vertical lines).

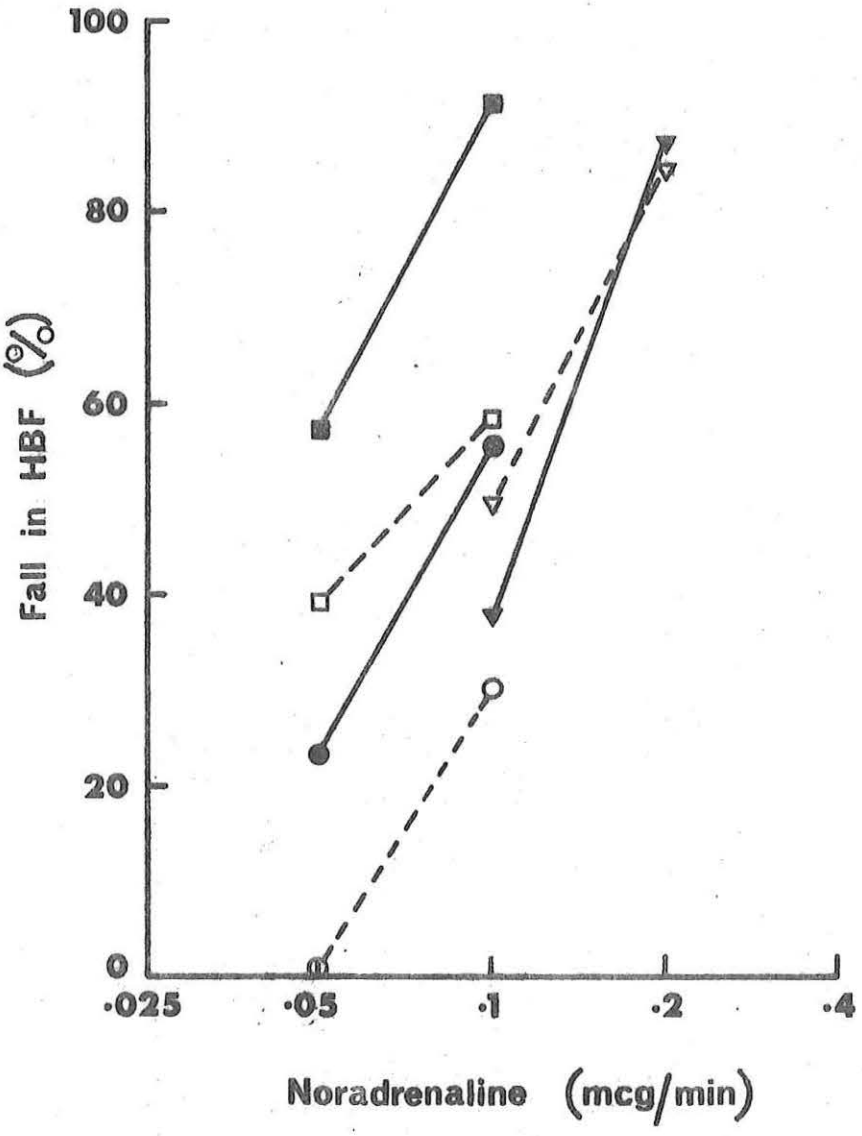


Fig. 2.12 Effect of 2 doses of I.A. noradrenaline on the hand blood flow in three subjects before (open symbols, interrupted lines) and after (closed symbols, solid lines) I.V. clonidine 150 mcg.

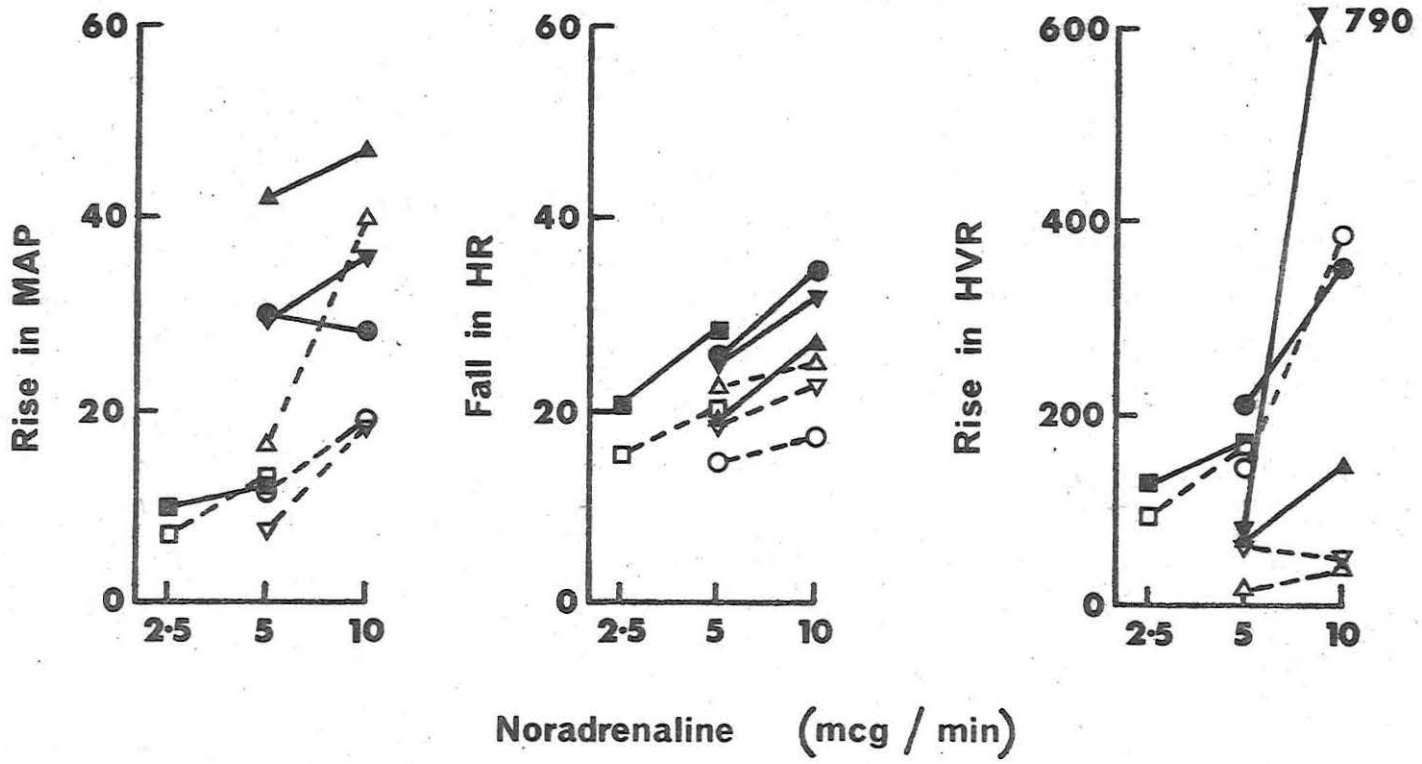


Fig. 2.13 Effect of 2 doses of I.V. noradrenaline on mean arterial pressure, heart rate and calculated hand vascular resistance in four subjects before (open symbols, interrupted lines) and after (closed symbols, solid lines) I.V. clonidine 150 mcg.

Subject	Noradrenaline (mcg/min)	M.A.P.	H.R.	H.V.R.
S.M.R.	5	2.6	1.7	1.4
	10	1.5	2	0.9
J.A.D.	2.5	1.4	1.3	1.2
	5.0	0.9	1.3	1.02
N.C.	5	2.5	0.9	3.6
	10	1.2	1.1	3.8
G.G.	5	3.8	1.3	1.3
	10	1.9	1.4	1.4

Table 2.3 Effect of I.V. clonidine (150 mcg) on the responses of mean arterial pressure (M.A.P.), heart rate (H.R.) and calculated hand vascular resistance (H.V.R.) to two doses of I.V. noradrenaline in four subjects. Results are expressed as the degree of potentiation (treatment response ÷ control response).

hypertension, and during the period of clonidine therapy were under the care of Dr R. L. Hodge.

K.D. (born 15.9.12) essential hypertension

1961 - August, hypertension first recorded, 210/130 mm Hg.

1969 - October 14, selected for treatment with clonidine because therapy with other drugs unsatisfactory. All drugs but valium stopped.

- November 4, cardiovascular sensitivity to noradrenaline I.A. (0.1, 0.2 and 0.4 mcg/min for four min) and I.V. (2.5, 5 and 10 mcg/min for five min) tested. Supine direct arterial blood pressure, 214/102 mm Hg. Started on clonidine 75 mcg t.d.s. and later hydrochlorthiazide added 25 mg b.d.

1970 - January to March, clonidine intake interrupted while patient in another city.

- April 4, clonidine restarted, 75 mcg q.i.d. plus chlorothiazide 0.5 gm b.d.
- May 12, clonidine increased to 150 mcg t.d.s.
- May 14, noradrenaline sensitivity tested again, i.e. after about five weeks of continuous clonidine intake. Supine direct arterial pressure 166/82 mm Hg.

D.F.A. (born 29.10.29) essential hypertension

1964 - September, first seen in medical outpatients, blood pressure 220/130 mm Hg seated and 180/126 mm Hg standing despite

treatment with perolysen 3 mg t.d.s., hydrochlorthiazide 25 mg b.d., reserpine 0.25 mg mane and neostigmine 30 mg b.d.

1970 - January, treatment with guanethidine, methyldopa and chlorothiazide which were controlling blood pressure at 170/100 mm Hg seated and 130/95 mm Hg standing.

- March 31, selected for treatment with clonidine because of troublesome side effects with the current therapy. All drugs stopped.
- April 14, cardiovascular sensitivity to noradrenaline I.A. (0.025, 0.05 and 0.1 mcg/min for four min) and I.V. (1.25, 2.5 and 5 mcg/min for four min) tested. Supine direct arterial pressure 236/137 mm Hg. Starting dose of clonidine 75 mcg t.d.s.
- June 30, clonidine dose now 300 mcg t.d.s. and cyclopenthiazide 0.5 mg b.d.
- July 31, dose of clonidine 375 mcg t.d.s.; treatment had been continuous for approximately 15½ weeks; noradrenaline sensitivities retested. Direct supine blood pressure 205/101 mm Hg.
- August and September, complaining of abnormal pallor and coldness of his hands in the winter weather.

1971 - January and February, clonidine dose 750 mcg t.d.s., but control still inadequate. Guanethidine 10 mg mane added and

blood pressure then 155/105 mm Hg seated and 130/95 mm Hg standing.

- September, complained that circulation in fingers troublesome again (winter); clonidine stopped abruptly and guanethidine dose increased; one week later, aches and pains in leg muscles and a slight headache, sleeplessness and anorexia; home measurement of blood pressure showed a rise to 180/120 mm Hg. These symptoms were dramatically relieved by taking a few tablets of clonidine (patient's own initiative). Also volunteered that hair loss during clonidine had increased - diminished again when clonidine stopped. Blood pressure control later re-established with bethanidine, guanethidine and propranolol, 190/110 mm Hg supine and 140/95 mm Hg standing.

Table 2.4 gives the mean resting values for the circulatory variables which were measured or calculated on the two occasions of testing the cardiovascular sensitivity to noradrenaline. It shows that both patients had significant reductions in H.R., M.A.P. and H.B.F., but the calculated H.V.R. was only significantly increased in D.F.A.

Noradrenaline sensitivity

I.A. Noradrenaline (Fig. 2.14): In patient K.D. the three doses of noradrenaline caused greater percentage reductions in H.B.F. during clonidine than during the control period. The potentiation

Subject	State	H.R. (beats/min)	M.A.P. (mm Hg)	H.B.F. (ml/100ml/ min)	H.V.R. (units)
K.D.	Control (Mean)	96	140	20.2	7.2
	Treated (Mean)	69	113	12.1	9.2
	p	<0.001	<0.001	<0.05	>0.05
D.F.A.	Control (Mean)	112	164	15.7	10.7
	Treated (Mean)	54	138	6.5	21.3
	p	<0.001	<0.01	<0.02	<0.01

Table 2.4 Effect of chronic clonidine treatment on heart rate (H.R.), mean arterial pressure (M.A.P.), hand blood flow (H.B.F.) and calculated hand vascular resistance (H.V.R.) in two patients. The values (average over five minutes) were measured at the times of noradrenaline sensitivity testing. (Student's paired t-test.)

of the percentage fall in flow varied from 1.04 times for 0.4 mcg/min to 2.2 times for the 0.1 mcg/min infusion.

In patient D.F.A. the three control doses of noradrenaline (0.025, 0.05 and 0.1 mcg/min) caused reductions in H.B.F. of 61, 69 and 67% in order of increasing concentration. During retesting only 0.025 and 0.1 mcg/min doses were infused. With the larger dose a potentiation of approximately 1.1 times was recorded, but the response to the smaller dose was less than that elicited prior to clonidine.

I.V. Noradrenaline (Tables 2.5 and A.5, and Fig. 2.15): During clonidine therapy the pressor effects of 2.5 and 5 mcg/min noradrenaline infusions in K.D. were potentiated 2.6 and 1.9 times, respectively. However, the reflex bradycardia was less during each infusion. The increase in calculated H.V.R. caused by 2.5 mcg/min noradrenaline infusion was potentiated during clonidine treatment, but that to 5 mcg/min was depressed by clonidine.

All of the circulatory effects of 1.25 and 2.5 mcg/min noradrenaline (I.V.) given to D.F.A. were potentiated during clonidine therapy, the augmentation of the pressor effect and the increase in calculated hand vascular resistance to the smaller dose being 3.3 and 4.3 times, respectively.

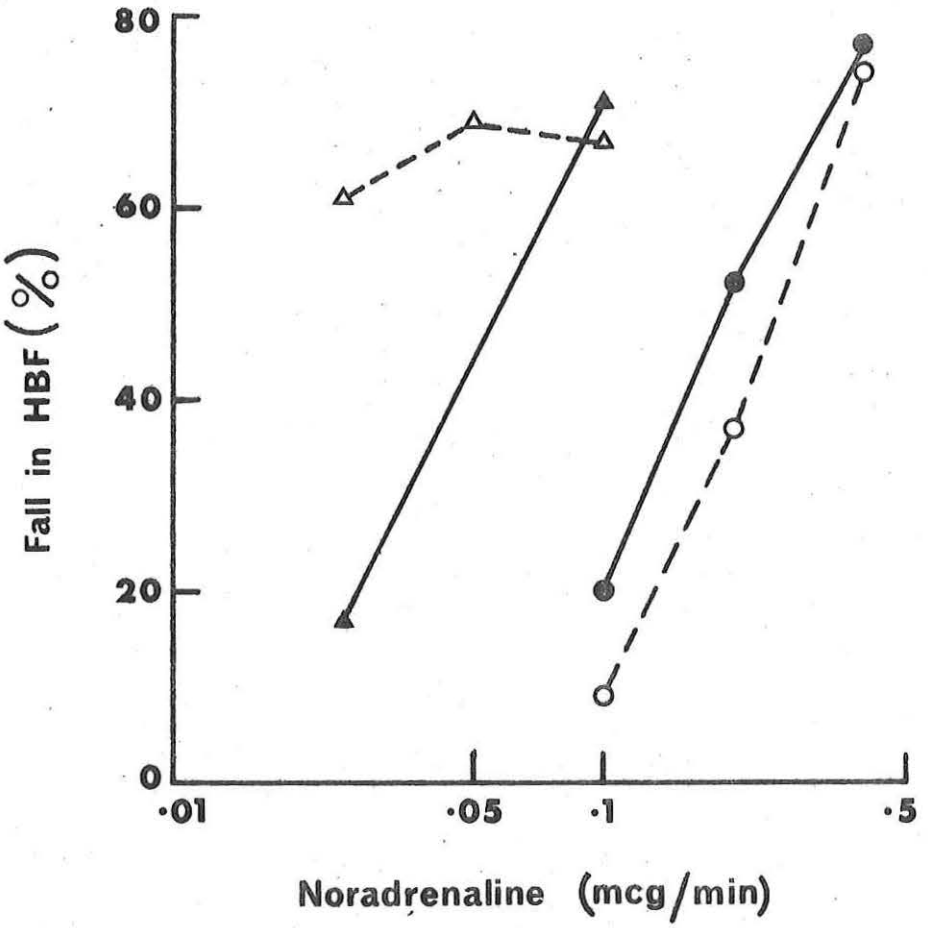


Fig. 2.14 Effect of 2 or 3 doses of I.A. noradrenaline on the hand blood flow in two patients before (open symbols, interrupted lines) and during (closed symbols, solid lines) chronic clonidine treatment.

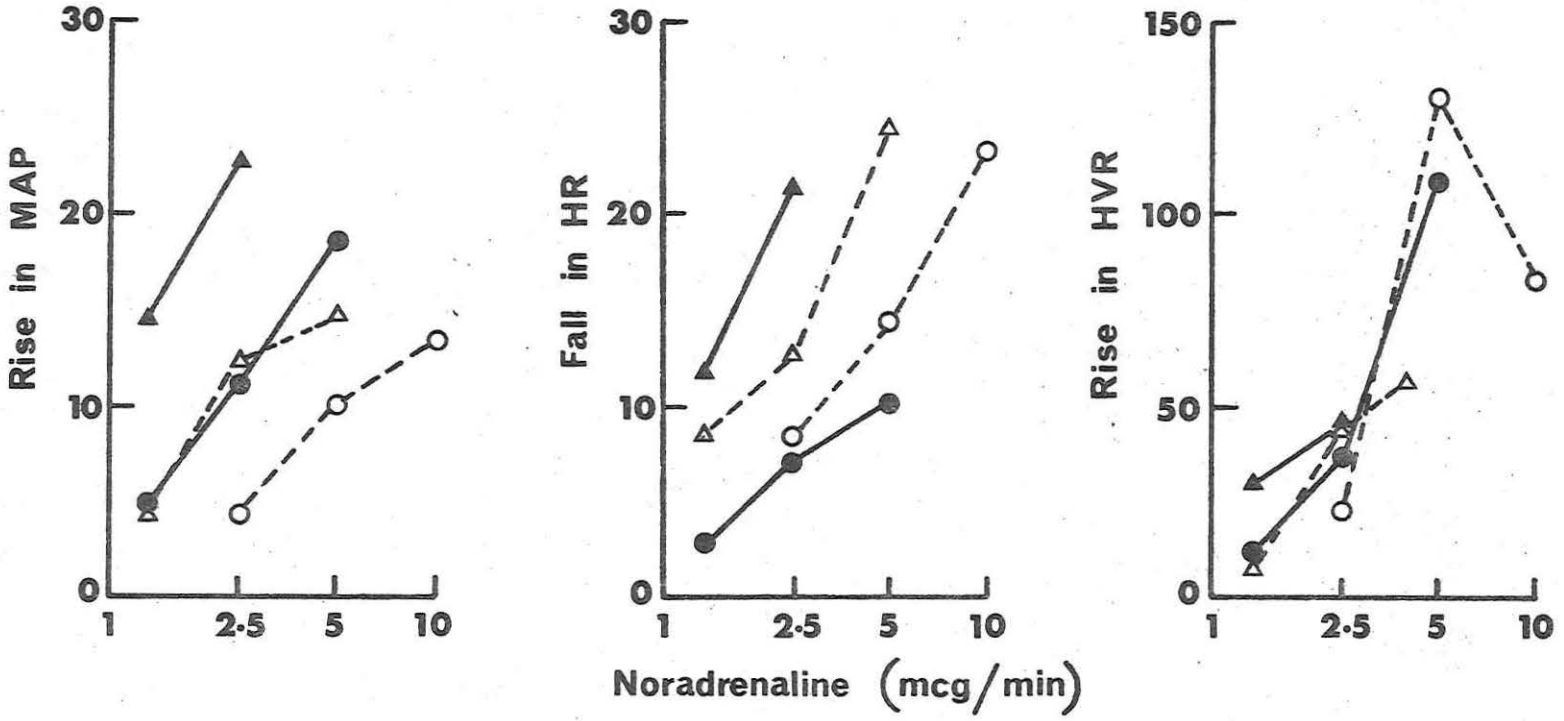


Fig. 2.15 Effect of 2 or 3 doses of I.V. noradrenaline on mean arterial pressure, heart rate, and calculated hand vascular resistance in two patients before (open symbols, interrupted lines) and during (closed symbols, solid lines) chronic clonidine treatment.

Subject	Noradrenaline (mcg/min)	M.A.P.	H.R.	H.V.R.
K.D.	2.5	2.6	0.8	1.6
	5.0	1.9	0.7	0.8
D.F.A.	1.25	3.3	1.4	4.3
	2.5	1.8	1.7	1.02

Table 2.5 Effect of chronic clonidine treatment on the responses of mean arterial pressure (M.A.P.), heart rate (H.R.) and calculated hand vascular resistance (H.V.R.) to two doses of intravenous noradrenaline in two patients. Results are expressed as the degree of potentiation (treatment response ÷ control response).

DISCUSSION

Vasoconstriction with Acute Clonidine

I.A. infusions of clonidine were used by Ehringer (1966) to examine its local vascular effects. He found that it caused a dose dependent constriction in the foot and the calf which was more marked in the former. Vasoconstriction also occurred in one subject after lumbar sympathectomy and persisted for more than 10 minutes. However, the role of the adrenergic alpha receptors was not investigated, nor were the effects on skin and muscle circulation differentiated. The vasoconstrictor effect in the hand and forearm observed in the present studies was also dose related and was completely abolished by adrenergic alpha receptor blockade. Thus, in man, as in animals, the local effect of clonidine is alpha receptor stimulation. Since a prolonged vasoconstriction also occurred in denervated vessels (Ehringer, 1966), there is circumstantial evidence that the receptor stimulation is a direct one. From the fact that vasoconstriction was more prominent in the foot (largely skin) than in the calf (largely muscle) Ehringer concluded that clonidine has different effects in cutaneous and skeletal muscle vascular beds. The conclusion has now been verified by measurement of superficial and deep venous oxygen saturation during I.A. clonidine which showed that the vasoconstriction was limited to the skin, and blood flow in muscle did not change. This local effect

of clonidine is undoubtedly the basis for the initial cutaneous vasoconstriction noted during I.V. administration in the present and other human studies (Bock *et al.*, 1966; Ehringer, 1966; Merguet *et al.*, 1968).

Any conclusions about the other effects of I.A. clonidine observed in the present study must be more tentative as they are based on single experiments only. However, the persistence of hand vasoconstriction during a thirty minute infusion may indicate that acute tachyphylaxis to the drug does not occur. The relevance of this is that tachyphylaxis would thereby be eliminated as a possible contributor to the decline in calculated H.V.R. seen with I.V. clonidine. Furthermore, in this experiment the flow fluctuated in phase with that on the control side, though the magnitude was reduced, which suggests that the vessels were still at least partially responsive to sympathetic and other influences. In addition, the maintenance of the ice response, which is reduced or abolished by guanethidine, bretylium and bethanidine (Cooper *et al.*, 1963; Fewings, Hodge, Scroop & Whelan, 1964), is further evidence that acute clonidine does not impair adrenergic transmission. It is probable that sympathetic cholinergic transmission is likewise unimpaired since the response of F.B.F. to mental arithmetic also persisted during I.A. clonidine.

Effects of I.V. Clonidine

Most of the previous reports of the effect of doses of clonidine similar to the I.V. dose given in this study have noted an initial rise in arterial pressure of 8-40 mm Hg occurring within 0.5 to 1 minute of the injection and lasting 1-2 minutes (Bock *et al.*, 1966; Barnett & Cantor, 1968; Merguet *et al.*, 1968; Muir *et al.*, 1969). However, Ehringer (1966) infused 150 mcg I.V. over five minutes and noted only a slight rise in diastolic pressure. The present findings confirmed that a slow rate of I.V. infusion reduces the pressor response, and indicate that in hypertensive emergencies a detrimental rise in pressure may be avoided by giving the drug slowly over five to ten minutes yet not delay its hypotensive effect. Although at a rate of 25 to 30 mcg/min there was little or no pressor response, in each subject the calculated H.V.R. rose and attained a peak during or shortly after the infusion. Two factors probably explain the lack of rise in pressure: firstly, with this rate of infusion the drug would be diluted to a greater extent than the initial dilution of a bolus injection and therefore would elicit a smaller degree of vasoconstriction; and, secondly, the drug would start to exert its hypotensive effect before the infusion was completed and thus offset the pressor effect of the increased cutaneous vascular resistance. The initial sympathetic tone in blood vessels may also be a determinant of the vascular effect. The two subjects who had the

highest resting resistance (S.M.R. and J.A.D.) not only had smaller initial percentage increases in H.V.R. (i.e. the local effect), but the resistance also later declined to a value less than control (Fig. 2.11 and Table A.3). In contrast, the other two subjects with lower initial resistance had a more marked percentage rise in resistance which did not subsequently fall below resting values. Barnett & Cantor (1968) made a similar observation, that in subjects who had low initial digital blood flow, clonidine (I.V.) caused a gradual flow increase, whereas subjects with high resting flow showed no change after the drug.

The degree of hypotension caused by clonidine in the normotensive subjects in the present study is similar to that reported in other series (Ehringer, 1966; Barnett & Cantor, 1968; Muir *et al.*, 1969). While the falls in H.R. in the four subjects were relatively small in absolute terms, they were statistically significant, and the degree of bradycardia may again reflect the importance of the resting autonomic tone in determining the degree of change caused by clonidine. For instance, the resting heart rates fell from an average of 70 to about 66 beats/min, whereas Ehringer (1966) shows an example of a subject whose pulse rate fell from 85 to 78 beats/min.

Noradrenaline sensitivity

The potentiation of the pressor response (1.2 to 3.8 times) to seven of the eight infusions of I.V. noradrenaline is an indication

of an overall increase in cardiovascular reactivity which has also been noted in various animal studies in which less than 5-10 mcg/kg clonidine has been given (Kundig *et al.*, 1967; Rand & Wilson, 1968; Bentley & Li, 1968; Boissier, Giudicelli, Fichelle, Schmitt & Schmitt, 1968). In contrast to the present findings, Merguet *et al.* (1968) found that the pressor effects of noradrenaline (5-14 mcg) were not significantly altered by clonidine (150 mcg I.V.). However, the method of giving the noradrenaline in the two studies differed in that Merguet *et al.* (1968) gave a bolus injection and not an infusion over several minutes. An injection does not permit a stable response to develop and, furthermore, the total dose of noradrenaline used by these workers was usually smaller.

The potentiation of both the rise in H.V.R. with I.V. noradrenaline and the vasoconstrictor effect of I.A. noradrenaline by clonidine indicates an increase in vascular sensitivity to the catecholamine which must be playing at least some part in the increased pressor responsiveness to I.V. noradrenaline. The results also indicate that the reported decrease in vascular responsiveness to vasoconstrictors and vasodilators after several weeks of clonidine administration to cats (Zaimis & Hanington, 1969) cannot explain the hypotensive effect of clonidine in man after acute administration.

An interpretation of the potentiation of the reflex bradycardia

produced by I.V. noradrenaline may be aided by referring to Fig. 2.16, where the degree of bradycardia is plotted in relation to the magnitude of the pressor response before and after clonidine. The slope of this dose response line is steeper in every case after clonidine, i.e. the same increment of arterial pressure produced by I.V. noradrenaline resulted in a greater reflex bradycardia after clonidine than during the control period. This indicates a facilitation of the autonomic reflex control of cardiac slowing, in the absence of which the pressor response would probably have been greater.

Effects of Chronic Clonidine

Although the arterial pressure of D.F.A. at the time of retesting remained inadequately controlled, both patients had a significant reduction in pressure during clonidine treatment. The fall in H.R. recorded in both hypertensives was more marked than in the normotensives and persisted throughout the treatment period. In the case of D.F.A., the H.R. was halved, and he also had a similar reduction in hand blood flow with a significant rise in calculated H.V.R. The reduction in blood flow was probably the basis of his complaint that the circulation in his hands was more sensitive to the cold than previously, an observation which is consistent with the reports of circulatory insufficiency noted by other workers (Iisalo & Laurila, 1967; Ebringer, Doyle, Dawborn, Johnstone & Mashford,

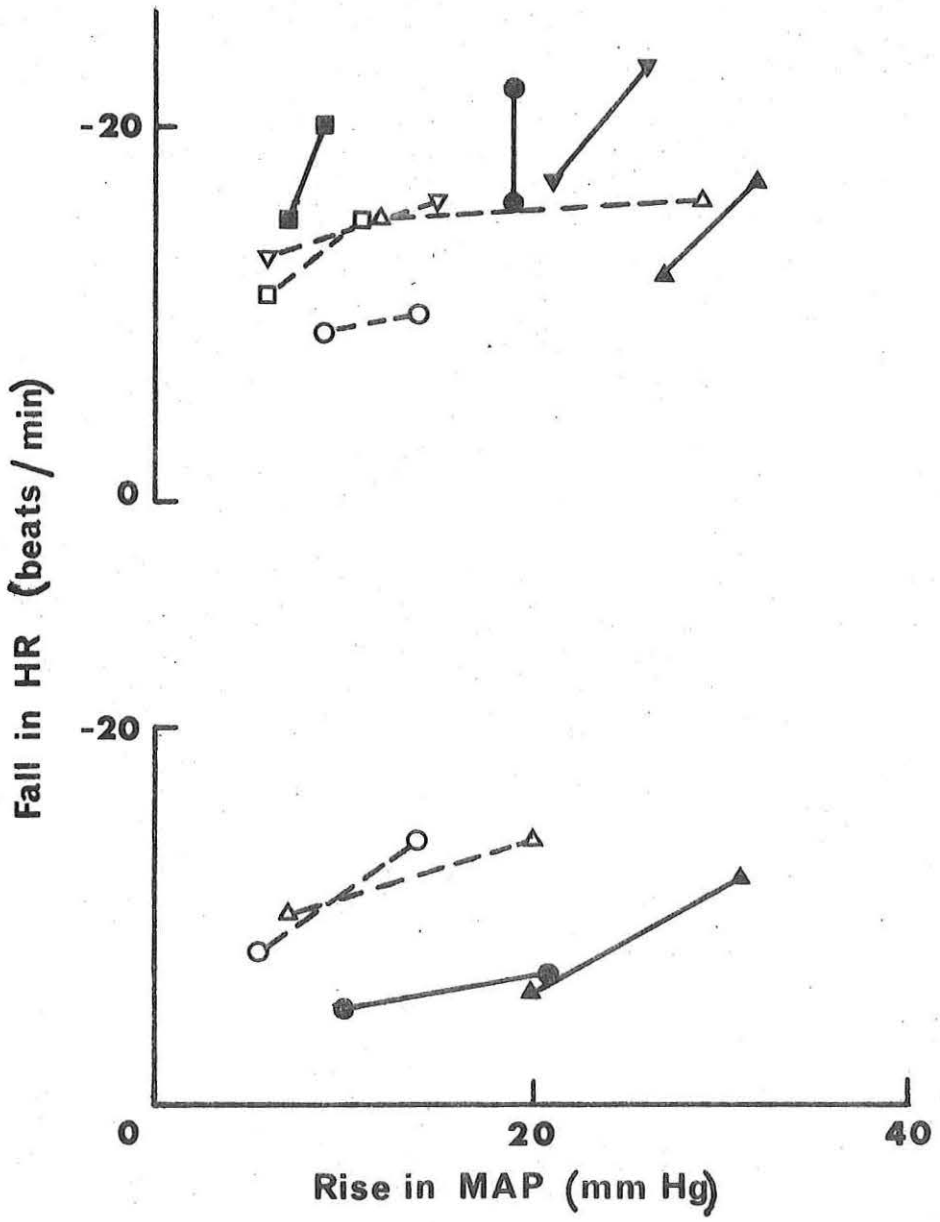


Fig. 2.16 Effect of I.V. clonidine (upper frame) and chronic clonidine (lower frame) on the relationship between the mean arterial pressure response and reflex fall in heart rate to 2 doses of I.V. noradrenaline. Open symbols, before clonidine; closed symbols, after clonidine.

1970; Kellet & Hamilton, 1970; Winchester & Kennedy, 1971). The possible association between treatment with clonidine and the increased loss of scalp hair, which may be a further indication of impaired skin circulation, does not appear to have been reported before. The sympathetic overactivity associated with abrupt withdrawal of clonidine experienced by D.F.A. has been observed by others (e.g. Hökfelt, Hedeland & Dymling, 1970).

Noradrenaline sensitivity

The effect of I.V. noradrenaline on arterial pressure in the hypertensive patients receiving chronic clonidine followed the pattern found in the subjects given I.V. clonidine; that is, there was a potentiation which was greater for the smaller dose of noradrenaline than the larger, and it is interesting that the potentiation with 5 mcg/min was greater in the normotensive than in the hypertensive subjects. The clonidine potentiation of both the increase in the H.V.R. during I.V. noradrenaline and the vasoconstrictor effect of I.A. noradrenaline indicates that, as was the case in the acute studies, part of the pressor potentiation of I.V. noradrenaline results from increased vascular sensitivity. Though the response of D.F.A. to I.A. noradrenaline (0.025 mcg/min) was depressed after clonidine, there was no other evidence of a depressed vascular response to which the hypotensive effect could be attributed. The relationship between the magnitude of the pressor effect of

noradrenaline and the reflex bradycardia in the hypertensives was the reverse of that seen in the normotensives, i.e. clonidine depressed the slope of the dose response line (Fig. 2.16), which may reflect a difference in the dose and duration of clonidine administration or be related to differences in the cardiovascular control mechanisms in hypertensive and normotensive man.

The results with both acute and chronic clonidine administration to man show that a variable degree of increase in sensitivity to noradrenaline does occur. Such an increase in sensitivity has a rapid onset and does not appear to be any greater after 15½ weeks treatment with doses up to 1.125 mg/day than after a single I.V. dose, and thus it is perhaps not likely to cause the tolerance which has been reported in some clinical trials (e.g. Davidov *et al.*, 1967; Ng *et al.*, 1967). However, such an increase in cardiovascular reactivity may be associated with undesirable and dangerous hypertensive episodes if a patient being treated with clonidine is concurrently exposed to other vasoactive drugs, e.g. sympathomimetics. Such an interaction and loss of control of the blood pressure response to treatment has been reported during the use of some other antihypertensives, e.g. the adrenergic neurone blockers.

SUMMARY

Intra-arterial infusion of clonidine (125 to 500 ng/min) into the brachial artery caused a rapid and dose dependent constriction

of the skin vessels of the forearm and the hand which was absent during alpha adrenergic receptor blockade. This sympathomimetic vasoconstriction is the most probable cause of the brief rise in calculated hand vascular resistance which was observed during, and shortly after, intravenous clonidine (150 mcg).

Both intravenous infusion and prolonged oral treatment with clonidine caused significant reductions in mean arterial pressure and the heart rate, the latter being markedly affected in the two hypertensives, and a significant reduction in hand blood flow during the chronic intake. The cardiovascular sensitivity to both local and systemic noradrenaline was increased to about the same degree by both acute and chronic clonidine administration. On the basis of this phenomenon a detrimental interaction with other vasoactive drugs can be predicted.

CHAPTER 3

INTRODUCTION

The local vasoconstrictor action of clonidine has been attributed partly to direct adrenergic alpha receptor stimulation and partly to neuronal noradrenaline release (Kobinger & Walland, 1967; Boissier *et al.*, 1968; Constantine & McShane, 1968; Nayler, Price, Swann, McInnes, Race & Lowe, 1968; Rand & Wilson, 1968). Other evidence suggests that the drug has alpha receptor blocking properties (Ng *et al.*, 1967; Boissier *et al.*, 1968; Bentley & Li, 1968). In addition to these local vascular actions after acute administration, Zaimis & Hanington (1969) suggested that prolonged oral intake of clonidine results in a diminished vascular sensitivity to catecholamines and angiotensin. This is in contrast to the cardiovascular supersensitivity to catecholamines and other vasoactive agents which can be induced by a number of antihypertensive drugs including reserpine, guanethidine, bethanidine, and methyldopa. The specific aims of the studies described in this chapter were:

1. To clarify the roles which adrenergic receptors and sympathetic nerves play in the response to acute clonidine;
2. To determine arterial and venous smooth muscle sensitivity to noradrenaline and other vasoconstrictor agents following chronic clonidine administration;
3. To examine the effects of chronic clonidine on catecholamine stores in vascular sympathetic nerves.

Four isolated animal vascular smooth muscle preparations from two animal species were used in the studies. The perfused segment from the rabbit central ear artery (de la Lande & Rand, 1965) has been well characterised, and the double cannulated modification (de la Lande, Cannell & Waterson, 1966) was used to obtain information about clonidine's mechanism of action on arterial smooth muscle and the role of the sympathetic nerves in this action. The perfused segment from the ventral rat tail artery (Nicholas, 1969) has not been as extensively documented as the rabbit ear artery preparation, but was used to obtain information from another species about the role of the sympathetic nerves in clonidine's local vascular action. Complete dose response relationships from threshold to maximum effects cannot be studied in perfused segments, and therefore a helical strip preparation of the rat tail artery was developed for this purpose. The preparation was then used to obtain further information about clonidine's adrenergic action and its effects on arterial smooth muscle sensitivity to vasoconstrictor agents. The effect of chronic clonidine on venous smooth muscle sensitivity was studied in the unperfused rat portal vein segment (Johansson, Jonsson, Axelsson & Wahlstrom, 1967) which, like the rabbit artery, has been well characterised.

METHODS

The four isolated preparations were obtained from semi-lop-eared

rabbits weighing 1.5 to 2.7 kg and albino Wistar rats weighing 160 to 340 gm. To remove the vessels, the animals were anaesthetized with intraperitoneal (I.P.) injections of either 25% urethane in a dose of 8 ml/kg (rabbits) or pentobarbitone 50 mg/kg (rats).

Pretreatment

Six to fourteen days prior to the experiment four of the rabbits used in this study were anaesthetized with halothane and diethyl ether and the ear artery of the left side sympathetically denervated by removal of the homolateral cervical ganglion. The effectiveness of the denervation was confirmed subsequently by the absence of response of the isolated artery to stimulation by field electrodes (de la Lande & Rand, 1965) or by the absence of specific catecholamine fluorescence in sections of the artery examined by the histochemical method of Falck (1962), as modified and applied to the ear artery by Waterson and Smale (1967). See appendix for details.

Three groups of rats were used for the studies on the effects of chronically administered antihypertensive drugs on vascular sensitivity to vasoactive agents and the vascular catecholamine fluorescence. Within each group pairs of animals were matched for weight and assigned to control or treatment groups. In series 1, treated animals were given drinking water containing clonidine (1.58 mM) which resulted in a drug intake of 37 to 56 mcg/kg/day (mean 47.3 ± 2.8 mcg/kg/day); the treated animals in series 2 were given reserpine in

the form of serpasil by I.P. injection in a dose of 1 mg/kg on the first day and 0.3 mg/kg for six further days; the treated rats in series 3 were given guanethidine 7.5 mg/kg daily by I.P. injection for one week. Control animals of each pair of rats were treated in an identical manner with the exception that they did not receive any active agent. Vascular preparations from pairs of animals were removed and studied simultaneously 6 to 8 weeks after clonidine intake started in series 1 animals and 24 hours after the last injection in the animals of the other two series. Portions of these vessels (the distal end of the artery and the mesenteric end of the vein) were also histochemically prepared to show monoamine fluorescence.

In the rats given clonidine chronically, arterial blood pressure in the tail was measured plethysmographically in the unanaesthetized state before and during drug administration. At least three determinations of blood pressure were made on each occasion. (See appendix.)

Dissection and Mounting of Preparations

Perfused artery segments

The methods of obtaining and perfusing the rabbit ear arteries were as described by de la Lande & Rand (1965) with the modification of double cannulation (de la Lande *et al.*, 1966). Cannulating both ends of the segment prevents mixing of the perfusate and bath solution and enables drugs to be applied to intimal and adventitial surfaces of the vessels separately. Vasoactive drugs in the perfusing fluid must

pass through the smooth muscle of the media before reaching the nerves situated at the medial adventitial border, whereas from the adventitial surface the drugs must first diffuse through these to reach the smooth muscle (de la Lande & Waterson, 1967). The role of the nerves in drug action can therefore be analysed by using this technique.

The vessels were dissected out and cannulated *in situ* and then placed in an organ bath of 15 or 20 ml capacity containing a modified Krebs solution (Appendix) at 37^{o}C through which a gas mixture of 5% carbon dioxide and 95% oxygen was continuously bubbled. The arteries were also perfused with a similar solution which was pumped through the artery at a constant rate of 5-6 ml/min and at a pressure of 3 to 30 mm Hg above zero flow pressure. The proximal (lower) cannula was fixed, and the vertically positioned artery had the distal (upper) cannula suspended under a tension of one gram which prevented the artery from kinking as it lengthened during contraction. A mercury manometer attached to a side arm between the pump and the artery recorded on a smoked drum the changes in arterial resistance as changes in perfusion pressure.

The tail artery of the rat was more difficult to remove and prepare because the neurovascular bundle lies in a ventral groove formed by tendons and covered by a firm connective tissue layer. Furthermore, there were numerous arterio-venous anastomoses between the vessels in the bundle that precluded the isolation of arterial

segments much longer than 1 to 2 cm which were free of side branches. To obtain longer segments it was necessary to remove vein and artery together, tying the former off at each end to prevent leakage from the cannulated preparation. Cannulation and perfusion of the vessels was identical with that described for rabbit ear arteries. A diagrammatic representation of the technique is shown in Figure 3.1.

Rat tail artery strips

A length of tail artery was removed and, by means of tension on a cotton thread tied to each end, it was maintained under slight stretch whilst the strip was cut. Using fine pointed scissors, a small cut was made in the wall of the artery at one end and one blade of the scissors inserted into the lumen. When the scissors were held and operated at an angle of about 45^{o} to the vessel, it slowly rotated producing a spiral cut and finally a helical strip. Throughout these manoeuvres the artery was prevented from drying by intermittently dripping Krebs solution onto it. The strips were not trimmed and averaged about 55 mm length. Strips were mounted either singly in 12 ml organ baths for studies of the acute application of clonidine or, in the case of arteries from the animals in the chronic series, two strips (one each from control and treated rats) were mounted in the same 30 ml organ bath. Each strip was attached at the bottom end to a hook and the upper end was attached to a Harvard isotonic strain gauge by means of cotton thread. The muscle was under a tension of

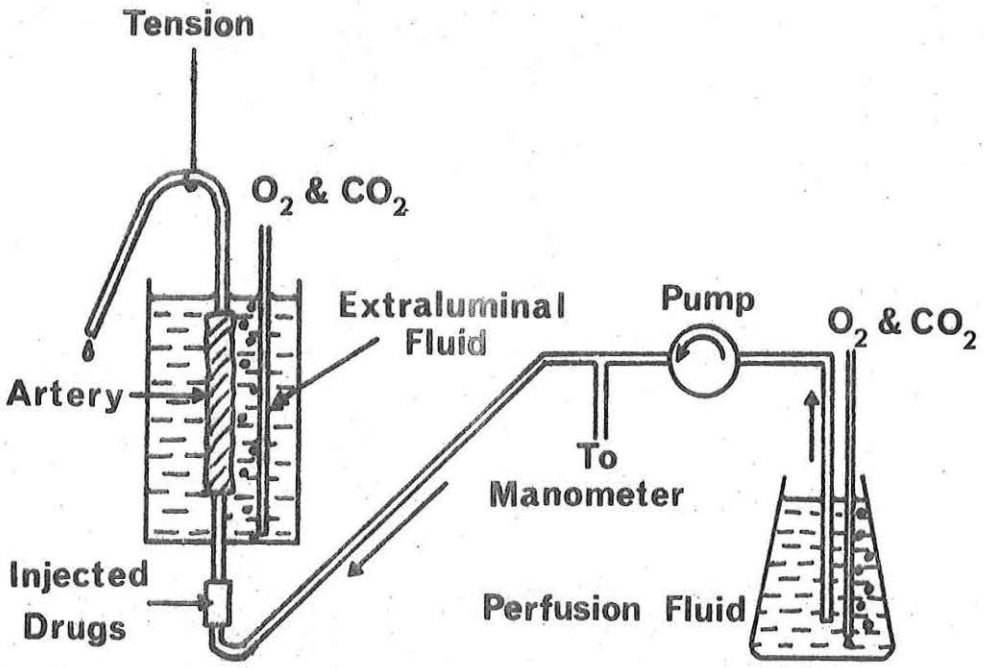


Fig. 3.1 Diagrammatic representation of the apparatus used to perfuse the isolated arteries of the rabbit ear and rat tail. Double cannulation method.

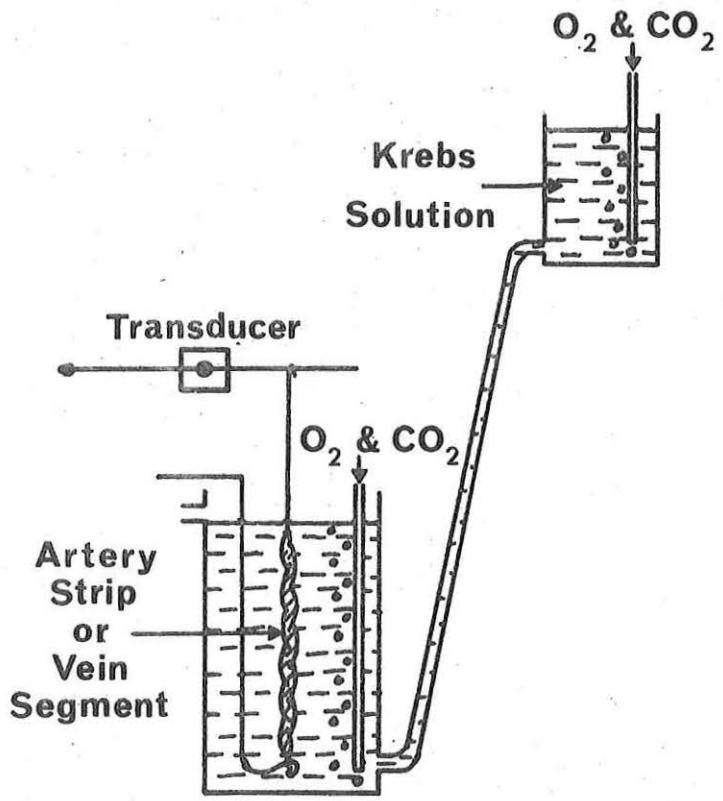


Fig. 3.2 Diagrammatic representation of the apparatus used to measure the contractions of the rat tail artery helical strip and the rat portal vein.

one gram after exploratory experiments had established that this was a suitable compromise between detecting extraneous vibrations and muscle sensitivity.

Rat portal vein segments

In the chronic administration series a portal vein segment was also obtained from the rats. After removing the tail artery and setting up the strip, the abdomen of the animal was opened and, by gentle retraction, the extra-hepatic portion of the portal vein was exposed and dissected free. A length of about 10 mm was tied off, removed, and the intact segment suspended in the same 30 ml organ bath as the vessel from the other rat of the pair, i.e. vein segments from a control and a treated rat were studied simultaneously. The method of mounting the vessel and recording its contractions, and the bathing solution were as described for the artery strips, and, again, one gram tension provided the best recording conditions. The vein exhibits spontaneous regular contractions which are superimposed on a steady baseline, and responses were measured as changes in this baseline value. Figure 3.2 diagrammatically illustrates the technique used for both the helical strips and the vein segments.

Experimental Procedure and Expression of Results

All preparations were allowed to equilibrate in Krebs solution for at least one hour before the addition of drugs. Doses of drugs and KCl added to the bath, i.e. extraluminally in the case of the

perfused arteries, were contained in 0.1 to 0.3 ml of solution. Drugs applied intraluminally to the perfused arteries were added to the reservoir of perfusion fluid or injected as a bolus into the perfusion system proximal to the artery; the doses for injection were also contained in volumes of 0.3 ml or less. In the chronic administration series the effects of noradrenaline, KCl and methoxamine on the preparations were tested in that order of application. Concentration response curves were derived from duplicate responses to cumulative doses of all agents except methoxamine for which sometimes only one response to cumulative doses was obtained.

Perfused artery segments

For each of the perfused arteries the concentration response curves to intraluminal (I.L.) and extraluminal (E.L.) application of drugs were approximately parallel, and therefore the concentration required to produce a response of 50 mm Hg was used to determine the relative sensitivities of that artery to the drug applied by the two routes. This relative sensitivity is expressed as the I.L./E.L. concentration ratio; for example, when the artery was more sensitive to I.L. than to E.L. application of a particular drug, the ratio was less than one. The mean values of I.L./E.L. ratios were calculated as the geometric means (Fleming, Westfall, de la Lande & Jellett, 1972).

Artery strips and vein segments

Acute studies: Responses of strips are expressed as a percentage of the maximum response to noradrenaline, except for the responses to clonidine in the presence of cocaine when they are expressed as a percentage of the clonidine maximum.

Chronic studies: Artery strip and vein segment responses to noradrenaline, methoxamine and KCl are expressed as a percentage of their respective maximum responses. Geometric means of the agonist concentrations causing half maximal responses (E.D. 50) were calculated (Fleming *et al.*, 1972). Where the maximum responses to an agonist differed significantly between control and treated groups, statistical analysis of the absolute response at each concentration has been used as an indication of sensitivity changes.

RESULTS

Acute Studies

Histochemistry

Typical examples of transverse sections for normal and sympathectomized rabbit ear arteries examined by fluorescent histochemistry are shown in Figures 3.3 and 3.4. In agreement with earlier descriptions (de la Lande & Waterson, 1967; Waterson & Smale, 1967), the artery shows the characteristic distribution of sympathetic nerve terminals localised at the medial adventitial border. Transverse sections of the rat tail artery and portal vein after treatment by

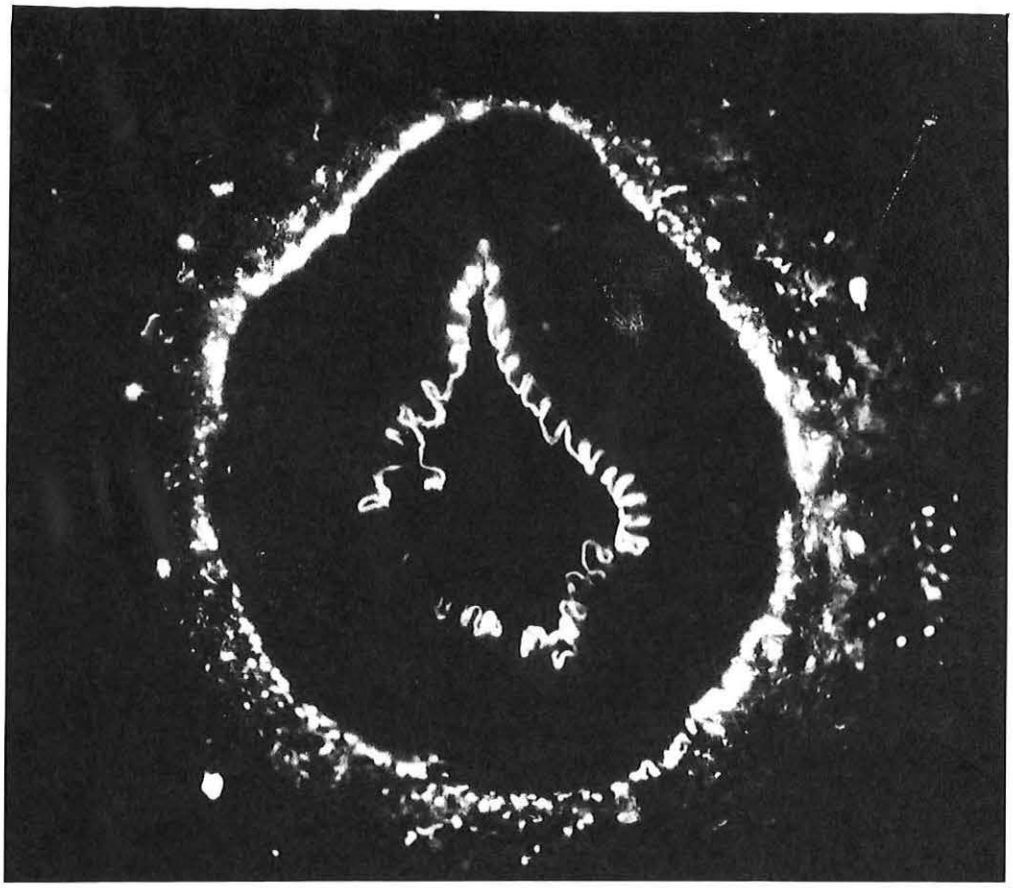


Fig. 3.3 Transverse section of the central artery from a rabbit ear prepared to show monoamine fluorescence at the medial adventitial border and intimal autofluorescence. Magnification x 140.

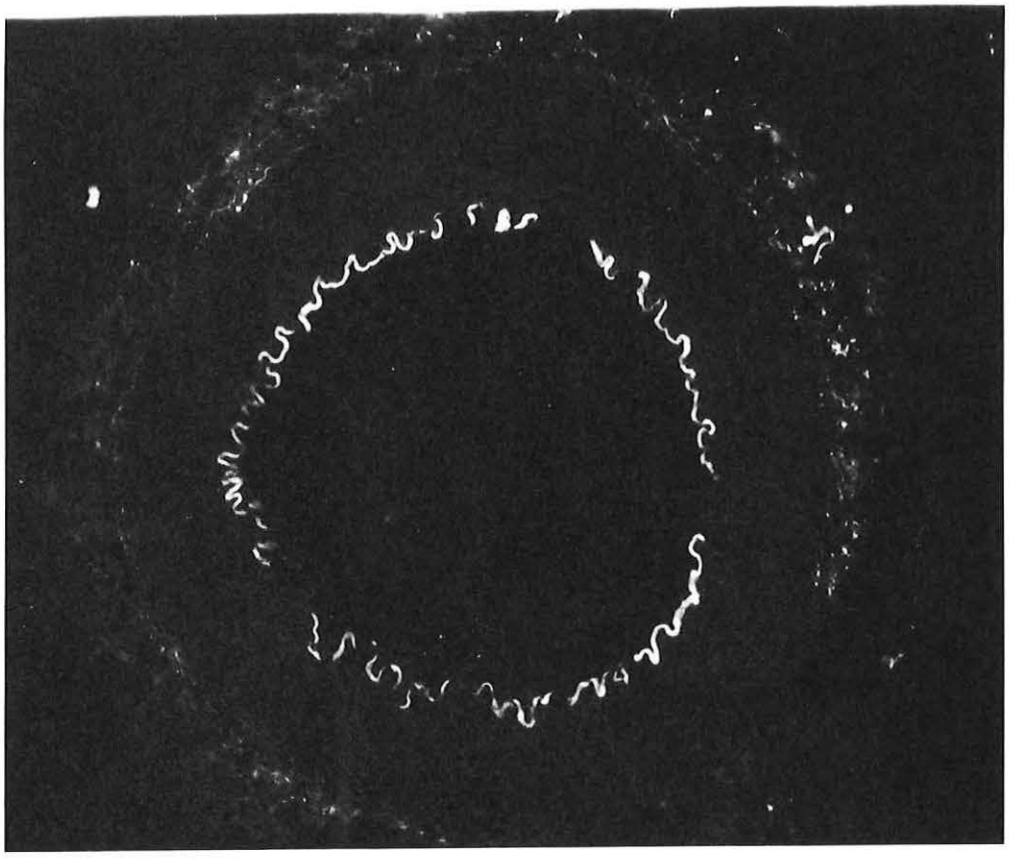


Fig. 3.4 Transverse section of the central artery from a rabbit ear six days after removal of the homolateral cervical ganglion. Intimal autofluorescence is present but medial adventitial monoamine fluorescence is absent. Magnification x 135.

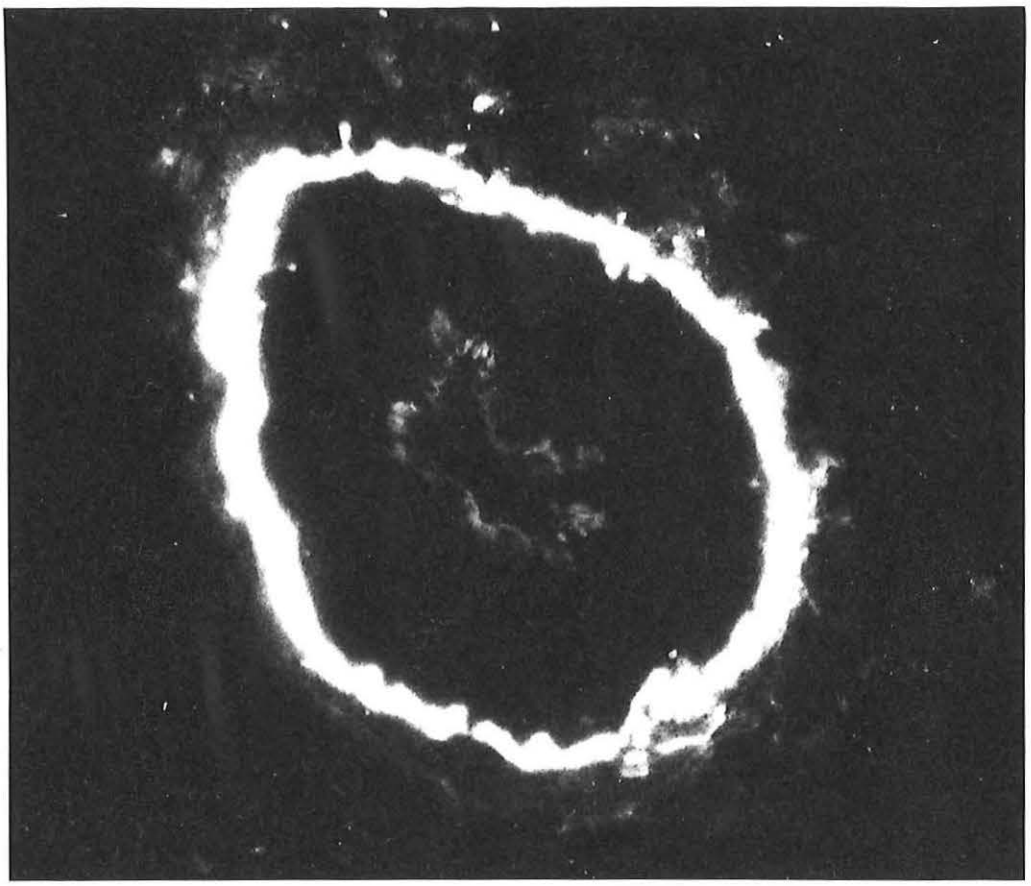


Fig. 3.5 Transverse section of the ventral artery from a rat tail showing monoamine fluorescence at the medial adventitial border and intimal autofluorescence. Magnification x 270.

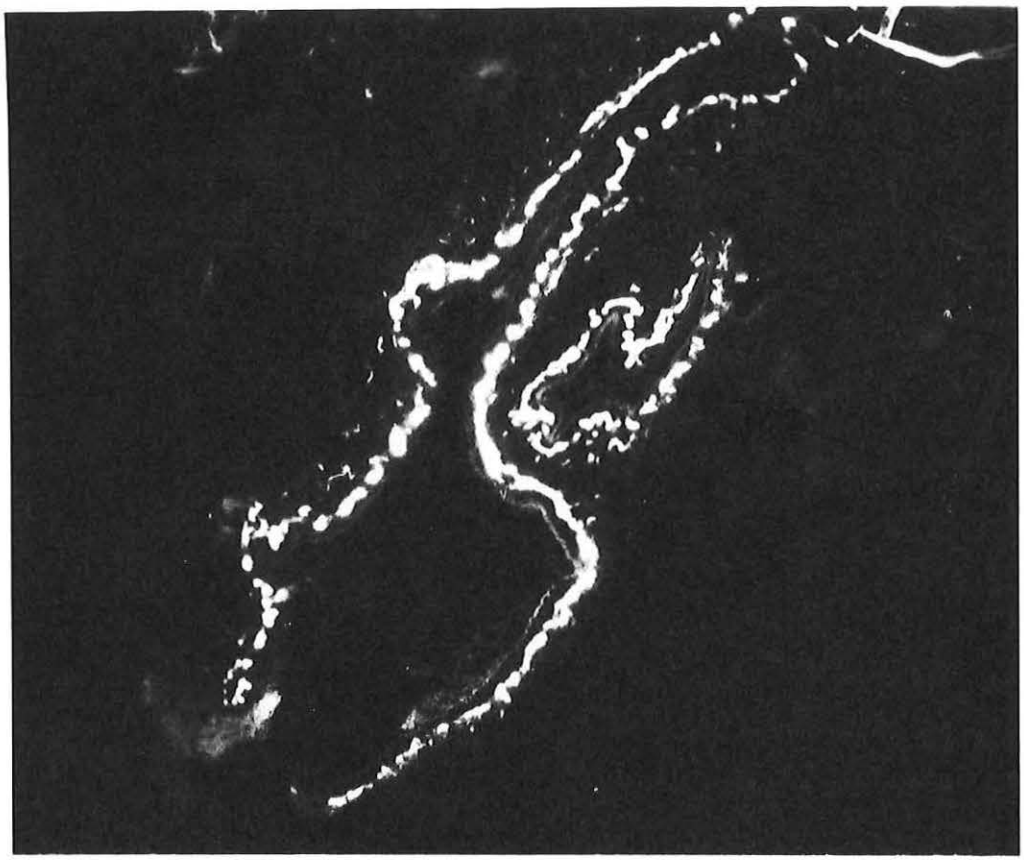


Fig. 3.6 Section of the portal vein from a rat showing monoamine fluorescence at the medial adventitial border and intimal auto-fluorescence. Magnification x 120.

fluorescent histochemistry are shown in Figures 3.5 and 3.6. The rat artery has a smaller diameter than the rabbit artery and the monoamine fluorescence tends to be more dense than in the rabbit vessel but, as in the case of the latter, does not penetrate into the media. The portal vein of the rat is much more thin walled than the artery, but, like it, shows considerable monoamine fluorescence localised to the medial-adventitial border.

Perfused artery segments

Nature of clonidine's action: There was a rise in perfusion pressure in cannulated arteries when clonidine was applied I.L. or E.L. which was maintained for the duration of the application (up to a maximum of 30 min). Single or cumulative doses of clonidine caused a pressure rise which was slower in onset and offset than that seen with noradrenaline (Fig. 3.7). These differences in response were more marked for E.L. drug application and, furthermore, the slower offset of the effect of clonidine was more pronounced after larger doses. Possibly because of the slower onset of action, relatively higher doses of clonidine were required to give responses equal to those of noradrenaline when the drugs were given I.L. by bolus injections instead of by continuous infusions.

Adrenergic alpha receptor blockade: The pressor responses in two rabbit ear arteries to both noradrenaline and clonidine injection were abolished by phentolamine (2.65×10^{-6} M) added to both the

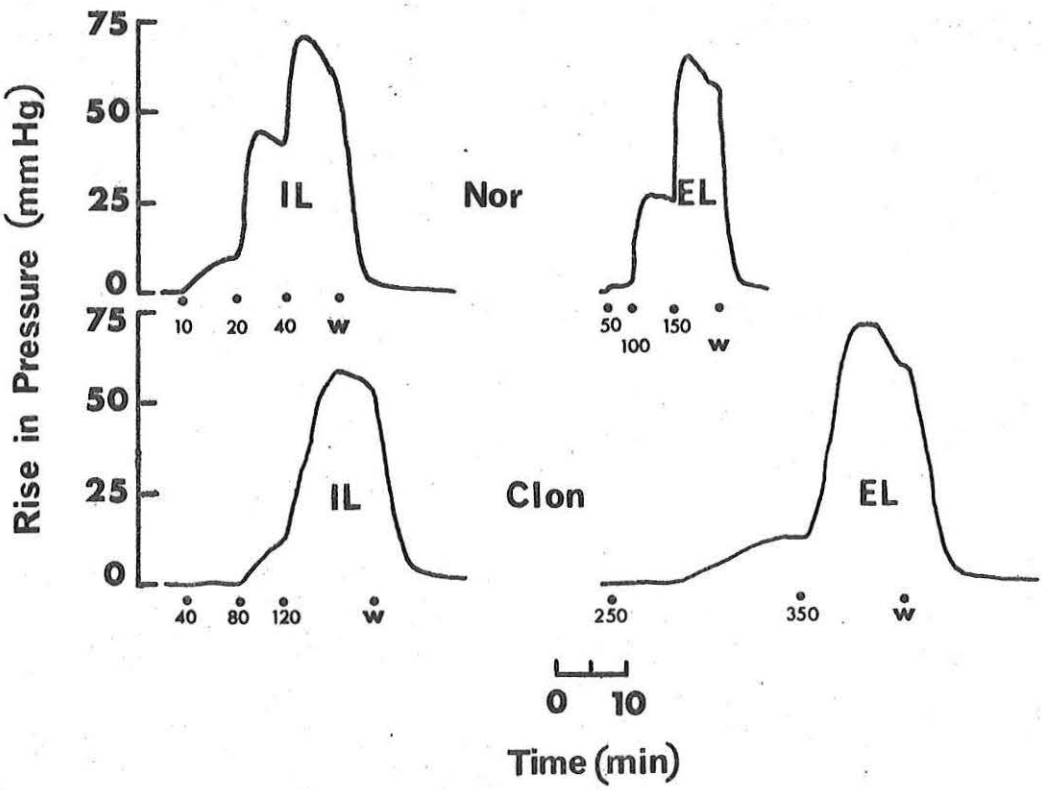


Fig. 3.7 Response of a perfused rabbit ear artery segment to cumulative doses of noradrenaline (Nor) and clonidine (Clon) applied intraluminally (IL) and extraluminally (EL). Drugs were added at • and the figures refer to the concentration in ng/ml. Drug washed out at W.

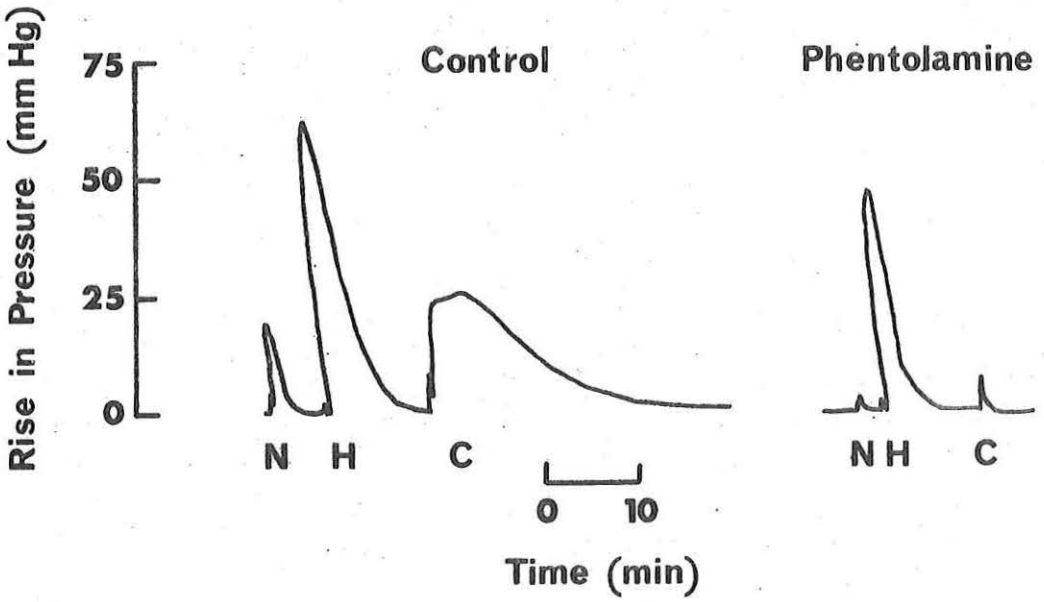


Fig. 3.8 Response of a perfused rabbit ear artery segment to intraluminal injections of noradrenaline (0.1 mcg at N), histamine (5 mcg at H), and clonidine (20 mcg at C) before and after blockade of adrenergic alpha receptors with phentolamine. Injection artefacts as well as vessel responses can be seen.

perfusate and bath fluid. Phentolamine had relatively little effect on the responses to injections of histamine. Figure 3.8 shows the results in one of the two arteries.

Role of the sympathetic nerves: The innervated perfused rabbit ear arteries were much more sensitive to an I.L. perfusion of noradrenaline than to E.L. application with a concentration ratio I.L./E.L. of approximately 0.1 which is similar to previously reported values (de la Lande *et al.*, 1966). Figure 3.9 shows typical concentration response curves derived from an experiment with a nonsympathectomized artery. The same pattern of differential sensitivity to noradrenaline was found in the single rat tail artery preparation with a concentration ratio I.L./E.L. of 0.06 (Fig. 3.10). In contrast, the differential sensitivity was much less evident in the case of clonidine (Figs. 3.9 and 3.10) and the mean clonidine concentration ratio I.L./E.L. in seven rabbit ear arteries was 0.77 (Table 3.1). The single rat tail artery had a clonidine I.L./E.L. ratio of 0.6.

The four denervated rabbit arteries had a greater sensitivity to noradrenaline than control which was more marked for E.L. than I.L. application, as is illustrated by the responses of one artery shown in Figure 3.11. The mean I.L./E.L. concentration ratio for noradrenaline was 1.15 (S.E. +0.2, -0.17), i.e. a tenfold difference existed between control and denervated arteries. However, the denervated arteries were only slightly more sensitive to clonidine than the control

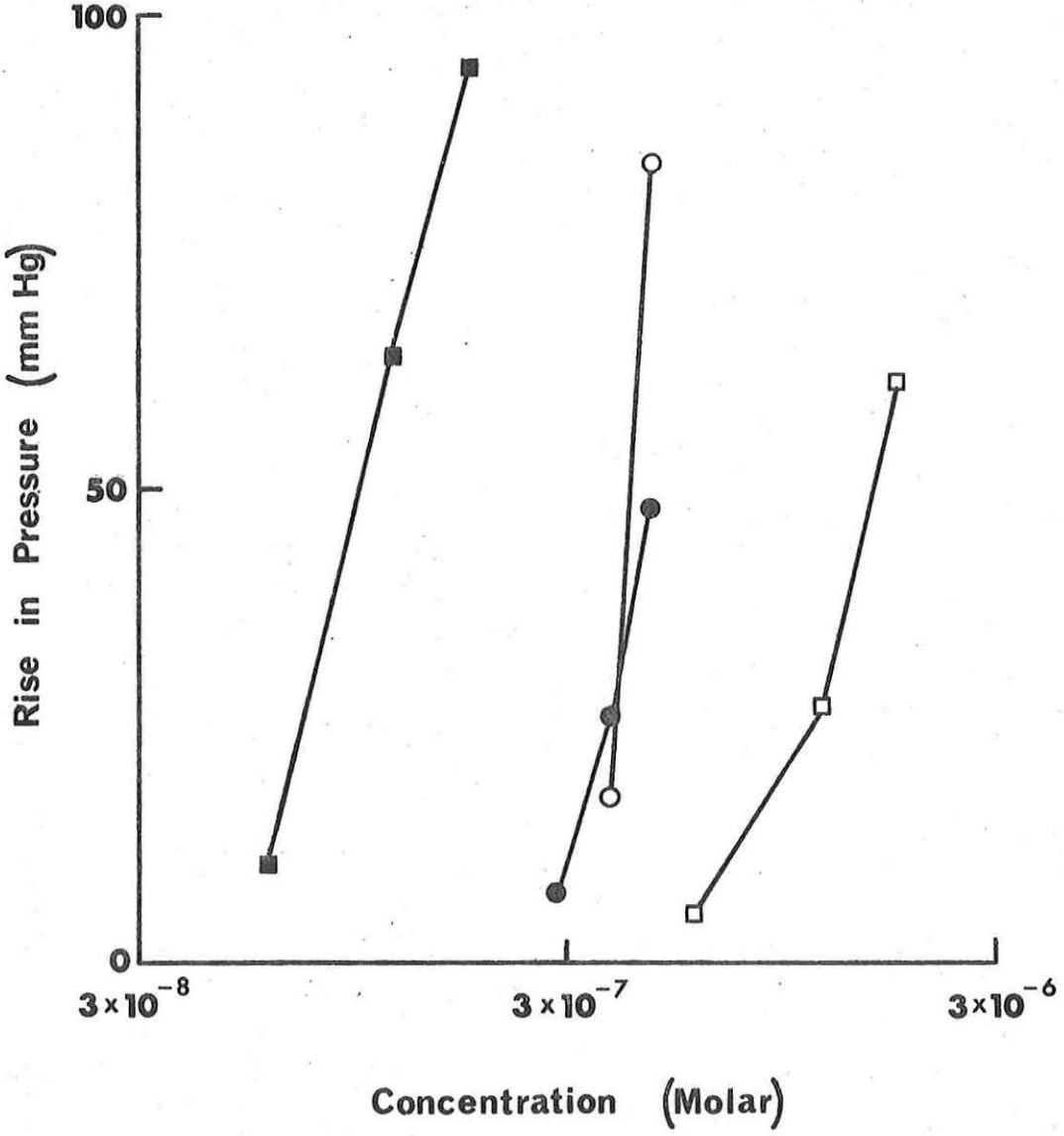


Fig. 3.9 Concentration response curves of a perfused rabbit ear artery segment to noradrenaline (squares) and clonidine (circles). Extraluminal drug application, open symbols; intraluminal application, closed symbols.

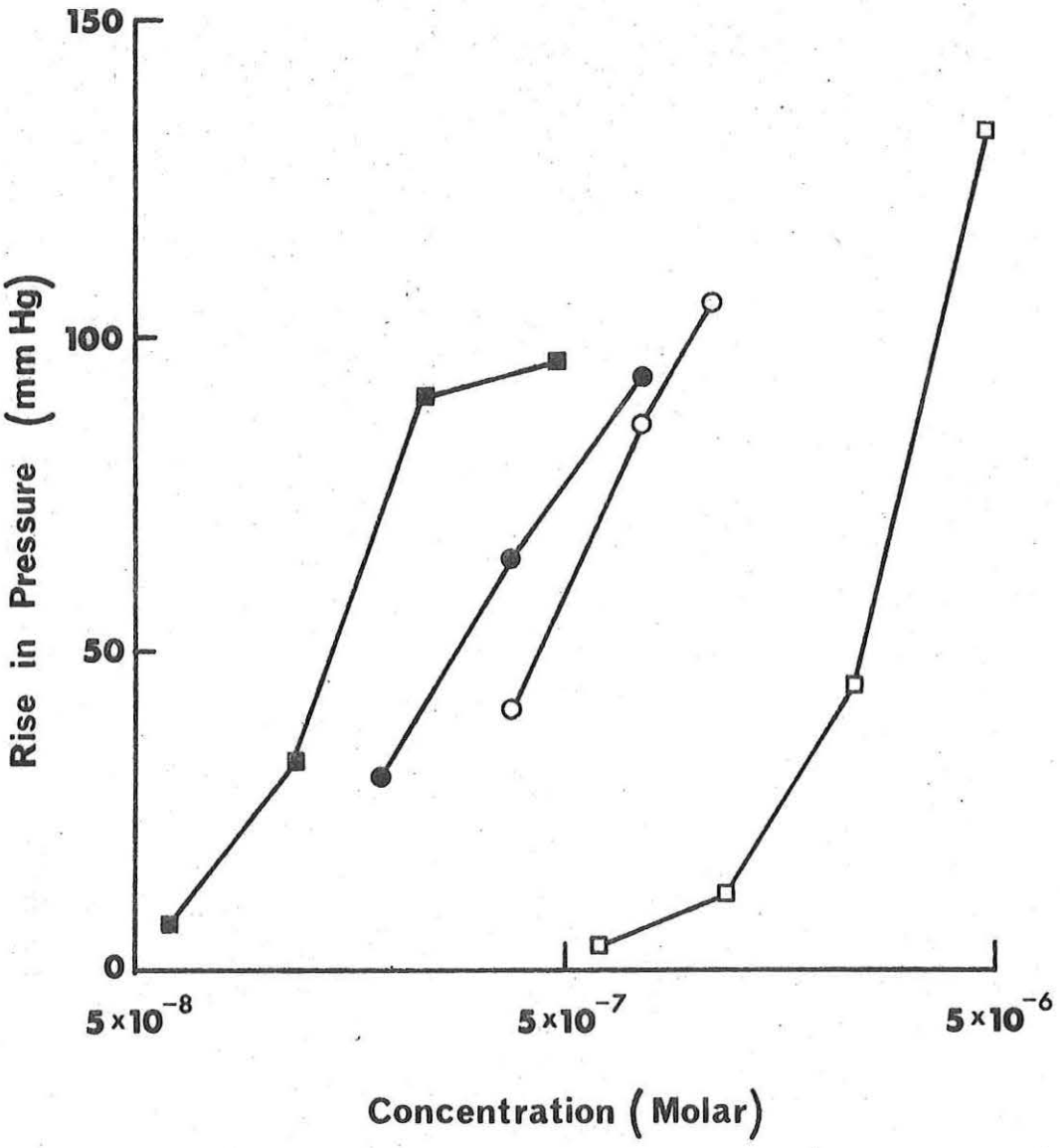


Fig. 3.10 Concentration response curves of a perfused rat tail artery segment to noradrenaline (squares) and clonidine (circles). Extraluminal drug application, open symbols; intraluminal application, closed symbols.

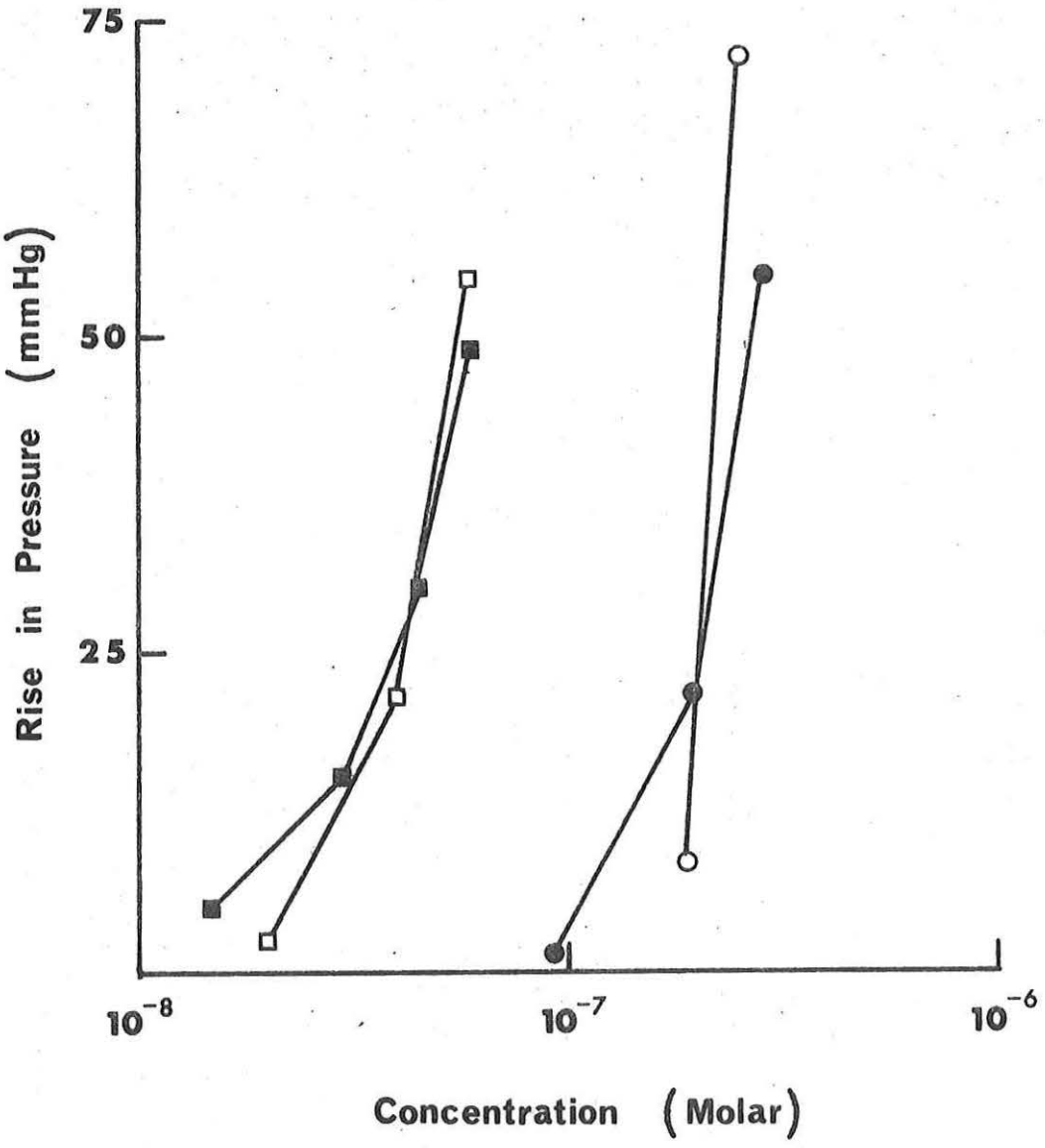


Fig. 3.11 Concentration response curves of a denervated perfused rabbit ear artery segment to noradrenaline (squares) and clonidine (circles). Extraluminal drug application, open symbols; intraluminal application, closed symbols. (The results illustrated in Figs. 3.9 and 3.11 were obtained from arteries removed from the same animal.)

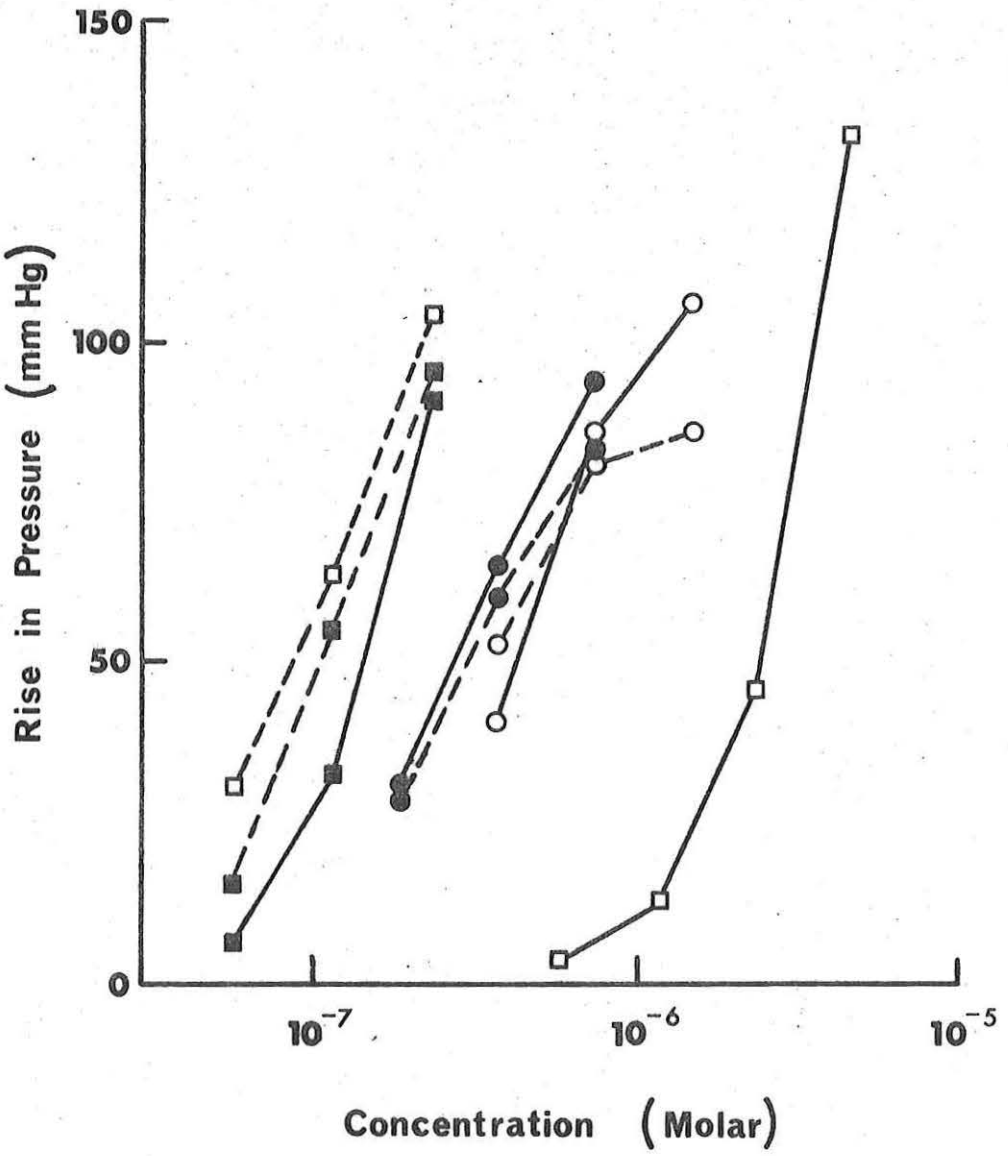


Fig. 3.12 Concentration response curves of a perfused rat tail artery segment to noradrenaline (squares) and clonidine (circles). Extraluminal application, open symbols; intraluminal application, closed symbols. Control responses, solid lines; cocaine (2.94×10^{-4}M) present, interrupted lines.

Control		Sympathectomy			Cocaine	
I.L./E.L. $Mx10^{-7}$	Ratio	Period of denervation (days)	I.L./E.L. $Mx10^{-7}$	Ratio	I.L./E.L. $Mx10^{-7}$	Ratio
4.13/6.00	0.69	12	4.43/5.44	0.81	18.01/14.26	1.26
5.00/5.85	0.85	6	2.63/2.25	1.17	4.69/6.38	0.74
1.16/2.03	0.57	7	3.94/8.44	0.47		
4.77/4.20	1.13	14	3.75/6.12	0.61		
4.20/12.00	0.35					
20.63/18.01	1.15					
9.01/8.33	1.08					
Mean ± S.E.	0.77 +0.14 -0.12			0.72 +0.16 -0.13		0.97

Table 3.1 Ratio of intraluminal (I.L.) to extraluminal (E.L.) clonidine concentrations causing a 50 mm Hg rise in perfusion pressure in seven control, four sympathectomized and two cocaine treated rabbit ear arteries. Individual ratios and geometric means (± S.E.).

arteries (Fig. 3.11) and the mean I.L./E.L. concentration ratio of 0.72 was not significantly different from the ratio in the seven control vessels (Table 3.1).

The effects of cocaine (2.94×10^{-6} M added to the perfusate and the bath fluid) on two rabbit arteries and one rat artery were similar to the effects of chronic denervation, i.e. a small increase in the intraluminal sensitivity and a much greater increase in extraluminal sensitivity (Fig. 3.12). As with chronic denervation, cocaine caused only a slight increase in sensitivity to clonidine, and the mean I.L./E.L. concentration ratio for the three preparations (0.93 + 0.16, -0.14) was not significantly different from the ratio in control vessels.

Rat artery strips

In the first hour after being set up all preparations relaxed to some degree but thereafter a stable resting length was usually maintained. If this state was not achieved the preparation was omitted from the analysis of results. At no time was any spontaneous activity of the strip detected.

Effects of noradrenaline: The effects of noradrenaline have been examined in five untreated and 14 control (chronic study) strip preparations and Figure 3.13 contains an example of the responses to cumulative doses. The contractile response to noradrenaline was rapid in onset and in attaining a plateau, but relaxation despite

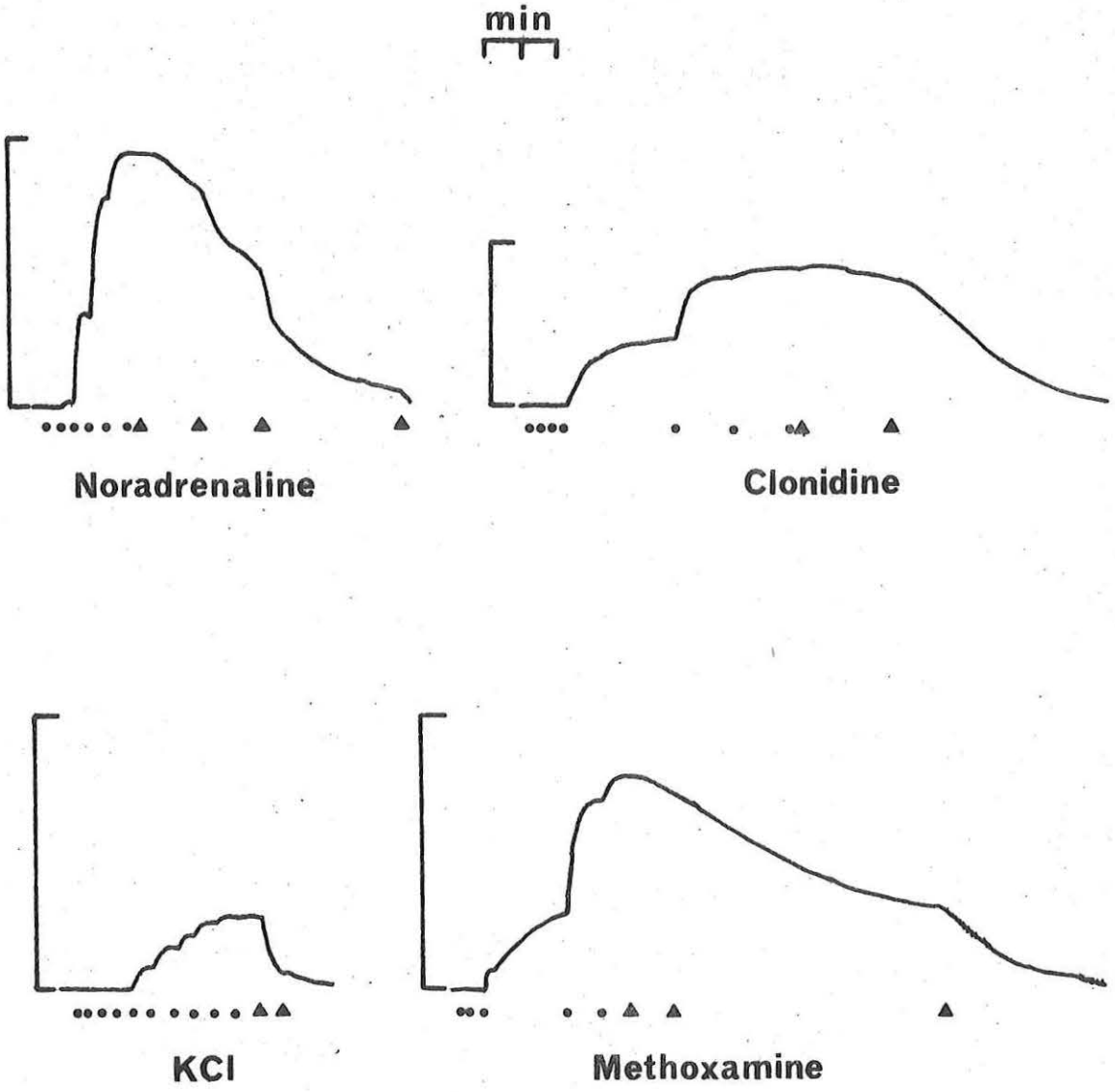


Fig. 3.13 Responses of tail artery helical strips from two rats to cumulative concentrations of four agonists. Responses of one of the four strips used in the acute study to clonidine (3.2×10^{-10} M to 3.2×10^{-5} M and 1.2×10^{-4} M). Responses of a strip from a control rat in the chronic clonidine series to noradrenaline (5.91×10^{-9} M to 5.91×10^{-4} M), to methoxamine (4.04×10^{-8} M to 4.04×10^{-4} M) and to KCl (10^{-2} M to 10^{-1} M). Drugs were added at • , and washed out at ▲ . Vertical scale 1 mm contraction.

Artery Strips %		Vein Segments %	
101.4		67.6	
82.8		71.4	
115.2		67.4	
101.8		81.3	
135.5		78.0	
65.5		62.7	
110.3		73.7	
99.0		70.2	
		82.8	
Mean	101.4	72.8	
	n.s.		p<0.001
± S.E.	7.4	2.3	

Table 3.2 Reproducibility of noradrenaline responses in 8 rat tail artery strips and 9 portal vein segments from control rats in the chronic studies. Maximum contractions to a third sequence of cumulative concentrations applied at the end of the experiment expressed as a percentage of the first two maximum contractions obtained at the beginning of the experiment. Significance of the difference between first two and third response is shown. (Student's paired t-test.)

repeated washouts was consistently slower. Threshold concentrations were usually between 5.91×10^{-8} M and 5.91×10^{-7} M, and maxima were attained at 5.91×10^{-5} M. The mean E.D. 50 (± S.E.) for the fourteen control preparations was $1.24 + 0.9, -0.52 \times 10^{-6}$ M.

Responses to noradrenaline were reproducible and in eight of the fourteen control strips a third sequence of cumulative concentrations was applied at the conclusion of the experiment (usually 5-6 hours). The maximum of the third response was not significantly different from the first two and responses to submaximal doses of noradrenaline were essentially unchanged (Table 3.2).

The responses of two strips to noradrenaline in the presence of cocaine (2.94×10^{-6} M) were determined. The maximum contraction was not increased but there was a 2.3 fold shift to the left of the E.D. 50 (Fig. 3.14). This increase in sensitivity is less than that to E.L. noradrenaline (27 x) but greater than that to I.L. application (1.3 x) in the rat perfused segment.

Effects of methoxamine and KCl: The effects of cumulative doses of these agonists were recorded in 13 control strips (e.g. Fig. 3.13). The time course of the contraction with both was usually slower than that with noradrenaline, and the relaxation after washout was more rapid following KCl and noradrenaline than methoxamine. The mean (± S.E.) maximum response to noradrenaline (0.52 ± 0.07 mm) was approximately equal to that to methoxamine (0.52 ± 0.09 mm) but

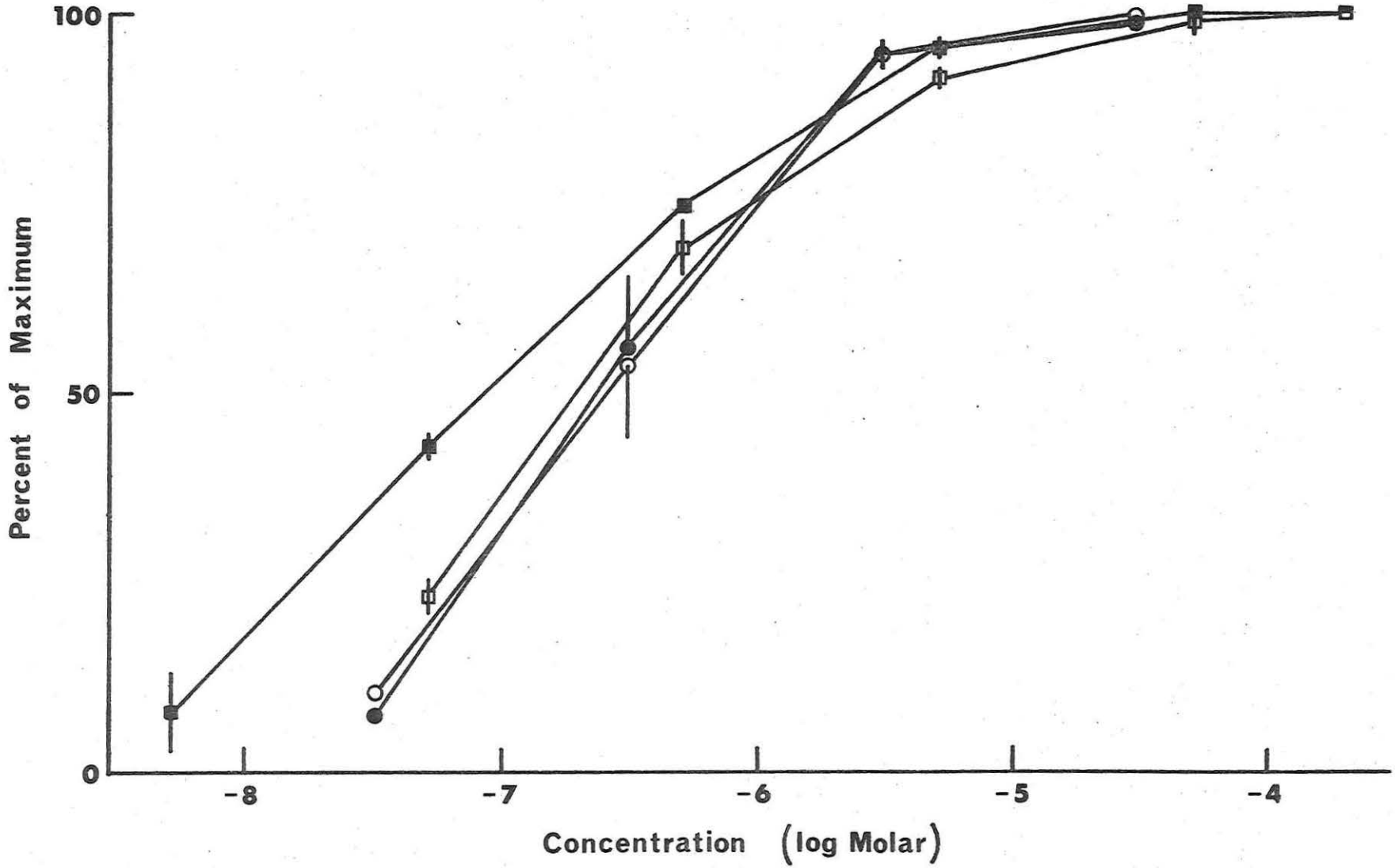


Fig. 3.14 Responses (mean ± range) of two rat tail artery helical strips to cumulative doses of noradrenaline (squares) and clonidine (circles) expressed as a percentage of the maximum contraction produced by each agonist. Open symbols, control responses; closed symbols, cocaine (2.94×10^{-4} M) present.

considerably greater than that to KCl (0.22 ± 0.04 mm). However, the strips were less sensitive to methoxamine (mean E.D. 50, 6.43 x 10^{-6} M) and also to KCl (mean E.D. 50, 5.83 x 10^{-2} M) than to noradrenaline.

Effects of clonidine: In four preparations the effect of clonidine was determined after responses to noradrenaline had been obtained (e.g. Fig. 3.13). The contraction produced by clonidine developed more slowly than that to noradrenaline, and the maximum contraction produced (at 1.2 x 10^{-4} M) was only about 25% of that caused by noradrenaline (1.8 x 10^{-4} M) (Fig. 3.15). In two of these strips a sustained maximum contraction with noradrenaline (1.7 x 10^{-4} M) was produced and then cumulative doses of clonidine applied. This resulted in a concentration dependent relaxation of the strip to 56.5% of the maximum contraction (Fig. 3.16). Conversely, when these two strips were in sustained maximum contraction with clonidine first cumulative doses of noradrenaline resulted in further contraction (Fig. 3.16).

The effect of cocaine on the responses to clonidine was determined in two strips. As with noradrenaline, the maximum contraction was not increased, but unlike the noradrenaline response, there was no shift of the E.D. 50 (Fig. 3.14).

Rat portal vein segments

These preparations exhibited spontaneous contractions whose

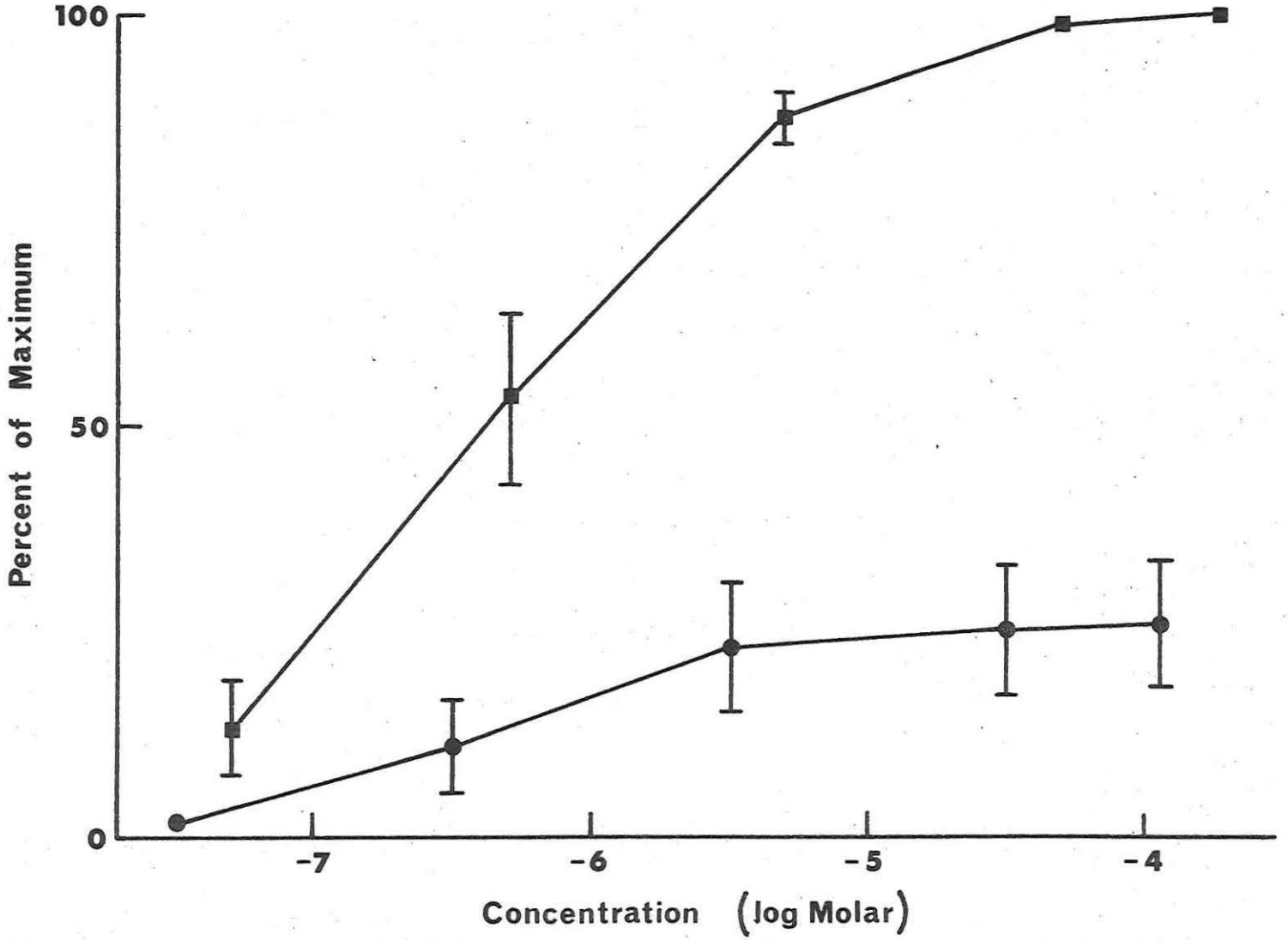


Fig. 3.15 Responses (mean ± S.E.) of four rat tail artery helical strips to cumulative doses of noradrenaline (squares) and clonidine (circles) expressed as a percentage of the maximum contraction produced by noradrenaline.

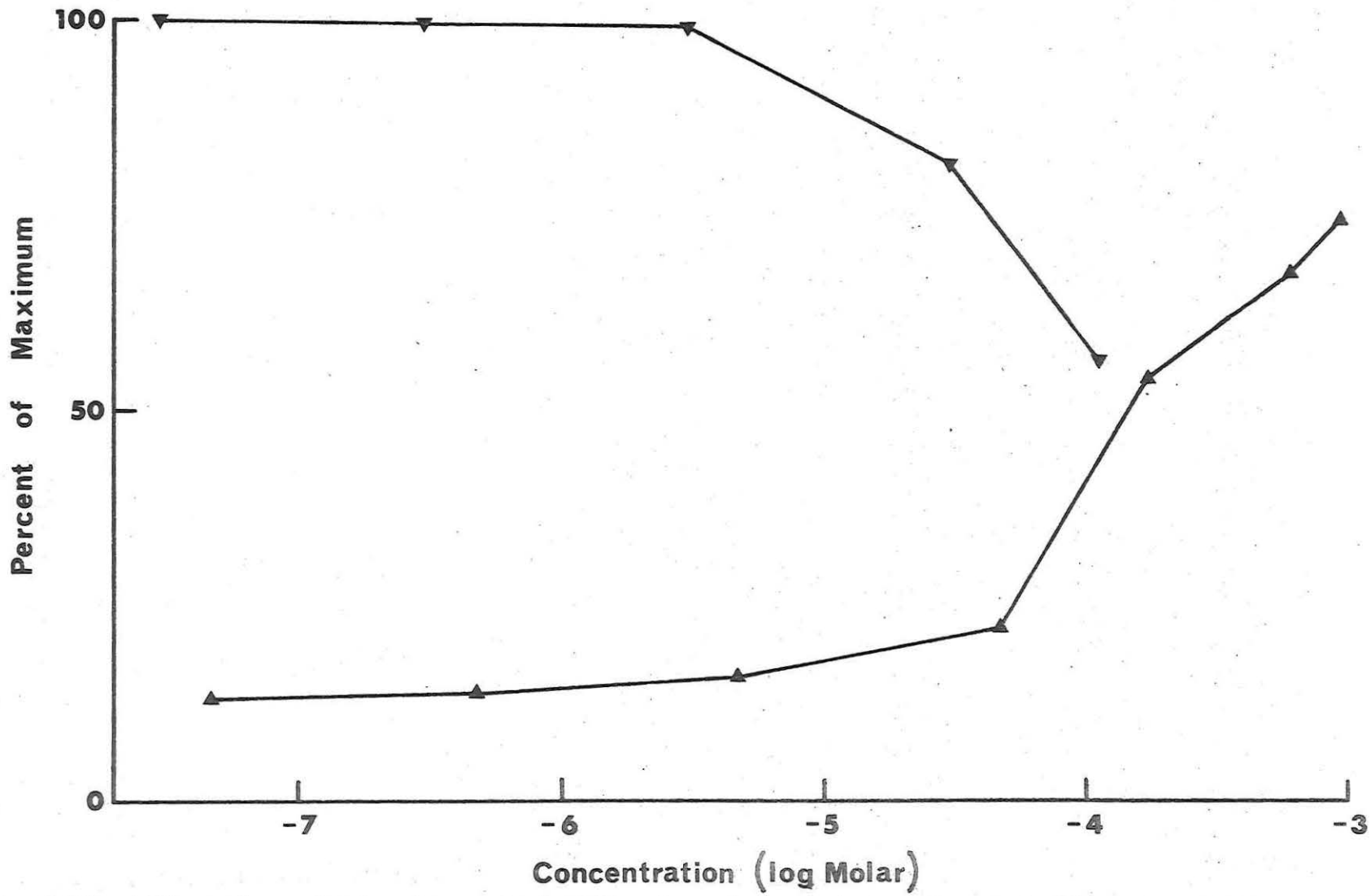


Fig. 3.16 Effect of cumulative doses of clonidine (▼) on artery strips maximally contracted to noradrenaline (1.7×10^{-4} M). Effect of cumulative doses of noradrenaline (▲) on artery strips maximally contracted to clonidine (1.1×10^{-4} M). Means of results in two rat tail arteries expressed as a percentag of the maximum contraction produced by noradrenaline.

frequency was greatest (approximately 15/min) in the first 10 to 15 minutes. At the end of an hour of equilibration in Krebs solution the preparation displayed a stable resting length and a relatively low frequency of contraction (3-5/min) which was maintained for four to six hours (e.g. Fig. 3.17). The amplitude fluctuated, but usually diminished during an experiment.

Effects of noradrenaline, methoxamine and KCl: Low concentrations of 5.91×10^{-8} M noradrenaline, 4.04×10^{-7} M methoxamine and 2.0×10^{-2} M KCl caused an increase in the frequency of contractions as well as a baseline contraction (e.g. Fig. 3.17). With higher concentrations the amplitude of spontaneous contractions rapidly diminished and then ceased as the baseline contraction increased further. After washout of the drug, the segment relaxed more rapidly following noradrenaline and KCl than following methoxamine. Spontaneous contractions reappeared during relaxation, initially at a greater than pre-drug frequency. Contraction amplitude often did not completely regain its control magnitude. Good duplicates with each agent were consistently obtained, but a third noradrenaline response at the end of the experiment was significantly less than the initial two (Table 3.2). In sixteen veins the mean (± S.E.) maximum responses to noradrenaline (1.58 ± 0.11 mm) were significantly greater than those to both methoxamine (1.36 ± 0.09 mm) and KCl (1.20 ± 0.1), $p<0.001$. The sensitivity of the veins to noradrenaline (mean E.D. 50,

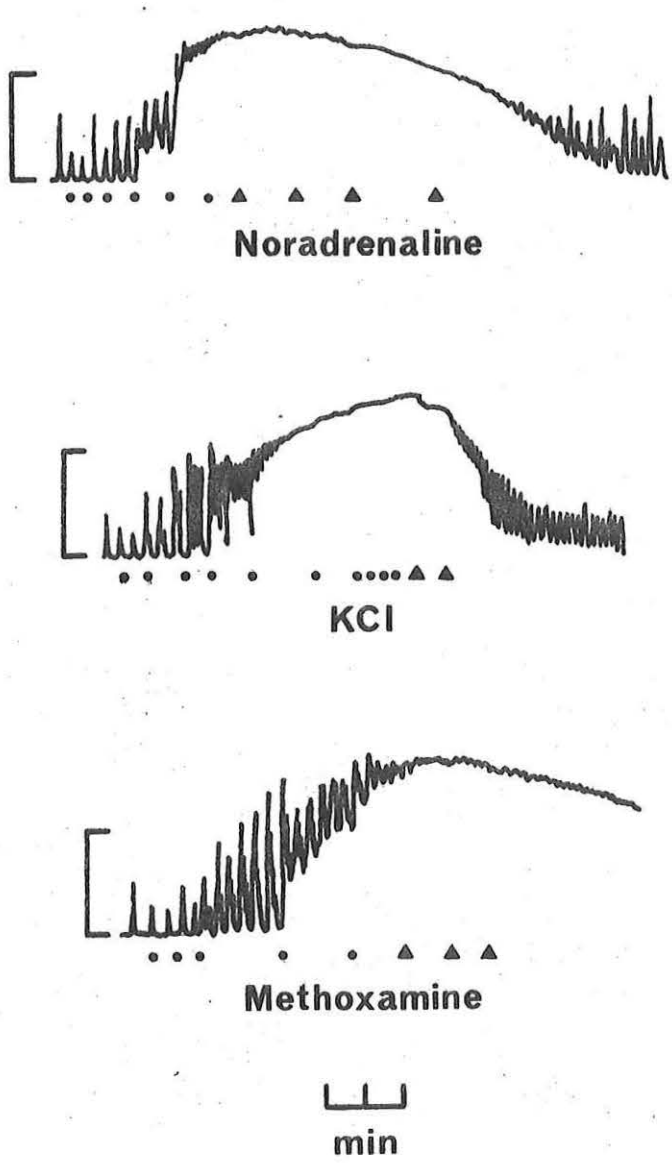


Fig. 3.17 Responses of the portal vein segment from a control rat in the chronic guanethidine series to cumulative doses of noradrenaline (5.91×10^{-9} M to 5.91×10^{-4} M), to KCl (10^{-2} M to 10^{-1} M) and to methoxamine (4.04×10^{-8} M to 4.04×10^{-4} M). Drugs were added at • , and washed out at ▲ . Vertical scale 1 mm contraction.

3.93×10^{-6} M) was greater than that to both methoxamine (mean E.D. 50, 5.99×10^{-6} M) and KCl (mean E.D. 50, 3.70×10^{-2} M).

Chronic Studies

Rat blood pressures and body weights

The blood pressures of the control and clonidine treated groups of rats before and during the treatment were not significantly different. Similarly, clonidine did not significantly affect the gain in weight during the pretreatment period (6-8 weeks).

Blood pressures were not measured in the animals of the other two series. Seven of the eight reserpine treated animals lost weight as a result of the pretreatment and the mean loss of 28 gm was significant (Table 3.3). Weight changes in the treated and control groups of animals in the guanethidine series were not significant.

Histochemistry

Specific monoamine fluorescence was markedly depleted or completely absent from the tail arteries and portal veins of rats after one week of parenteral reserpine or guanethidine (e.g. Figs. 3.18 to 3.21). In contrast, clonidine administered for six to eight weeks did not produce any significant change in intensity or distribution of monoamine fluorescence (e.g. Figs. 3.22 and 3.23).

Vascular sensitivity to noradrenaline, methoxamine and KCl

Chronic reserpine: Reserpine pretreatment did not affect the magnitude of the maximum responses of the arterial strips to

BODY WEIGHT (gms)				
Rat Pair	Control		Treated	
	Before	After	Before	After
1	210	212		
2	205	205	205	180
3	215	220	210	200
4			200	180
5			190	165
6	210	215	215	220
7	190	193	190	155
8	235	225	240	173
9			220	170
Mean ± S.E.	210.8 ± 6.0	211.7 ± 4.7	208.8 ± 5.9	180.4 ± 7.3
p	>0.9		<0.01	

Table 3.3 Effect on body weight of daily injections for one week of reserpine vehicle to control rats and of reserpine to treated rats. Individual weights and group means (± S.E.) before and after treatment. (Student's paired t-test.)

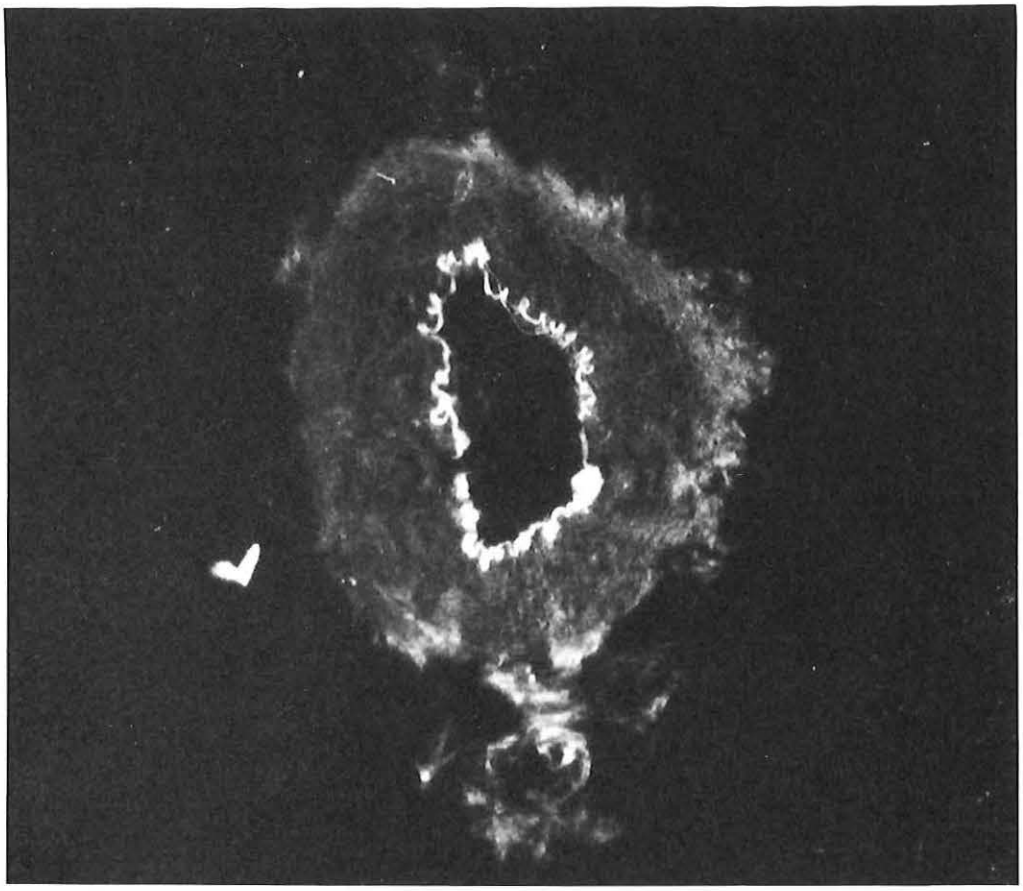


Fig. 3.18 Transverse section of the tail artery from a reserpine treated rat showing complete absence of medial adventitial monoamine fluorescence (c.f. Fig. 3.5). Magnification x 200.

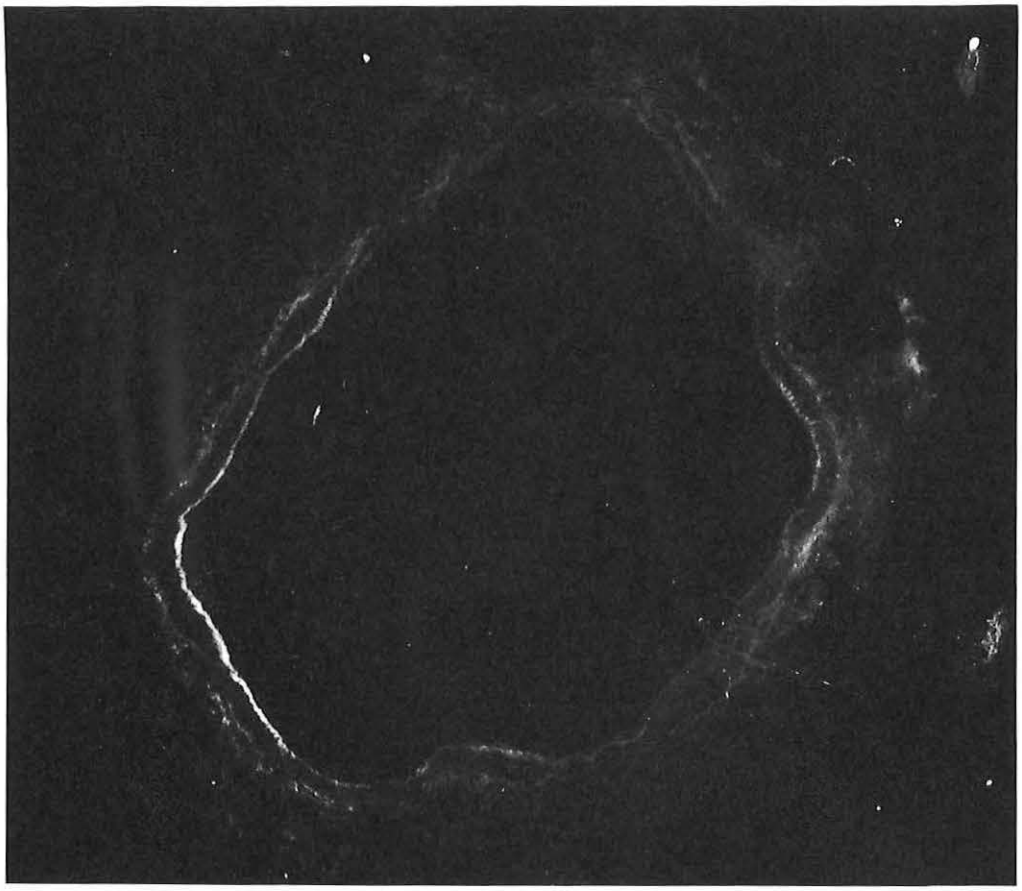


Fig. 3.19 Transverse section of the portal vein from a reserpine treated rat showing complete absence of medial adventitial monoamine fluorescence (c.f. Fig. 3.6). Magnification x 150.

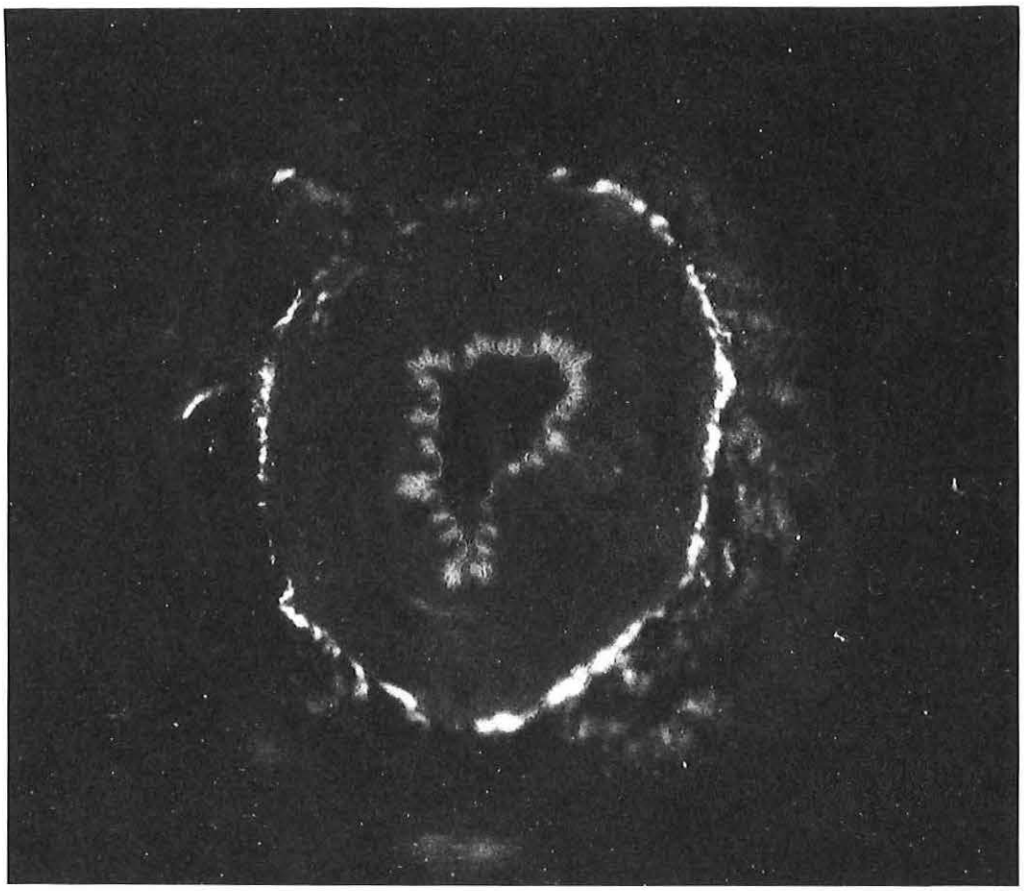


Fig. 3.20 Transverse section of the tail artery from a guanethidine treated rat showing a reduction in the intensity and the distribution of monoamine fluorescence at the medial adventitial border (c.f. Fig. 3.5). Magnification x 230.

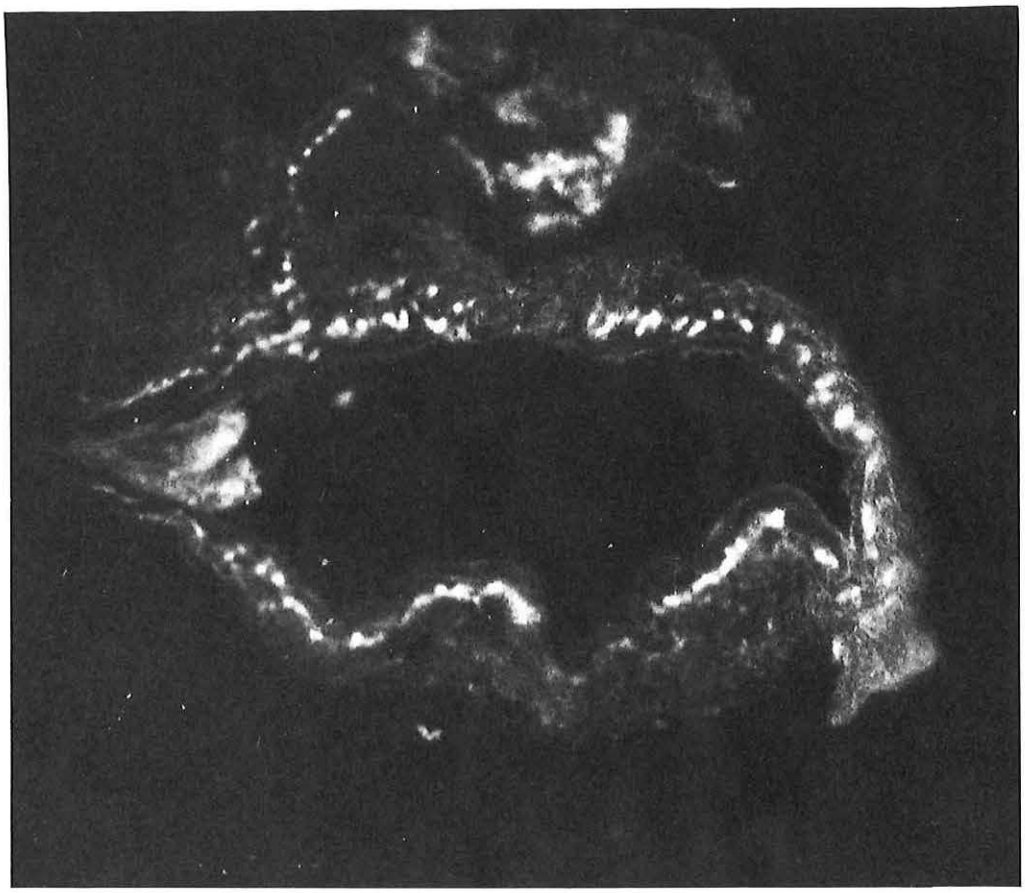


Fig. 3.21 Transverse section of the portal vein from a guanethidine treated rat showing a reduction in the intensity and the distribution of monoamine fluorescence at the medial adventitial border (c.f. Fig. 3.6). Magnification x 300.

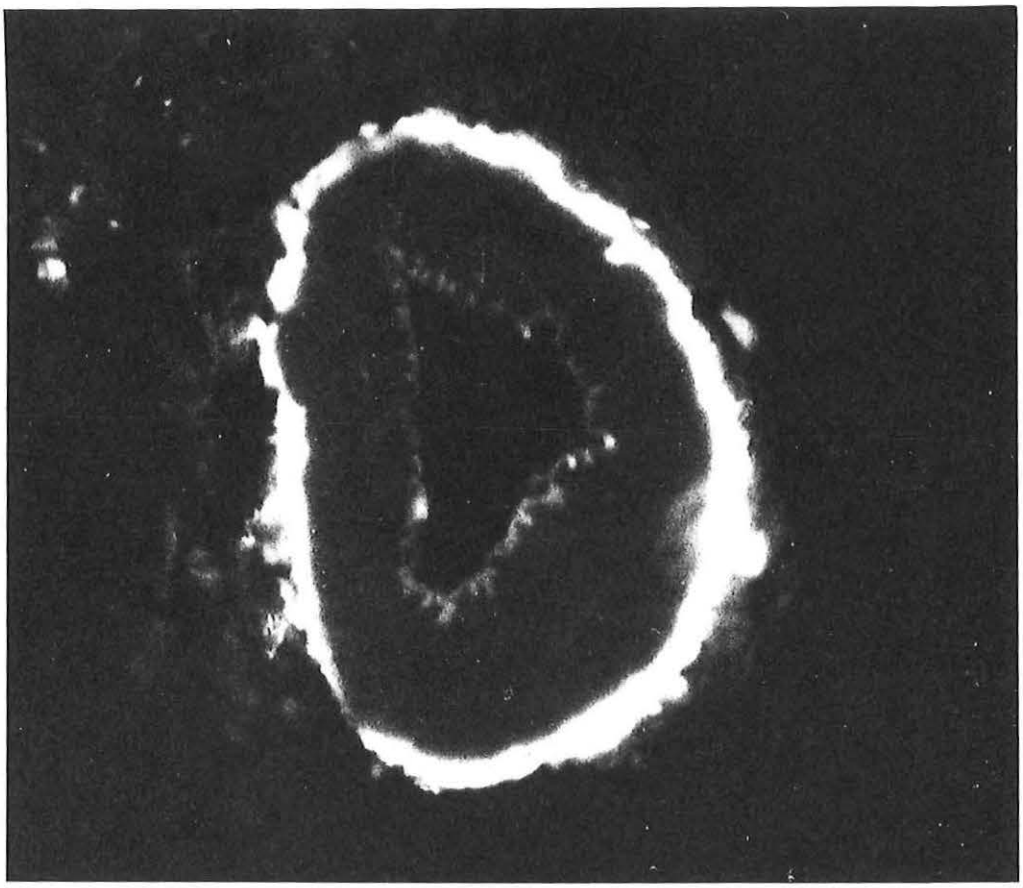


Fig. 3.22 Transverse section of the tail artery from a clonidine treated rat showing normal intensity and distribution of monoamine fluorescence at the medial adventitial border (c.f. Fig. 3.5). Magnification x 210.

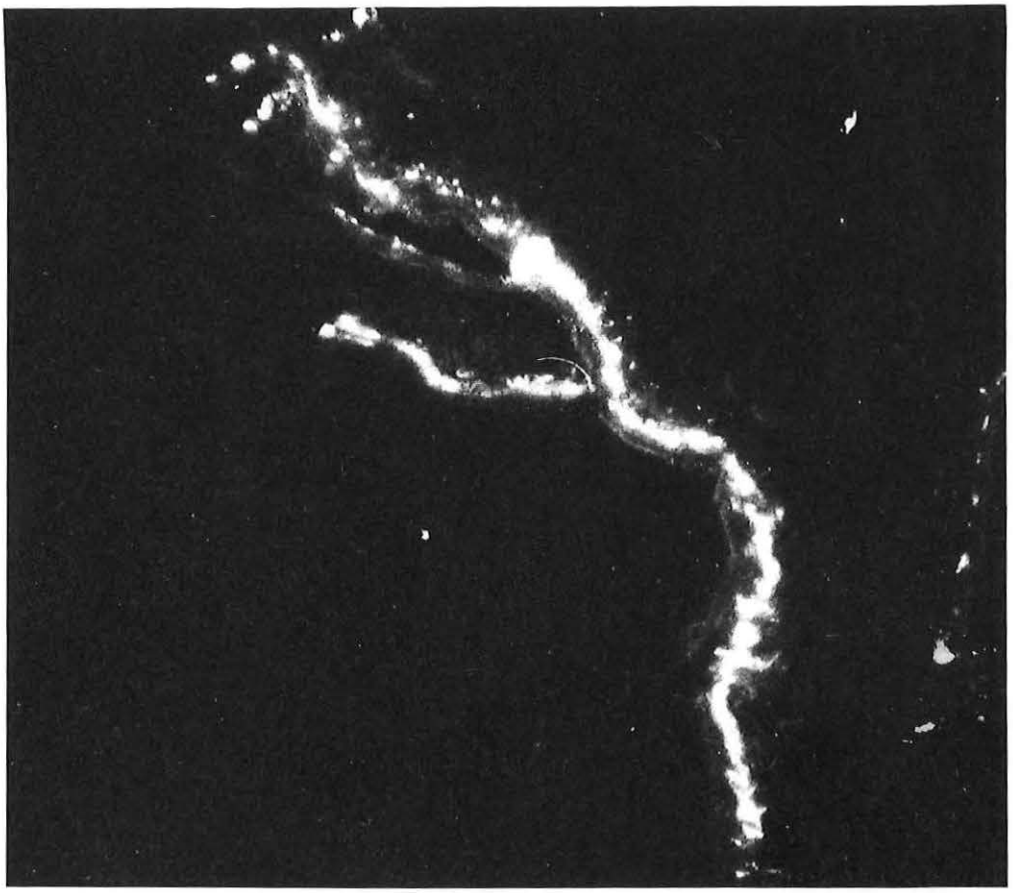


Fig. 3.23 Section of the portal vein from a clonidine treated rat showing normal intensity and distribution of monoamine fluorescence at the medial adventitial border (c.f. Fig. 3.6). Magnification x 160.

noradrenaline, but did increase the sensitivity as manifested by a decrease in both the E.D. 50 and the threshold dose of noradrenaline (Table 3.4 and Fig. 3.24). In the cases of methoxamine and KCl neither the maximum responses or the E.D. 50's were affected by the reserpine pretreatment (Table 3.4).

In the vein preparations neither the mean maximum response nor the mean E.D.50 of each of the three agonists (noradrenaline, methoxamine and KCl) was affected by reserpine pretreatment (Table 3.5).

Chronic guanethidine: Arterial preparations from two pairs of rats (pretreated and control) were discarded because relaxation continued at an unacceptable rate and responsiveness to noradrenaline was rapidly lost. The three other pairs of arterial preparations were satisfactory. The mean of the maximum responses to noradrenaline, methoxamine and KCl and the mean E.D. 50's were not significantly affected by guanethidine pretreatment (Tables 3.4 and A.7). The methoxamine threshold, however, was significantly less in the guanethidine treated than control strips.

In vein preparations from all five pairs of animals the mean maximum responses to noradrenaline, methoxamine and KCl were significantly greater in pretreated than control preparations. The concentration response curves (absolute values) of the treated veins lay to the left and above those of the control veins (Fig. 3.25) and the shift was significant at all but lowest concentrations for each

Pretreatment Series	Noradrenaline Concentration ($Mx10^{-6}$)		Methoxamine Concentration ($Mx10^{-6}$)		KCl Concentration ($Mx10^{-2}$)	
	Control	Treated	Control	Treated	Control	Treated
Clonidine (n=12)	1.59 +0.52 -0.39	1.40 +0.33 -0.27	6.79 +3.68 -2.39	5.68 +2.12 -1.55	5.74 ±0.13	5.53 ±<0.001
p	>0.7		>0.7		>0.1	
Reserpine (n=12)	0.97 +0.18 -0.15	0.55 +0.09 -0.08	6.63 +1.63 -1.31	6.31 +0.88 -0.77	6.12 +0.51 -0.47	5.56 ±0.18
p	<0.05		>0.8		>0.2	
Guanethidine (n=6 for noradr.) (n=4 for methox. and KCl)	1.13 +0.20 -0.17	0.47 +0.46 -0.26	5.12 +2.16 -1.52	6.52 ±0.15	5.58 ±<0.002	5.50 ±0.13
p	>0.3					

Table 3.4 Effect of chronic administration of clonidine, reserpine and guanethidine on the responses of rat tail artery strips to noradrenaline, methoxamine and KCl. Results expressed as the geometric mean (± S.E.) of the $E.D._{50}$ of each agonist. n = number of strips (control plus treated). Student's unpaired t-test.)

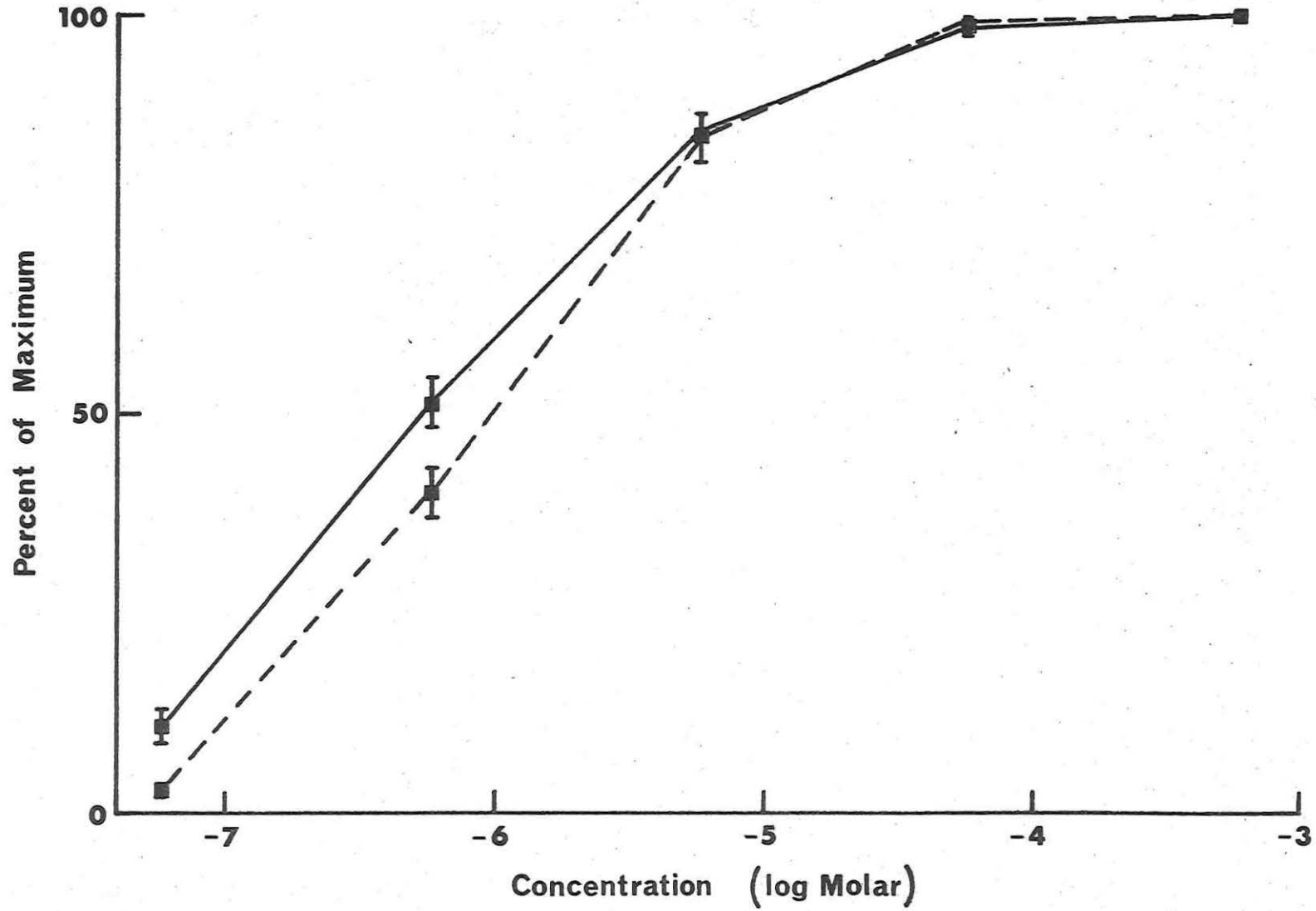


Fig. 3.24 Responses (mean ± S.E.) of rat tail artery strips to cumulative doses of noradrenaline expressed as a percentage of the maximum contraction. Control animals (n = 5), interrupted lines; reserpine treated animals (n = 7), solid lines.

Pretreatment Series	Noradrenaline Concentration ($Mx10^{-6}$)		Methoxamine Concentration ($Mx10^{-6}$)		KCl Concentration ($Mx10^{-2}$)	
	Control	Treated	Control	Treated	Control	Treated
Clonidine (n=11)	3.90 +1.73 -1.14	1.56 +0.68 -0.47	8.96 +1.27 -1.11	3.02 +3.51 -1.62	3.70 +0.25 -0.23	3.71 +0.39 -0.35
p	>0.1		>0.1		>0.1	
Reserpine (n=13)	4.38 +1.42 -1.07	2.79 +1.06 -0.77	7.10 +2.04 -1.58	4.32 +1.18 -0.92	3.31 +0.21 -0.19	3.12 ±0.16
p	>0.3		>0.2		>0.4	
Guanethidine (n=10)	3.66 +1.53 -1.08	1.83 +0.24 -0.23	3.12 +0.81 -0.64	3.19 +1.99 -0.75	4.12 +0.14 -0.13	3.82 +0.13 -0.12

Table 3.5 Effect of chronic administration of clonidine, reserpine and guanethidine on the responses of rat portal vein segments to noradrenaline, methoxamine and KCl. Results expressed as the geometric mean (± S.E.) of the $E.D._{50}$ of each agonist. n = number of segments (control plus treated). (Student's unpaired t-test.)

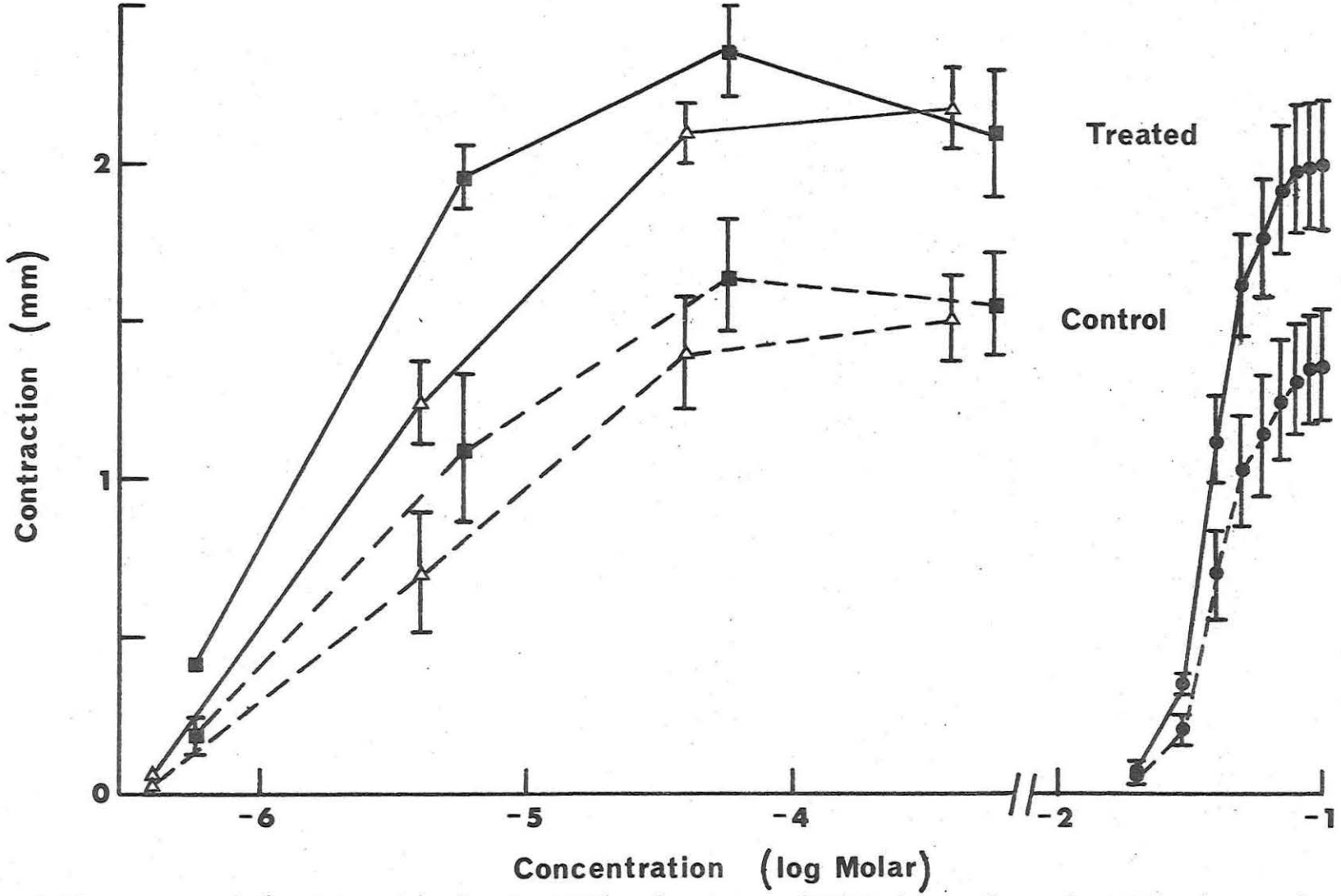


Fig. 3.25 Responses (mean ± S.E.) of rat portal veins to cumulative doses of noradrenaline (squares), methoxamine (triangles) and KCl (circles) expressed as absolute contraction in mm. Control animals (n = 5) interrupted lines; guanethidine treated animals (n = 5), solid lines.

agonist.

Chronic clonidine: Clonidine had no significant effect on the maximum responses of artery and vein preparations to noradrenaline, methoxamine and KCl and the slight shift to the left of their E.D. 50's were not significant (Tables 3.4, 3.5 and A.8).

DISCUSSION

Properties of the Isolated Vessels

The pattern of sympathetic innervation in the rabbit artery described by de la Lande & Waterson (1967) was confirmed in the present histochemical studies. These also showed that noradrenergic structures of the rat tail artery and rat portal vein, as in the rabbit artery, were confined to the medial adventitial border. The density of fluorescence in the rat artery appeared greater than that in the rabbit artery. However, the rat vessel is the smaller of the two and an inverse relationship between intensity of fluorescence and vessel diameter has been described by Falck (1962) and Norberg & Hamberger (1964). Monoamine fluorescence appeared only at the medial adventitial border in the portal vein, which contrasts with the demonstration of fluorescence between the inner circular and outer longitudinal smooth muscle layers by Johansson, Ljung, Malmfors & Olson (1970). The explanation of this discrepancy probably lies in the fact that in the present study segments for histochemistry were taken from the mesenteric end of the vein where the longitudinal

layer becomes thin or disappears (Johansson *et al.*, 1970) and all the fluorescence therefore appeared in the adventitia.

The adventitial location of the sympathetic nerves in the isolated rabbit artery preparation has been shown to be the major factor responsible for its tenfold greater sensitivity to I.L. than to E.L. noradrenaline, and also for the striking potentiation by cocaine of E.L. compared to I.L. noradrenaline (de la Lande *et al.*, 1966; de la Lande & Waterson, 1967; de la Lande, Frewin & Waterson, 1967). In the present study it has been demonstrated that the perfused rat artery segment exhibits similar properties and it was also found that the noradrenaline dose response curve of the new helical strip preparation was shifted to the left by cocaine. The latter increase in sensitivity was intermediate in value between those of I.L. and E.L. noradrenaline on the segment, reflecting the fact that in the strip noradrenaline reaches the smooth muscle from intimal and adventitial (and cut) surfaces simultaneously. Johansson *et al.* (1970) have shown that cocaine caused a marked increase in the noradrenaline sensitivity of the unperfused rat portal vein. Thus the four isolated preparations retained functional sympathetic post-ganglionic innervation, a characteristic which makes them eminently suitable for studying the mode of action of vasoconstrictor drugs.

The responses of the preparations were reproducible over some hours, except in the case of the portal vein in which the maximum

contraction diminished gradually so that after four to six hours it was approximately 73% of the initial maximum. As a consequence of this, methoxamine maximum responses were significantly less than initial noradrenaline maxima. However, they were of the same order of magnitude as the final noradrenaline response. Sensitivity of the vein preparation in terms of threshold agonist concentrations did not show this gradual decline in response.

The comparison of the effects of chronic antihypertensive drug administration on vascular sensitivity utilized only the artery strip and the portal vein, since full dose response relationships can be obtained with these preparations. Noradrenaline, methoxamine and KCl were chosen as representative agonists in the expectation that responses to them would indicate whether supersensitivity of the pre-junctional or post-junctional type had been induced by the antihypertensive agents. Increased responses to methoxamine and KCL occur when supersensitivity is of the post-junctional or non-specific type (Trendelenberg, Maxwell & Pluchino, 1970; Hudgins & Fleming, 1966), whereas an increased noradrenaline response occurs in both pre- and post-junctional supersensitivity (Trendelenberg, 1963).

Clonidine

Acute effects

Clonidine caused vasoconstriction in the perfused rabbit and rat artery segments. The effect was abolished by phentolamine and

therefore is presumably mediated by adrenergic alpha receptors. This is in agreement with investigations on other tissues, e.g. isolated cat hindquarters (Kobinger & Walland, 1967), perfused dog forepaw (Constantine & McShane, 1968), and the isolated perfused whole rabbit ear (Boissier *et al.*, 1968). The sensitivities to E.L. and to I.L. clonidine were approximately equal in contrast to the approximate tenfold difference in sensitivity to noradrenaline by the two routes. Furthermore, denervation and cocaine had little effect on the sensitivity to clonidine applied by either route in contrast to their selective effect on the sensitivity to E.L. noradrenaline. Similarly, in the helical strip cocaine did not affect the sensitivity to clonidine, whereas the effects of noradrenaline were potentiated. This evidence is consistent with that obtained by Rand & Wilson (1968) of the lack of effect of cocaine on the pressor response and of denervation on the contraction of the nictitating membrane produced by clonidine in the cat. However, it is in conflict with the evidence of Boissier *et al.* (1968) who found that the clonidine pressor response in the dog was reduced by cocaine and therefore concluded that there was an indirect component in its alpha receptor stimulant action. These differences may reflect species differences, but, regardless of that possibility, it is clearly difficult to relate effects on a complex phenomenon like the blood pressure response to effects on an isolated vessel.

In the rat tail artery strip the maximum response to clonidine was only about a quarter of that to noradrenaline, and when added during a maximum response to noradrenaline, it relaxed the strip. These effects of clonidine are consistent with Constantine & McShane's evidence on the rabbit aorta (1968) that it has less efficacy at, but greater affinity for, adrenergic receptors than adrenaline, i.e. it behaves like a partial agonist. The fact that clonidine exerts a similar effect on two dissimilar arteries (small muscular from the rat; large elastic from the rabbit) suggests that its partial agonist effect may extend to most types of artery.

In summary, these results of acute application offered no evidence that clonidine effects are neuronally mediated in any way, but pointed to a direct effect on alpha adrenergic receptors.

Chronic effects

The oral route of administration was chosen since the rat readily absorbs clonidine when it is given by this route and the blood levels achieved are comparable to those resulting from I.V. injection (Rehbinder & Deckers, 1966). This route of administration seemed preferable to twice daily injection for 1½ to 2 months. The dose employed was more than double that which had resulted in a depression of vascular responses to catecholamines and angiotensin in the cat hindlimb (Zaimis & Hanington, 1969), but, nevertheless, chronic clonidine had no effect on either the sensitivity of the rat artery

or vein preparations to noradrenaline, methoxamine or KCl, or on the maximum responses to these agents. Although acute administration of large doses of clonidine causes depression of catecholamine sensitivity (Hoefke & Kobinger, 1966; Boissier *et al.*, 1968), it is conceivable that in the present study the dose may have been insufficient since there was no evidence of a hypotensive effect during the course of the treatment. On the other hand, the failure to reproduce either the increased vascular sensitivity found in man, or the depressed sensitivity found in cats, may indicate that there is a species difference in the effect of clonidine on the response to catecholamines. But further studies on both normotensive and hypertensive animals at a number of dose levels of clonidine will be needed to establish that possibility or indicate other explanations for the discrepant results.

Hoefke & Kobinger (1966) reported that 5 mg/kg of clonidine daily for three days did not deplete the noradrenaline content of the rat heart, and the present study indicates that a smaller dose over a longer period is similarly lacking in effect on vascular catecholamines.

Chronic Reserpine

The only significant effect of chronic reserpine detected in the present study was an increase in noradrenaline sensitivity of the rat artery strip without a change in its maximum response. The change in sensitivity is less than that which occurs with chronic reserpine in the rabbit aortic strip (Hudgins & Fleming, 1966) which may reflect

a species difference or even a difference between small muscular and large elastic arteries. The reason for the failure to demonstrate supersensitivity to methoxamine and KCl in the artery strip and the lack of supersensitivity to all three agonists in the vein segment is not obvious. Reserpine induced supersensitivity in other smooth muscle preparations is of the post-junctional type (Trendelenberg, 1966), i.e. it would be expected to occur with methoxamine and KCl as well as with noradrenaline, and it is unlikely that the rat artery and vein are exceptions to this. It is possible that the amount of trauma and handling to which the vessels were subjected was partly responsible for the failure to detect supersensitivity to methoxamine and KCl in the artery and to all three agonists in the vein. (This possibility has been invoked by Westfall & Fleming (1968) to account for their finding that the reserpine treated guinea pig pacemaker supersensitivity to noradrenaline and calcium was greater *in vivo* than in the isolated perfused heart and was completely undetectable in the isolated spontaneously beating right atrium.) The weight loss and possibility of a poor nutritional state of the animals receiving reserpine may have been additional factors involved in determining the absence of a demonstrable supersensitivity.

Chronic Guanethidine

In contrast to the case with reserpine, chronic guanethidine induced a venous supersensitivity to noradrenaline, methoxamine and

KCl (i.e. it was of a post-junctional type) manifested mainly as an increase in the maximum response to these agonists though there was a slight shift to the left of their dose response curves. The failure to demonstrate a similar supersensitivity in the artery strip is puzzling. Most other studies have recorded a small increase (1.8 to 2 fold) in vascular sensitivity to catecholamines (e.g. Maxwell, Plummer, Schneider, Povlaski & Daniel, 1960; McCubbin, Kaneko & Page, 1961; Boura & Green, 1962; Zimmerman & Harris, 1963), although Altura & Zweifach (1966) found that in the rat mesenteric vessels sensitivity was depressed. The guanethidine treated rats, unlike the reserpine treated ones, did not lose any weight or show any gross ill effects with the drug treatment, so this cannot be invoked as a cause for the lack of supersensitivity in the artery. The dose of guanethidine used was the same as that which Zimmerman & Harris (1963) used when they demonstrated supersensitivity in mesenteric vessels. However, the preparation they used was not subjected to the same degree of trauma as the tail artery strip and this factor may have rendered the latter preparation incapable of detecting a small change in sensitivity.

Sites of Vascular Supersensitivity

The findings with reserpine and guanethidine are of additional interest since they extend the observations on the sites in the cardiovascular system which may develop supersensitivity. Most of

the information in earlier investigations related to the development of supersensitivity in the heart and larger elastic arteries such as the aorta and femoral artery (e.g. Burn & Rand, 1958; McCubbin *et al.*, 1961; Fleming & Trendelenberg, 1961; Boura & Green, 1962; Hudgins & Fleming, 1966; Westfall & Fleming, 1968). The present results indicate that venous supersensitivity, not only to sympathomimetic drugs but also to potassium, can be induced by guanethidine, and the importance of this phenomenon for the clinical use of this and related drugs needs to be investigated further. With the demonstration of supersensitivity in a small artery after reserpine there is now evidence that most of the arterial tree and the microcirculation can develop supersensitivity during chronic reserpine administration. Further research is needed to determine why venous supersensitivity developed with guanethidine but not with reserpine. The doses of guanethidine and reserpine used in most studies, including the present one, have been considerably greater than those likely to be used therapeutically and therefore the direct relevance of such findings to clinical practice needs to be explored.

The present study also confirms an observation made by Fleming & Trendelenberg (1961) that there is no direct relationship between supersensitivity and catecholamine depletion. Reserpine was usually associated with complete absence of monoamine fluorescence in both the artery and vein and yet resulted in a significant supersensitivity

only to noradrenaline in the artery preparation. In contrast, the guanethidine depletion of catecholamines was often not complete, but it nevertheless caused consistent and significant increases in venous sensitivity.

SUMMARY

Clonidine constricted the isolated perfused artery preparations of the rabbit ear and the rat tail and this effect was abolished by adrenergic alpha receptor blockade. In the helical strip preparation of the rat tail artery it was found that the maximum contraction produced by clonidine was only 25% of that caused by noradrenaline, and clonidine also reduced the maximum contraction produced by noradrenaline. Both of these effects on the helical strip indicate that clonidine is an alpha receptor partial agonist.

From concentration response curves in the perfused arteries it was determined that the intraluminal to extraluminal concentration ratios for clonidine were not significantly affected by chronic sympathetic denervation or cocaine. In contrast, these procedures considerably augmented the response to extraluminal noradrenaline and caused a tenfold increase in the I.L./E.L. concentration ratio. These results indicate that clonidine's vasoconstrictor effect results from a direct rather than an indirect action on alpha receptors.

Oral administration of clonidine for six to eight weeks did not

lead to any significant change in the sensitivity of the rat isolated tail artery strip or portal vein segment to noradrenaline, methoxamine or KCl. The present study was therefore unable to confirm the depression of vascular sensitivity to vasoconstrictors which has been observed after chronic clonidine in the cat or the increased sensitivity found in the hypertensive patients of the present study. There was no obvious effect of chronic clonidine on the monoamine fluorescence in these vessels.

In contrast, pretreatment with guanethidine and reserpine caused partial to complete depletion of monoamine fluorescence in both the artery and the vein. Reserpine was also associated with significant arterial supersensitivity to noradrenaline, and guanethidine caused a marked increase in venous sensitivity to methoxamine and KCl as well as to noradrenaline. In these experiments there was no correlation between the degree of catecholamine depletion and the development of vascular supersensitivity.

CHAPTER 4

The results obtained in the present study confirm that clonidine causes cutaneous vasoconstriction in man by adrenergic alpha receptor stimulation. The increase in hand vascular resistance which occurred in the first few minutes after intravenous injection can probably be entirely explained on the basis of this local effect. Thus there is no need to postulate that the constriction was reflexly induced or dependent upon the release of vasoactive hormones. The effect on the rat artery strip indicates that clonidine is, however, a partial rather than a full agonist, but alpha receptor blockade is unlikely to occur with the low doses which are commonly used in clinical practice. Thus clonidine's adrenergic activity shows it has pharmacological as well as structural similarities to tolazoline and naphazoline.

The maximum hypotensive effect of clonidine is attained approximately thirty minutes after intravenous injection. This compares favourably with the delay of three to four hours before the maximum effect of parenterally administered reserpine occurs (Finnerty, 1966). Johnston & Aickin (1971) also found that clonidine's antihypertensive effect in the acute situation was greater and more consistent than that of methyldopa. Although diazoxide is often the drug of choice in hypertensive emergencies because of its rapid effect (2-5 min), in cases where it is contra-indicated or ineffective, intravenous clonidine could be a suitable alternative. However, the initial

pressor effect following an I.V. injection may have undesirable consequences. Adrenergic alpha receptor blockade prevents the rise in pressure, but the hypotensive effect is then also blocked (Merguet *et al.*, 1968). Others have suggested that the intramuscular or subcutaneous routes of administration be used. Though these injections avoid the pressure rise, they also delay the onset of the antihypertensive effect (Vorburger, 1970; Bock, 1970; Onesti, Schwartz, Kim, Paz-Martinez & Swartz, 1971). Furthermore, subcutaneous injection involves the risk that constriction of cutaneous vessels at the injection site could be of sufficient severity to cause tissue necrosis or ulceration. However, as the present study and that of Ehringer (1966) have shown, a pressor response does not occur if the intravenous injection is given slowly, e.g. over five or ten minutes, and the maximum hypotensive effect is still attained in approximately half an hour.

Although it was only possible to study two patients on long term clonidine treatment for hypertension, there is such a dearth of information about its chronic effects that the information obtained from them is of value. Both patients had a decrease in hand blood flow and an increase in hand vascular resistance, i.e. the cutaneous vasoconstriction was also present during chronic treatment. Evidently any homeostatic reduction in total peripheral resistance occurring with long term clonidine (Reubi *et al.*, 1970) does not involve

cutaneous vessels. Consequently, there must be considerable vasodilatation in some or all other vascular beds. One of the two patients complained that, while taking clonidine, the circulation in his hands was abnormally sensitive to cold weather, but this was not sufficiently troublesome to necessitate stopping the drug. However, hand circulation can be more severely reduced and may present as Raynaud's phenomenon (Winchester & Kennedy, 1971). Though remission occurred in this case, permanent vascular damage remains a possible risk of prolonged clonidine treatment. It may therefore be prudent to use alternative antihypertensive agents if a patient on clonidine displays evidence of severe or persistent vasospasm, and existing vasospasm or impaired cutaneous circulation (e.g. in diabetic patients) may be a relative contra-indication to its use. In the present study, chronic clonidine not only caused an increase in hand vascular resistance, but also increased the response of these vessels to intra-arterial noradrenaline. Both of these factors may have been involved in producing the increased sensitivity of the hand circulation to the stimulus of low temperatures. Acute clonidine also causes a rise in cerebrovascular resistance (Deisenhammer & Klausberger, 1966; James, Larbi & Zaimis, 1970), but there are no studies in which this has been looked for during chronic administration, nor have there been any reports of unwanted effects which might be related to it.

The heart rate fell during both acute and chronic clonidine

administration and bradycardia was particularly marked in the case of chronic treatment. Unless the stroke volume in these latter patients was considerably increased, it is difficult to see how the cardiac output could be increased as Reubi *et al.* (1970) have suggested is the case in long term treatment. In fact, two of the four patients in the study by Reubi and co-workers were being treated with guanethidine, which may well have influenced their results, as well as with clonidine. It is obvious that more investigation into the haemodynamic effects of chronic clonidine is needed before the issue can be clarified.

The pressor effect of I.V. noradrenaline and the vasoconstrictor effect of I.A. noradrenaline were increased by both acute and chronic clonidine. However, vascular supersensitivity could not be demonstrated in the isolated smooth muscle preparations. Therefore, supersensitivity is unlikely to be contributing to the increased vascular responsiveness to noradrenaline. Alternatively, clonidine and noradrenaline may be increasing reactivity by an additive effect on alpha receptors. The observation that acute clonidine and submaximal concentrations of noradrenaline had additive contractile effects on the rat artery strip is evidence supporting this explanation. Nevertheless, it is unlikely that the phenomenon of tolerance seen in therapeutic use of the drug can be explained in terms of the increase in cardiovascular effects of catecholamines. Increased

responses to noradrenaline were observed within thirty minutes of intravenous clonidine and were no greater during chronic administration. In contrast, tolerance develops one to ten months after starting treatment. The increased noradrenaline response may, however, aggravate any tolerance which occurs. An alternative hypothesis is that tolerance to clonidine's antihypertensive effect results from an adaptation of the central sympathetic structures to the depressant action of the drug. The only evidence to support this hypothesis is indirect, i.e. the occurrence of rebound overactivity of the sympathetic nervous system when clonidine treatment is abruptly terminated. Withdrawal of the drug thus unmasks a hyperexcitability which presumably had developed in these structures as an adaptation to clonidine's depressant action.

Chronic clonidine (1-2 mcg/kg/day) has proved to be an effective prophylactic agent in migraine (e.g. Sjaastad & Stensrud, 1971; Shafar *et al.*, 1972). The doses needed are smaller than those used in the treatment of hypertension and blood pressure does not usually fall. The mechanism of its action in this condition is unknown. A depressed vascular responsiveness to vasodilators and vasoconstrictors has been suggested as the basis of clonidine's action in migraine (Zaimis & Hanington, 1969). However, the doses used in this animal study were approximately ten times greater than those used in migraine, and other mechanisms may be more important at lower doses.

The evidence that acute clonidine (1-2 mcg/kg) causes cerebral vasoconstriction may be relevant here (Deisenhammer & Klausberger, 1966; James *et al.*, 1970). If this effect persists during chronic administration and counteracts the temporal vasodilatation, the headache characteristic of this phase of an attack would be prevented.

The increased cardiovascular effect of noradrenaline found in the present studies may also be relevant to the use of clonidine in the treatment of migraine. Other drugs acting on alpha receptors may, like noradrenaline, have an increased effect. Thus there is the possibility of an interaction with vasoconstrictors (e.g. ergotamine) used in the management of an acute attack of migraine. The enhanced vasoconstriction might be beneficial and terminate the attack. However, constriction in other vascular beds (e.g. the skin) would also be enhanced and could possibly result in detrimental effects.

The possibility that clonidine may interact with vasoactive drugs in other situations is also worth consideration. The responses to sympathomimetics can be expected to be increased by clonidine and, since there was no histochemical evidence of depletion of neuronal catecholamine stores, this includes the effect of indirectly acting sympathomimetics. A number of these drugs are present in proprietary medicines, e.g. cold remedies, which may be taken by the patient without the knowledge of his medical advisor and may lead to hypertensive episodes or inadequate control of the hypertension.

Theoretically, it is also possible for the cardiovascular effects of some general anaesthetic agents to be potentiated in the presence of clonidine. Diethyl ether and cyclopropane cause an increase in sympathetic activity and circulating catecholamines (Bhatia & Burn, 1933; Price, 1957; Price, Warden, Cooperman & Miller, 1969) which, in the presence of a clonidine-induced increase in sensitivity to noradrenaline, could be potentially hazardous. Of equal importance is the possible potentiation of the effects of these two agents in a case where clonidine has been withheld a day or two prior to the anaesthesia and rebound sympathetic overactivity develops. An increase in arterial carbon dioxide (e.g. from hypoventilation during general anaesthesia) also increases sympathetic tone and plasma catecholamines (Sechzer, Egbert, Linde, Cooper, Dripps & Price, 1960) which further increases the chances of producing hypertensive episodes during the anaesthetic and post-anaesthetic period.

None of these possible interactions with clonidine have been reported; however, the drug has not been extensively used up to the present time. With more widespread availability and greater use in the treatment of hypertension and migraine these, and probably a number of other interactions, can be expected to occur.

In summary, the cutaneous vasoconstriction, increased responses to noradrenaline and withdrawal sympathetic hyperactivity which are

induced by clonidine have some undesirable consequences for its use in the treatment of hypertension and the prophylaxis of migraine. The pressor effect of intravenous administration can be avoided by injecting the drug slowly without thereby delaying the onset of its antihypertensive effect. Chronic administration, particularly of larger doses, may cause troublesome and deleterious vasospasm, the long term effects of which are not known, and it may be contra-indicated in cases where the cutaneous circulation is already impaired. Control of blood pressure in hypertensive patients may become erratic, and hypertensive crises may be precipitated when a patient being treated with clonidine also takes other vasoactive drugs or agents capable of potentiating the effects of endogenous catecholamines. The potential for such interactions to occur also exists for several days after the interruption or abrupt termination of clonidine treatment.

PART II

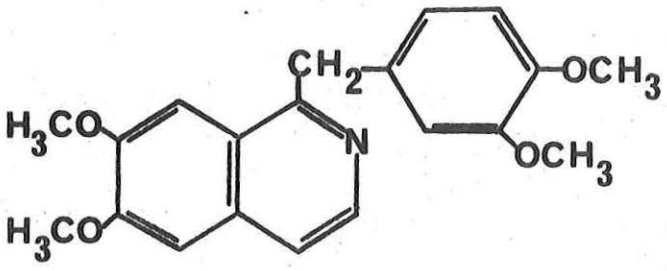
PAPAVERINE

CHAPTER 5

INTRODUCTION

Papaverine is an isoquinolone alkaloid (Fig. 5.1) present in crude opium and first synthesised in 1909. In animals, small doses stimulate the heart, but larger doses (more than 10 mg/kg) cause sinus slowing, A-V conduction block and ventricular premature beats by a direct action on the myocardium (Macht, 1916; Elek & Katz, 1942; Darby, Sprouse & Walton, 1958). Papaverine also has a non-specific vascular smooth muscle relaxant and spasmolytic action, with more marked effects on the cerebral and coronary vessels than other vascular beds (Macht, 1916; Eckenhoff & Hafkenschiel, 1947; Karlsberg, Elliott & Adams, 1963). When the alkaloid is given I.V. to man, in doses which are adequate to cause peripheral vasodilatation (100 to 200 mg), the arterial pressure usually falls and the blood flow in some vascular beds may be reduced as a result of the fall in perfusion pressure (Carpi & Giardini, 1972). Furthermore, the direct cardiac effect of such doses may give rise to serious cardiac arrhythmias and death (Gray, Riseman & Stearns, 1945; Sagall & Dorfman, 1945). In contrast, the cardiovascular effects of 100 to 200 mg given orally are minimal or absent (Russek, Urbach, Doerner & Zohman, 1953), despite relatively rapid and complete absorption from the gastro-intestinal tract (Axelrod, Shofer, Inscoe, King & Sjoerdsma, 1958).

Because of its brief action (Gray *et al.*, 1945; Carpi & Giardini, 1972), the deleterious cardiac effects after parenteral



Papaverine

Fig. 5.1 The structural formula of papaverine.

administration, and the lack of any significant effects during oral administration, the therapeutic use of papaverine has been minor and largely confined to the direct application onto, or local injection into, blood vessels in spasm. Despite these considerable disadvantages, a sustained release formulation of papaverine hydrochloride (Pavabid Plateau Caps) has recently been developed in an attempt to provide an orally effective preparation. There have been several clinical trials with this preparation (Stern, 1965, 1966, 1967 and 1968; Tibbs, 1969) which concluded that Pavabid was of value in improving the circulation in a variety of vascular diseases. These results are surprising in view of the previous experience of the ineffectiveness of papaverine by the oral route. The efficacy of Pavabid may result from more sustained levels of papaverine, an increased sensitivity of the blood vessels in these patients to the action of papaverine, or a combination of both factors.

The aim of the present studies was two-fold:

1. To determine the plasma papaverine concentrations after the I.V. infusion of doses which had cardiovascular effects;
2. To measure the plasma papaverine concentrations and cardiovascular effects occurring with 2-14 days of sustained release papaverine administration.

METHODS

Subjects and Papaverine Administration

The subjects for these experiments were sixteen healthy volunteers and one subject who suffered from migraine headaches but who was otherwise in good health. There were four experimental groups:

1. The effect of brachial artery infusions of papaverine hydrochloride (0.25 - 2 mg/min) on hand and forearm blood flow were measured in six subjects.
2. Intravenous infusions of papaverine hydrochloride (1 - 1.25 mg/min) were given to three subjects and the effect on blood pressure, heart rate, hand and forearm blood flow measured. Venous blood samples for papaverine assay were taken before, during and after the infusion.
3. Two subjects took papaverine hydrochloride as the non-sustained release preparation (150 mg four times daily) for three days and then substituted an identical dose of the sustained release preparation for the same period. Venous samples were taken before starting the drug, and at one and four hours after the second daily dose on the last two days of each schedule. No cardiovascular measurements were made.
4. Administration of sustained release papaverine hydrochloride in two dosage schedules:

(a) 150 mg twelve hourly was taken by four subjects for seven days. Venous samples were taken before starting the drug, at four days and at the end of the seven day period at approximately the same time of the day. Cardiovascular measurements were made in all subjects;

(b) 150 mg four or five times daily was taken by six subjects for 14 days. Venous samples were taken at approximately the same time of day before starting the drug, and after one and two weeks of continuous intake. Cardiovascular measurements were made in three of the six subjects.

The sustained release papaverine tablets used in the studies were prepared in Australia by the Mead Johnson Laboratories (Pavabid Plateau Caps were not used). All the subjects taking sustained release tablets kept records of times when drugs were taken and noted any doses which were missed. The records and tablet counts showed that only one subject forgot to take a tablet on more than one occasion. Venous sampling and cardiovascular testing were at least 12 hours after any such omission by a subject.

One subject participated in all the studies except the I.A. infusions, and another was involved in two of the oral administration studies. All subjects gave informed consent to all procedures undertaken.

Laboratory Conditions

The studies on the cardiovascular effects of papaverine were performed in a laboratory at ambient temperatures ranging from 24° C to 30° C, though the temperature during any particular study varied less than ± 0.5° C. The same laboratory temperature - usually 27° C - was used when subjects were studied on more than one occasion during chronic intake of papaverine. Subjects lay supine on a couch for at least thirty minutes before recordings began, and during this time the recording apparatus was applied and the appropriate catheters or infusion needles were inserted.

Systemic Arterial Pressure and Heart Rate (H.R.)

In two of the three subjects given I.V. papaverine, systemic arterial pressure was measured directly through an indwelling brachial artery catheter inserted percutaneously using the Seldinger technique (Seldinger, 1953). This catheter was connected to a Statham pressure transducer (P23 DC), the output of which was recorded by a Beckman Dynograph (type R). Sphygmomanometric determinations of arterial pressure were taken in the third subject given I.V. papaverine. The heart rate was read from an E.C.G. recorded by either the Beckman Dynograph or a Grass Polygraph (model 5D).

Hand and Forearm Blood Flow (H.B.F. and F.B.F.)

Hand or forearm blood flows were measured by the technique of venous occlusion plethysmography, usually employing water-filled

plethysmographs (Greenfield, 1954), but in some of the studies during chronic papaverine intake the mercury-in-rubber strain gauge (Whitney, 1953) was used to measure the forearm blood flow.

Cardiovascular Reflexes

The effect of chronic papaverine on circulatory reflexes was tested by measuring the cardiovascular responses to:

1. The Valsalva manoeuvre (35 mm Hg maintained for 30 seconds);
2. The performance of mental arithmetic (Blair *et al.*, 1959);
3. Passive postural changes, viz. a 60^{o} head up tilt for four minutes on a tilt-table and leg raising maintained for 1 to 2 minutes (Roddie & Shepherd, 1956).

Intravascular Infusions and Expression of Results

I.A. infusions were given into the brachial artery at the elbow through a 22 gauge short-bevel needle inserted percutaneously with local anaesthesia. I.V. infusions were given through a catheter placed in an antecubital vein. Needles and catheters were connected through polyethylene tubing to infusion pumps which delivered 2 ml of solution per minute. Saline (0.9% w/v) was infused during control periods and was used as a vehicle for the drugs.

The method of treating and expressing the results has been described in Chapter 2.

Determination of Plasma Papaverine

The determination of plasma papaverine concentration was performed

by Mead Johnson Laboratories (Sydney) using the method originally described by Axelrod *et al.* (1958) (Appendix). In summary, the technique involves isolating the papaverine from blood plasma at an alkaline pH by extraction into n-heptane. The drug is then returned to 0.1 N HCl and assayed spectrophotometrically at 251 and 266 mμ. Reading the optical density at two wavelengths serves to eliminate the effects of interfering substances in plasma. Duplicate determinations of papaverine concentration were done 'blind' by the analyst on coded samples.

RESULTS

Acute Parenteral Administration

Effects of I.A. papaverine

I.A. papaverine (0.25 - 2 mg/min) caused an increase in hand and forearm blood flow which was rapid in onset, achieved a maximum within one to two minutes, and returned to resting flows equally rapidly when the drug infusion ceased (e.g. Figs. 5.2 and 5.3). The forearm vasodilatation was dose dependent, but that in the hand was not (Table A.9, Fig. 5.4). For the same dose of papaverine the percentage increase in F.B.F. was two to eight times greater than was the percentage increase in H.B.F.

In one subject, papaverine was infused during a reduction in hand blood flow caused by angiotensin (0.1 mcg/min). Papaverine caused an increase in blood flow to control levels, but there was a

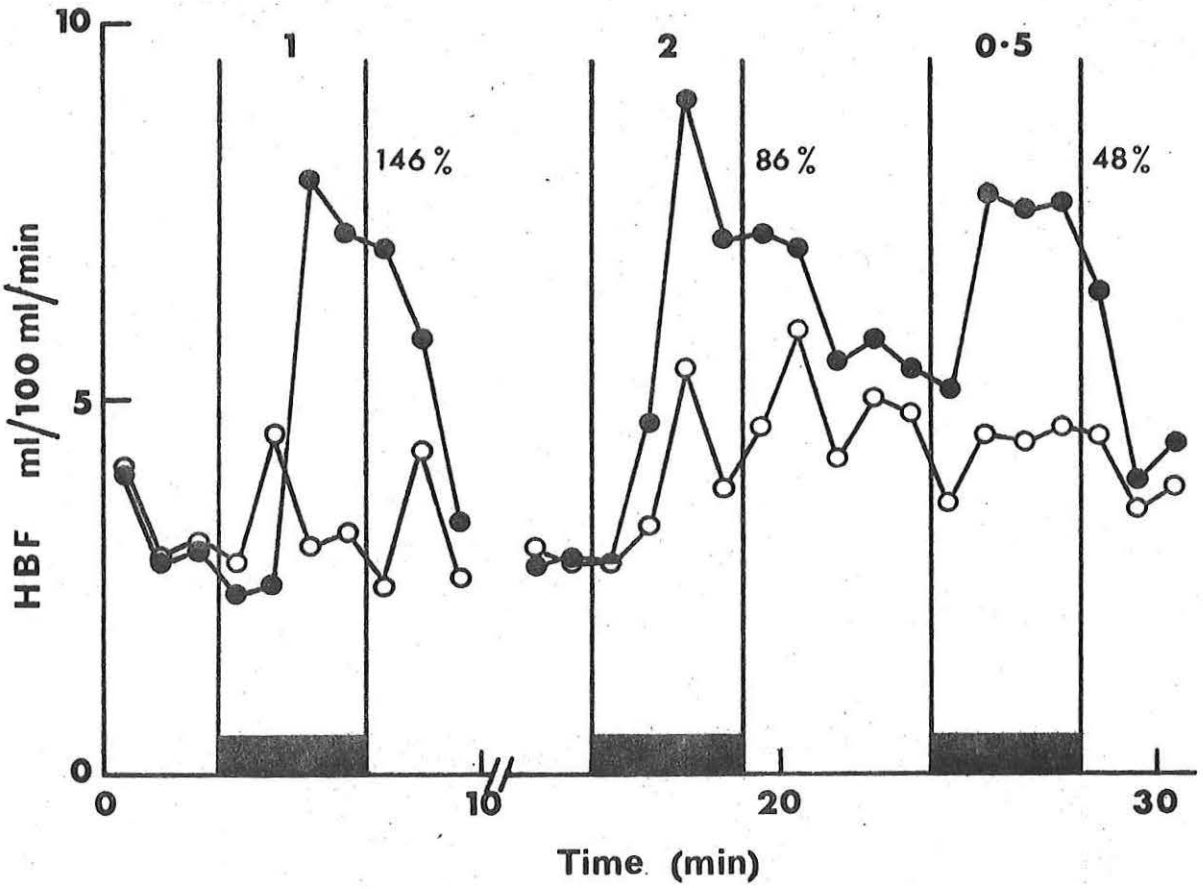


Fig. 5.2 Changes in hand blood flow in one subject (A.W.) in response to I.A. papaverine (0.5, 1 and 2 mg/min). The percentages indicate increases in flow on the infused side after corrections for fluctuations in flow on the control side. O , uninjected control side; ● , injected side.

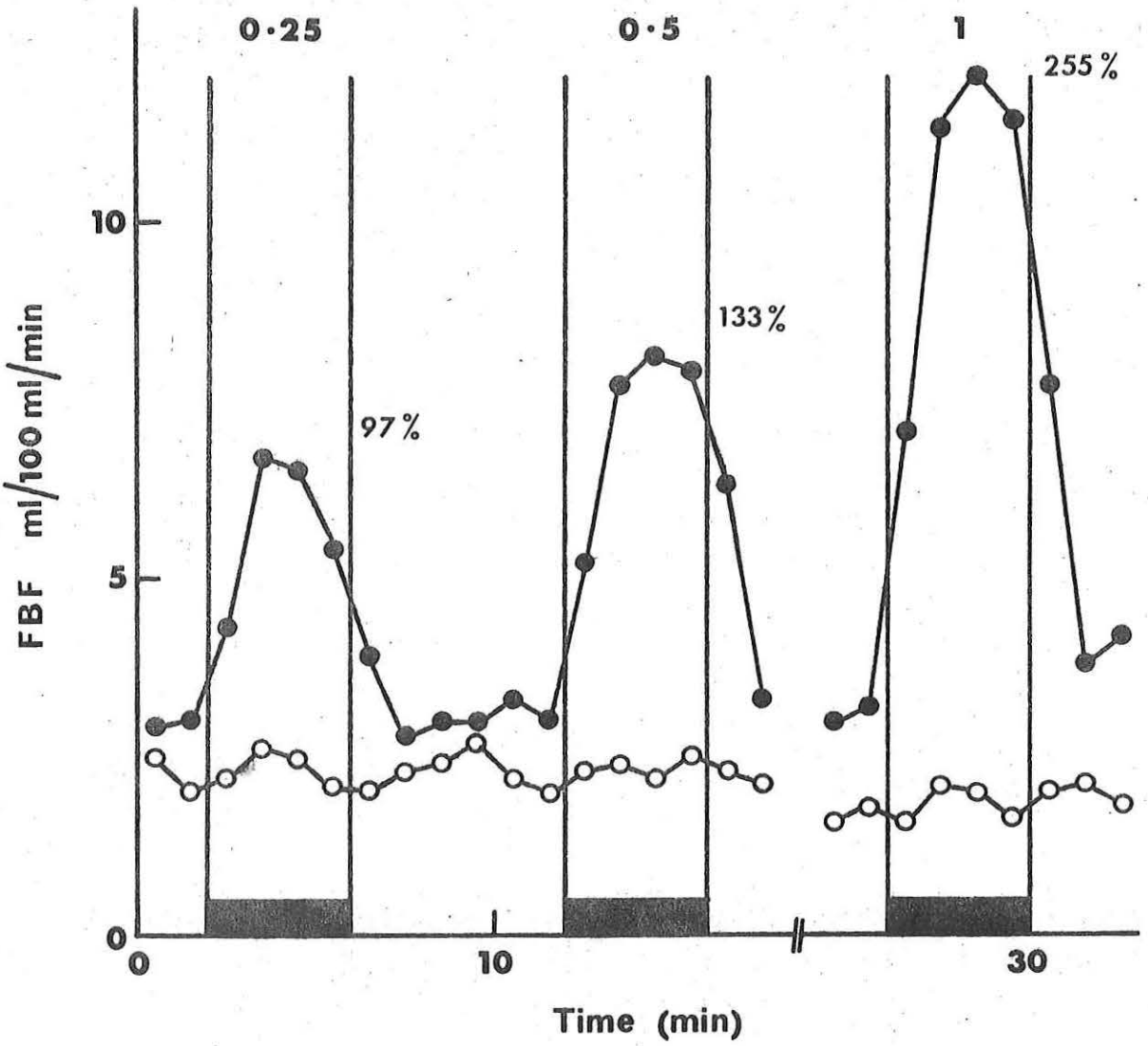


Fig. 5.3 Changes in forearm blood flow in one subject (N.B.) in response to I.A. papaverine (0.25, 0.5 and 1 mg/min). The percentages indicate the increase in flow on the infused side after correction for fluctuations in flow on the control side. O , uninjected control side; ● , injected side.

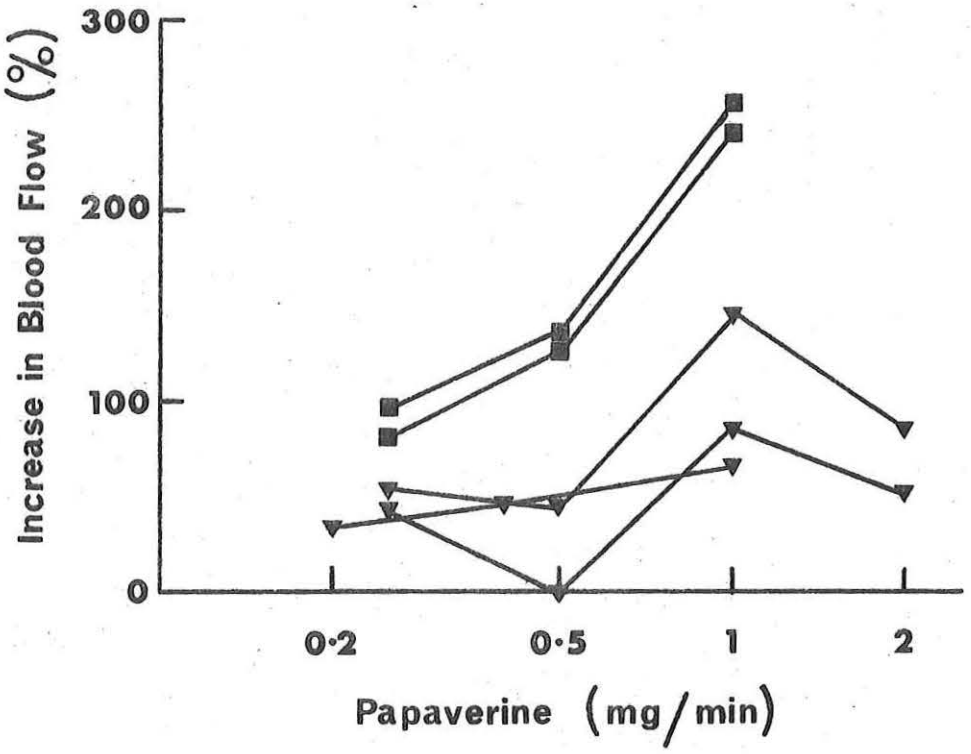


Fig. 5.4 Effect of I.A. papaverine on hand and forearm blood flow. Individual responses in five subjects to three or four doses. ▼, hand blood flow; ■, forearm blood flow.

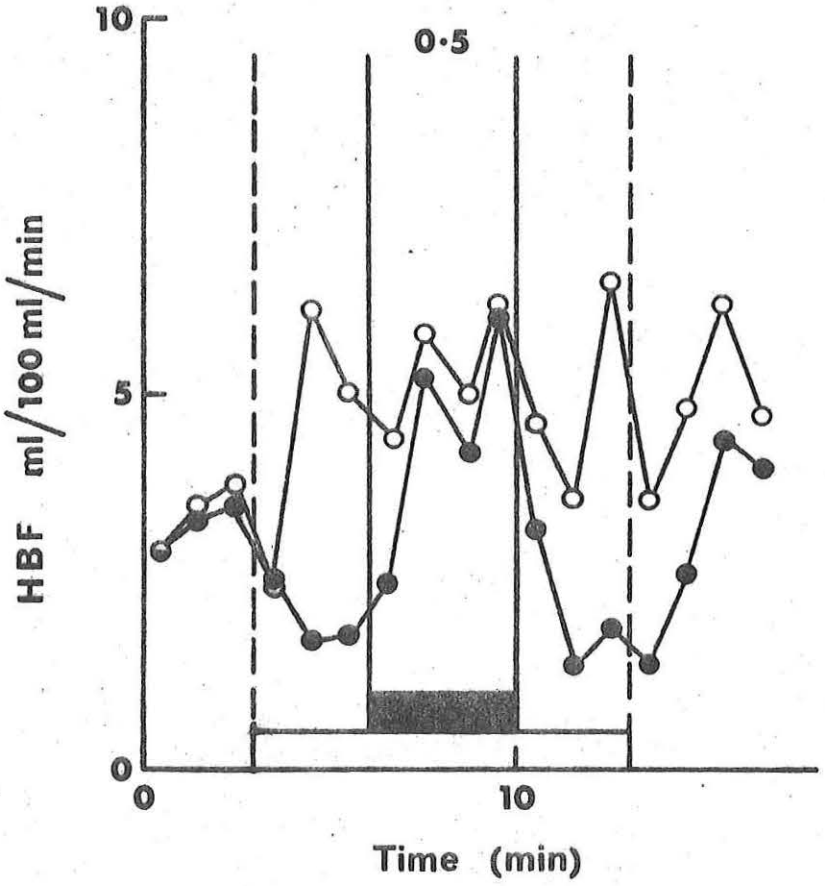


Fig. 5.5 Effect of I.A. papaverine, 0.5 mg/min (between solid vertical lines), on hand blood flow during an I.A. infusion of angiotensin (between interrupted vertical lines) in one subject (A.W.). O , uninjected control side; ● , injected side.

rapid reduction again when the papaverine infusion was terminated (Fig. 5.5).

Effects of I.V. papaverine

Papaverine 1 to 1.25 mg/kg (70 - 110 mg) was infused I.V. into three subjects over seven to ten minutes and the effect on the H.R., blood pressure, H.B.F. and F.B.F. determined (e.g. Fig. 5.6). An increase in H.R. occurred in all three subjects (mean increase 12 beats/min) within 2½ to 3 min of starting the infusion and returned to control values one to eight minutes after the infusion had finished (Table 5.1). During the infusion of 1.25 mg/kg in two subjects, occasional ectopic beats, which were not present during the control period, occurred but disappeared shortly after the end of the infusion.

During the papaverine infusion only one of the subjects had a fall in M.A.P. (7 mm Hg, 8%) which returned to control levels within eight minutes of the end of the infusion. An increase in F.B.F. (mean 39%) and a decrease in F.V.R. (mean 26%) occurred in all three subjects during the infusion and returned to control values two to three minutes after the end of the infusion (Table 5.1). However, the effects of the infusion on H.B.F. and H.V.R. were variable, and only one subject had a significant change which was a 31% fall in H.B.F. and a 34% rise in H.V.R. (Table 5.1). In this subject the effects on hand vessels lasted only three minutes after the end of the infusion.

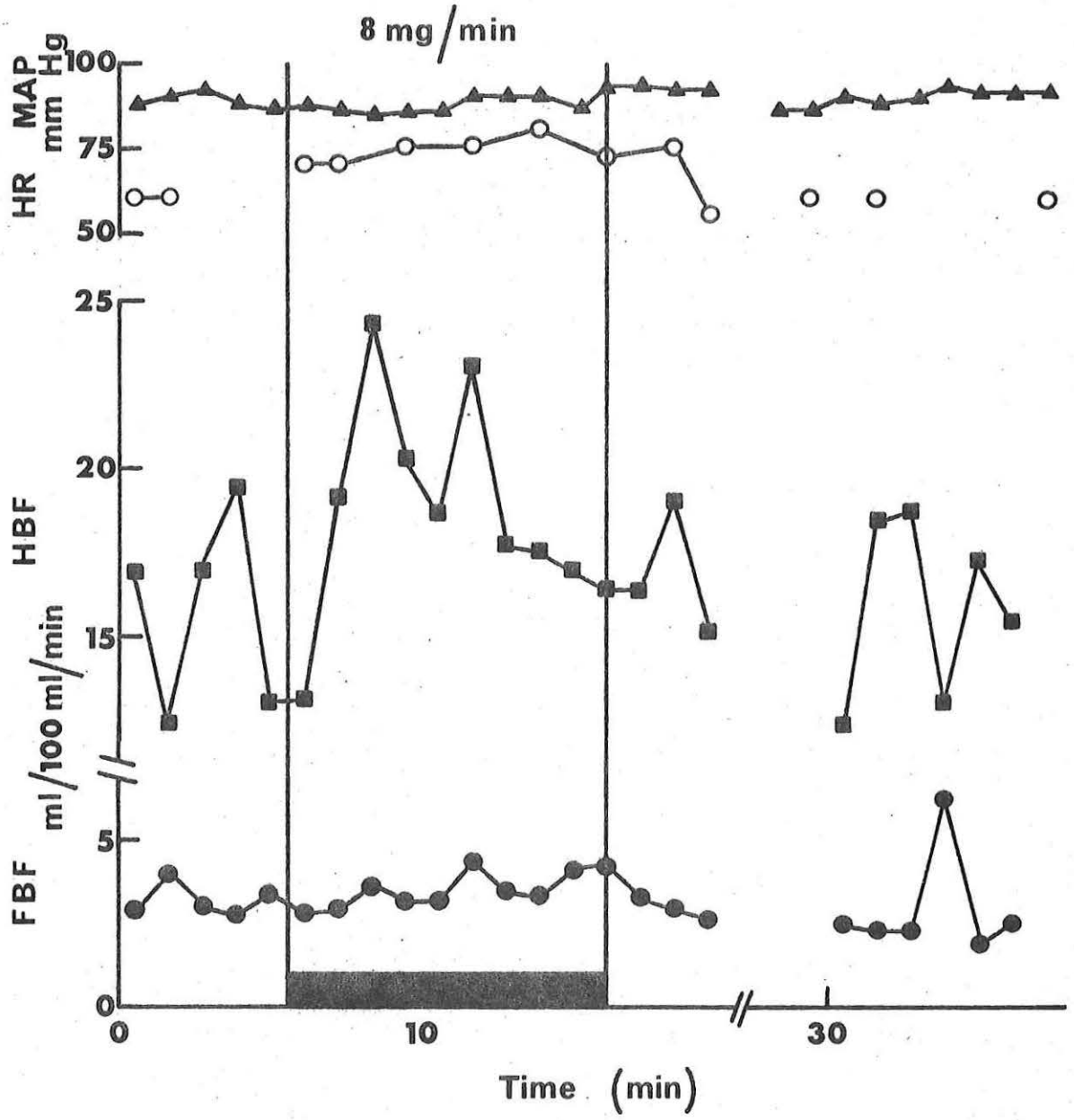


Fig. 5.6 Effect of I.V. papaverine, 8 mg/min (between vertical lines), on the mean arterial pressure (M.A.P.), heart rate (H.R.), hand blood flow (H.B.F.) and forearm blood flow (F.B.F.) in one subject (M.H.).

Subject and Dose (mg/kg)	H.R. beats/ min	H.R. % Change	M.A.P. mm Hg	M.A.P. % Change	H.B.F. ml/100 ml/min	H.B.F. % Change	H.V.R. units	H.V.R. % Change	F.B.F. ml/100 ml/min	F.B.F. % Change	F.V.R. units	F.V.R. % Change
S.M.R. B	60	167	109	0	8.8	-33	15.5	33.5	1.3	61.5	91	-34.4
(1) A	67		109		5.9		20.7		2.1		59.7	
A.C. B	60	16.7	90	-7.8	8.0	5	10	3.0	2.3	39	36	-27.8
(1.25) A	70		83		8.4		10.3		3.2		27	
M.H. B	60	30	88	0	16.4	5.5	5.3	-5.7	3.1	16.1	29	-16.2
(1.25) A	78		88		17.3		5.0		3.6		24.3	
Mean Change ± S.E.	11.7	19.4	-2.3	-2.6	-0.5	-7.5	1.7	10.3	0.7	38.9	-15.3	-26.1
	3.3	5.4	2.3	2.6	1.2	12.8	1.7	11.9	0.1	13.1	8.1	5.3
p	>0.05		>0.3		>0.7		>0.3		<0.05		>0.3	

Table 5.1 Effect of I.V. papaverine (1 or 1.25 mcg/kg) on heart rate (H.R.), mean arterial pressure (M.A.P.), hand blood flow (H.B.F.), calculated hand vascular resistance (H.V.R.), forearm blood flow (F.B.F.) and calculated forearm vascular resistance (F.V.R.) in three subjects. B, average of values in two minutes before papaverine; A, average of values in last two minutes of papaverine infusion. (Student's paired t-test.)

Venous blood samples were collected intermittently before, during and after the papaverine infusions. Determinations on the control blank plasma samples taken before the infusions gave readings of 0.02 to 0.06 mcg/ml with a mean concentration of 0.04 mcg/ml. The highest measured values resulting from the infusions were 1.16, 1.83 and 2.01 mcg/ml, and these occurred in samples taken 10, 3 and 12 minutes, respectively, after the start of the infusion (Fig. 5.7). After the infusion was stopped plasma concentration declined rapidly and final samples 20 to 38 minutes later were approximately half the peak concentrations.

Chronic Oral Administration

Plasma concentrations during non-sustained and sustained release tablets

The non-sustained release papaverine tablets were associated with greater fluctuations in plasma papaverine levels, but the average concentration achieved was higher than the average concentration reached with the sustained release tablets (Fig. 5.8 and Table A.10). However, with both preparations the plasma concentration was still showing an upward trend on the last day of intake. This was more marked with the sustained release tablets. The highest measured papaverine concentrations during the intake of non-sustained release tablets were 0.26 and 0.39 mcg/ml in the two subjects, whereas with the sustained release papaverine they were 0.14 and 0.1

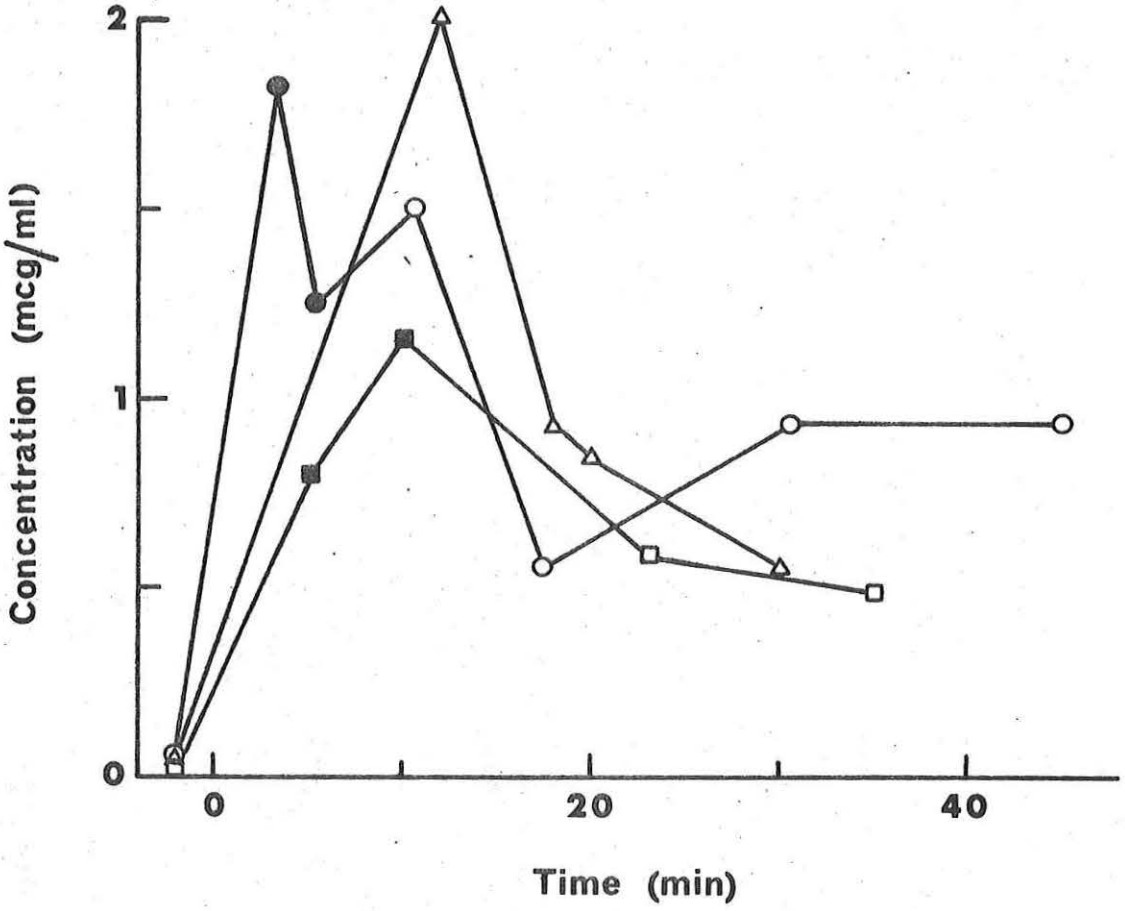


Fig. 5.7 Plasma papaverine concentrations in three subjects before, during and after an intravenous infusion of papaverine (1 or 1.25 mg/kg). Open symbols, before (control) and after the infusion; closed symbols, during the infusion.

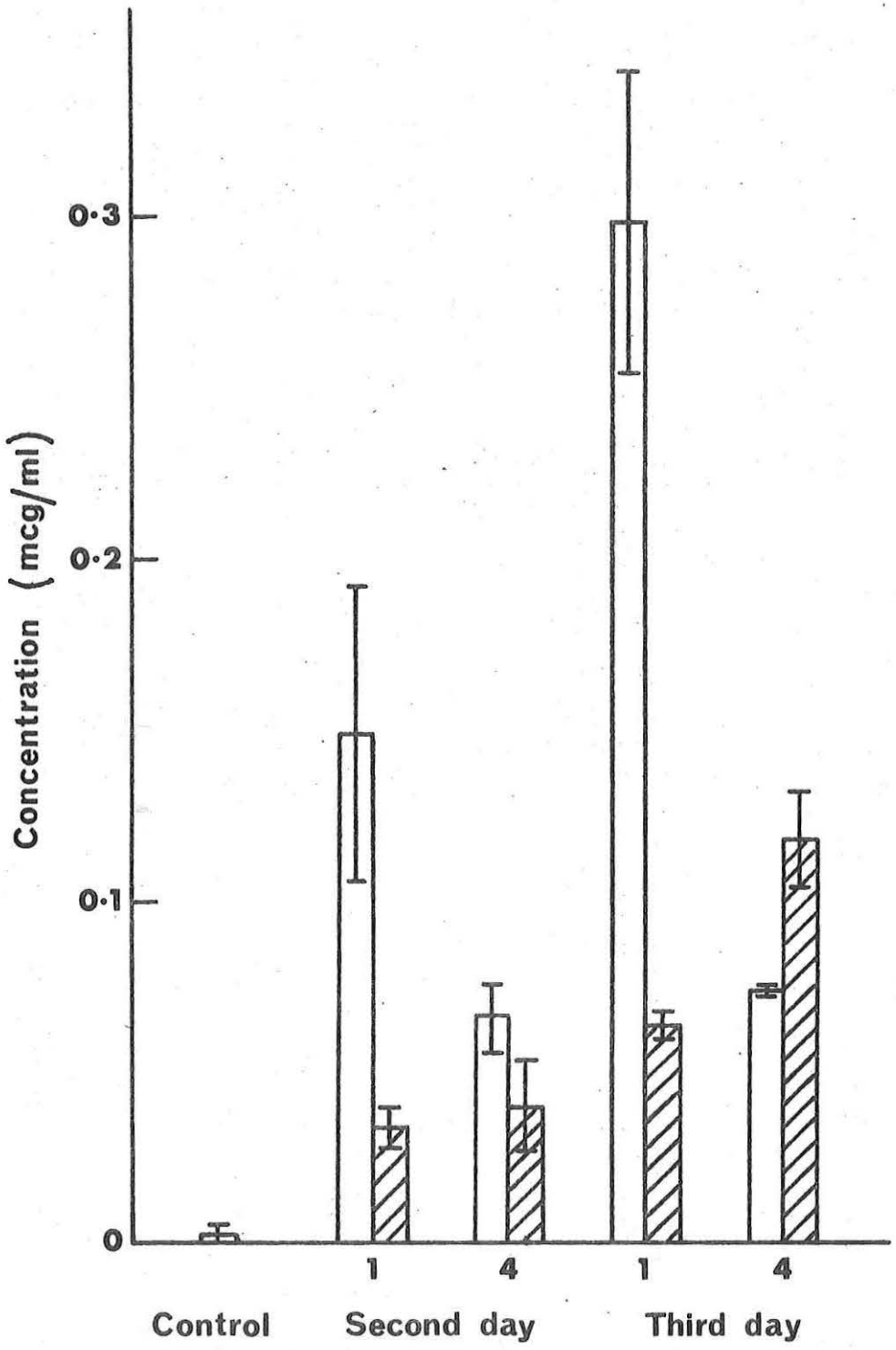


Fig. 5.8 Means (± S.E.) of the plasma papaverine concentrations in two subjects on the second and third days of oral papaverine administration (150 mg 4 times daily). Samples taken one and four hours after the second dose on each day. Open bars, concentration during non-sustained release tablet intake; cross-hatched bars, concentration during sustained release tablet intake.

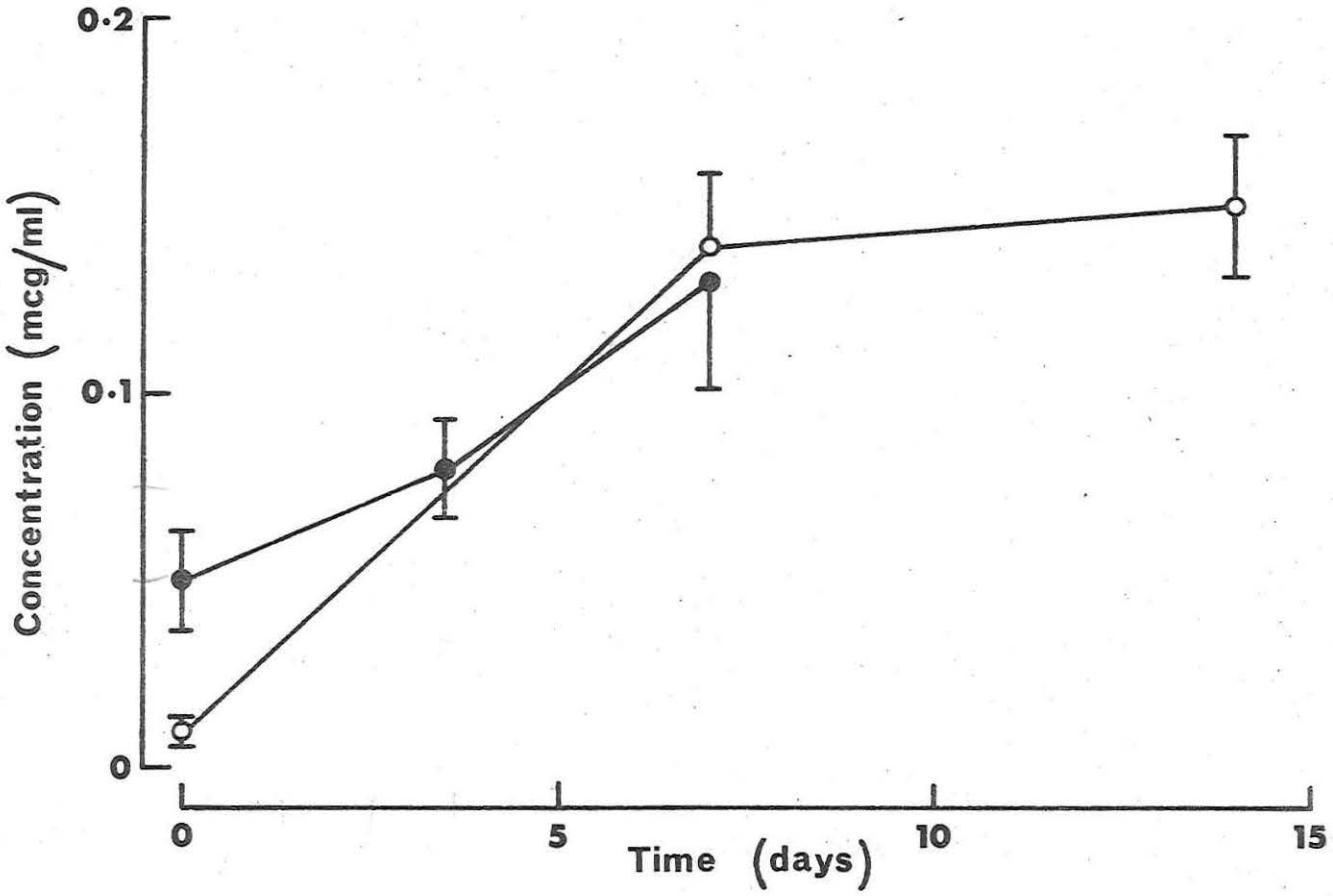


Fig. 5.9 Means (± S.E.) of plasma papaverine concentration during sustained release tablet intake. Closed symbols, concentration in four subjects taking 150 mg 12 hourly (1 week); open symbols, concentration in six subjects taking 150 mg four or five times daily (2 weeks).

mcg/ml, respectively.

Effects of sustained release tablets, 150 mg twice daily (1 week)

On a body weight basis, the papaverine intake varied from 4.15 to 5.1 mg/kg/day with a mean for the four subjects of 4.56 mg/kg/day. The mean control 'blank' plasma papaverine concentration was 0.05 mcg/ml, and after seven days of papaverine dosage the concentration had risen significantly to a mean of 0.13 mcg/ml (Table A.11 and Fig. 5.9).

There was no consistent effect of this dose of papaverine on the resting H.R., nor was there any consistent change in the pattern of the H.R. response to the Valsalva manoeuvre, leg raising or head-up tilt (Table 5.2). Two subjects had significantly greater resting H.B.F. (27 and 44%) during papaverine than in the control period, but the other two had minimal changes. All subjects had a greater fall in H.B.F. in response to the Valsalva manoeuvre while they were taking papaverine, but no consistent pattern of effect on the H.B.F. response to leg raising or head-up tilting was found (Table 5.2). Resting F.B.F. during papaverine varied from a fall of 35% to an increase of 69% when compared to control flows. No consistent change in the pattern of the F.B.F. responses to the three cardiovascular reflex tests was seen (Table 5.2).

Effects of sustained release tablets, 150 mg, 4 or 5 x daily (2 weeks)

Papaverine intake varied from 8.3 to 11.8 mg/kg/day (mean 10.08

Subject	H.R. beats /min	% Increase in H.R.			H.B.F. ml/100 ml/min	% Change in H.B.F.			F.B.F. ml/100 ml/min	% Change in F.B.F.		
		V	Leg Raise	Tilt		V	Leg Raise	Tilt		V	Leg Raise	Tilt
B	55	56	26	42	7.5	-67	-43	-55	1.7	-44	7	-22
S.M.R. A	-	-	-	-	7.9	-68	-64	-72	2.0	-4	13	9
A/B	-	-	-	-	1.05	1.01	1.49	1.31	1.18	0.09	1.86	-
B	70	45	-	23	15.5	-75	-	-40	2.9	-42	-	-30
R.L.H. A	69	53	24	27	14.9	-85	68	-27	2.9	-44	91	-14
A/B	0.99	1.18	-	1.17	0.96	1.13	-	0.68	0	1.05	-	0.47
B	61	48	14	42	4.5	-51	-44	56	1.6	-33	575	8
D.V.D. A	52	58	28	33	5.7	-67	-44	52	2.5	-15	54	-4
A/B	0.84	1.21	2.0	0.79	1.27	1.31	0	0.93	1.56	0.45	0.09	-
B	55	64	18	76	5.4	-83	22	49	1.7	-53	563	6
J.R.M. A	63	37	6	42	7.8	-98	41	8	1.1	-5	763	-8
A/B	1.15	0.58	0.33	0.55	1.56	1.18	1.86	0.16	0.65	0.09	1.36	-

Table 5.2 Effect of sustained release papaverine (150 mg twice daily) on heart rate (H.R.), hand blood flow (H.B.F.) and forearm blood flow (F.B.F.), and on responses to the Valsalva manoeuvre (V), passive leg raise and 60° head-up tilt. Individual values in four subjects before papaverine (B) and after one week of papaverine (A); A/B, potentiation of effects by papaverine.

mg/kg/day). The papaverine concentrations measured in venous samples taken from all six subjects at various intervals before and during papaverine are given in Table A.12. One subject (D.M.B.), taking a dose of 11.8 mg/kg/day, withdrew from the study after seven days because excessive sleep requirements, apparently induced by the drug, interfered with her work. No particular drowsiness occurred during the day, but she slept 2 to 3 hours longer than usual during the night and required more than the usual stimulus to waken her. The blood sample taken three hours after her last tablet had a papaverine concentration of 0.49 mcg/ml, which is more than double that measured in any of the other subjects. Her sleep needs rapidly returned to normal after papaverine intake ceased. The mean plasma concentration of papaverine in the other five subjects was not significantly higher after 14 days treatment than after seven days (Fig. 5.9), nor was there any significant difference in the mean concentrations at one week between papaverine 150 mg taken twelve hourly or four to five times daily (Fig. 5.9).

The circulatory effects of papaverine were measured in only three of the six subjects. All three showed a fall in the resting F.B.F. (4, 50 and 62%), though the effect was variable and significant in only two. The responses of the forearm vessels to mental arithmetic and the Valsalva manoeuvre were not consistently altered by chronic papaverine; in two subjects the reflex responses were impaired,

Subject	Mental Arithmetic % Increase in F.B.F.			Valsalva Manoeuvre % Fall in F.B.F.		
	B	A	A/B	B	A	A/B
J.N.Mc.	185	112	0.67	30	16	0.53
N.W.	248	130	0.52	54	43	0.80
B.J.D.	32	55	1.72	26	59	2.27

Table 5.3 Effect of sustained release papaverine (150 mg 4 or 5 times daily) on the forearm blood flow response to mental arithmetic and the Valsalva manoeuvre in three subjects. B, before papaverine; A, after 2 weeks of papaverine; A/B, potentiation of effects by papaverine.

while in the third they were enhanced (Table 5.3).

One subject (B.A.G.), taking 150 mg four times daily (10.5 mg/kg/day) for 14 days, commented that he had no migraine attacks during the period on papaverine and for two weeks thereafter. Previously, and subsequently, migraine headaches occurred at intervals of about 10 days. The plasma concentrations in this subject were between 0.19 and 0.21 mcg/ml during papaverine therapy.

One subject (S.M.R.) was given papaverine (I.V. and oral) on several occasions separated by intervals of from 2 weeks to five months and, despite an increase in the dose, each administration was accompanied by lower plasma papaverine concentrations. Thus concentrations during 150 mg twice daily (Table A.11) were considerably higher than those measured on a later occasion when 150 mg four times daily was being taken (Table A.12).

DISCUSSION

Acute Papaverine

The intra-arterial infusions confirmed that papaverine dilates normal and constricted human vessels, though the effects only lasted for the duration of the infusion. Increasing the dose did cause a greater dilatation of forearm vessels, but the duration of effect was not prolonged. Local infusions are therefore likely to be useful only in conditions where the vasoconstriction is brief, e.g. the spasm resulting from surgical handling, though direct application to the

external surface of a vessel may have a more prolonged effect. The effects on forearm blood flow were greater and more consistent than those on hand blood flow, which may indicate a greater effect on muscle than skin vessels. However, it is also possible that the difference merely results from a difference in the concentration of papaverine reaching the vessels of the two segments of the arm.

The patients in whom Gray *et al.* (1945) had noted the arrhythmic effects of papaverine (65 mg I.V.) were all being treated for angina pectoris and might be expected to be more sensitive to drugs capable of affecting cardiac rhythm. However, the occurrence of ectopic beats in the two subjects given 1.25 mg/min in the present study shows that cardiac arrhythmias can be produced in young healthy men. The other cardiovascular effects of note, namely a 19% increase in H.R. and 39% increase in F.B.F., were also of short duration. These were recorded at a time when the plasma concentration was approximately 1.2 mcg/ml. The fall in plasma levels after the end of the infusion was rapid and confirms a finding of a similar rapid decline after infusions of 3 mg/kg (Axelrod *et al.*, 1958). In the dog, papaverine (I.V.) is rapidly taken up by the liver and adipose tissue, where one hour after the infusion concentrations are 3-4 times those in plasma and other tissues (Axelrod *et al.*, 1958). There are no direct studies of distribution in man, but Axelrod found that less than 1% of the unchanged drug appeared in the urine, indicating almost complete

degradation in the body. The human liver, like that of the dog, may therefore concentrate papaverine and be partly or wholly responsible for the rapid fall in plasma concentration and consequently for the brevity of the cardiovascular effects after I.V. infusion.

Chronic Papaverine

The most notable findings in the chronic studies were the low plasma concentrations produced by the sustained release papaverine and the failure to demonstrate any marked cardiovascular effects.

The doses of oral papaverine were 5 to 10 times the I.V. dose used and yet the plasma concentrations were one-sixth to one-tenth those during I.V. papaverine. Using a non-sustained release preparation, Axelrod *et al.* (1958) showed that absorption from the human gastro-intestinal tract is relatively rapid and essentially complete. Absorption studies with the sustained release tablet have not been done, but it is possible that this tablet formulation results in more gradual, though less complete, absorption of the papaverine. However, the main cause for the marked disparity in plasma levels after I.V. and oral administration probably results from hepatic removal of substantial amounts of papaverine from the portal circulation before it reaches the systemic circuit. Even if the papaverine from sustained release formulation was completely absorbed, its slower rate of absorption would favour more efficient hepatic uptake. The role of the liver in determining the fate of oral papaverine in man needs

to be clarified, but the foregoing conjecture could explain the difference in plasma levels resulting from oral and I.V. routes of administration and from sustained and non-sustained release formulations.

In the subject taking the largest dose of papaverine (11.8 mg/kg/day), who withdrew from the study after one week, plasma concentrations were more than double those in the subject with the next highest levels. The concentrations were, in fact, similar to those reported by Axelrod *et al.* (1958) in two subjects taking 200 mg six hourly. However, there is no obvious explanation for the considerable difference from the other observations in the present study. The possibility of enzyme induction during prolonged use of papaverine is raised by the observation, in one subject, of a progressive decline in plasma concentrations with successive administrations.

In view of these studies on plasma papaverine concentrations after different oral preparations and dose schedules, it is apparent that any future investigations of dose response relationships should be based on plasma concentrations rather than the orally administered dose.

With respect to the cardiovascular measurements in the chronic studies, it must be noted that it is difficult to be certain that baseline or resting measurements of circulatory variables obtained at intervals of one week or more represent a sufficiently similar

cardiovascular status for a valid comparison to be made. However, the experimental conditions were standardized as completely as possible and the assumption was made that any significant change was the result of the drug.

Although the doses in the present study (300-750 mg daily) were similar to those used and recommended by previous workers (Stern, 1965, 1966, 1967; Tibbs, 1969), there was no consistent increase in upper limb blood flow. In fact, with 600-750 mg/day a reduction in forearm blood flow was recorded in the three subjects tested. However, previous claims that chronic papaverine increased limb blood flow were based on indirect assessments, such as an improvement in the distance walked before the onset of claudication (Stern, 1965, 1967; Tibbs, 1969). In a more recent trial, Batterman, Jensen & Jensen (1970) found that chronic papaverine increased digital pulse volume (measured plethysmographically) in patients with rheumatoid arthritis, but decreased it in patients with osteoarthritis and in normal subjects. They attributed this difference in effect to the relief of digital vasospasm, which occurs in rheumatoid patients but not in normal or osteoarthritic subjects. From this evidence it can be tentatively concluded that chronic papaverine has significant effects only on vessels in spasm and that studies on its efficacy need to be conducted using patients with such pathophysiology. The observation made by one subject in the present study that during chronic papaverine

the incidence of migrainous headaches was markedly reduced is a further indication that papaverine is only likely to be of therapeutic benefit in patients with constricted or hyper-reactive vessels.

Cardiovascular reflex responses were not consistently altered by chronic papaverine treatment. This may be evidence that the drug is without effect on the autonomic nervous system and that side effects, such as postural hypotension, are unlikely to be encountered during its therapeutic use. However, the production of unequivocal evidence that chronic papaverine does not affect cardiovascular reflexes must await studies in which a significant effect of the drug on the circulation of the subjects is present.

SUMMARY

Brachial artery infusions of papaverine caused vasodilatation in the hand and forearm and a similar, though less marked, effect in the forearm was observed during intravenous infusion. Cardiac effects, manifested as an increase in heart rate and the occurrence of occasional ectopic beats, were also observed during I.V. infusions. The cardiovascular effects of acute systemic administration of papaverine were brief, and the duration of this response correlated well with the plasma concentration which declined rapidly when the infusion was stopped.

Chronic papaverine was administered to normal subjects as a sustained release tablet in two dose schedules. The plasma level at

the end of a week with each of the dose levels was only about one-sixth of that associated with vasodilator effects after intravenous administration. The lower dose did not consistently alter hand or forearm blood flow, though reflex vasoconstriction in the hand caused by the Valsalva manoeuvre was potentiated. The higher dose consistently reduced forearm blood flow and reflex responses were not consistently changed. At neither dose was there any correlation between plasma concentration and the cardiovascular effect in an individual subject. It is concluded that papaverine lacks significant vasodilator effects when given orally, even in doses five to ten times those which cause vasodilatation and cardiac effects when given intravenously. The failure to attain adequate plasma concentrations of the drug is the most probable cause for this finding.

APPENDIX

METHODS

Venous Occlusion Plethysmography

Assumptions

There are three basic assumptions underlying the technique of venous occlusion plethysmography:

1. That the application of the pneumatic collecting cuff pressure does not affect arterial pressure inflow;
2. That complete venous occlusion is effected for long enough to measure flow;
3. That the resulting venous distension and increase in pressure do not initially reduce the rate of arterial inflow.

These assumptions have all been critically examined by a number of investigators (e.g. Landowne & Katz, 1942; Wilkins & Bradley, 1946; Greenfield & Patterson, 1954a; Formel & Doyle, 1957) who have shown that they are valid provided the technique is correctly and meticulously applied.

Apparatus

A variety of recorders have been developed to measure the rate of increase in limb volume. Two different types were chosen for the present studies, viz. the water-filled plethysmograph with temperature control described by Greenfield (1954) and the mercury-in-rubber strain gauge described by Whitney (1953).

The water-filled plethysmograph has some potential disadvantages

in that a hydrostatic pressure is imposed on the limb and large pressure changes may accompany the volume change. The effect of the latter is minimised by the wide bore chimney used in the Greenfield plethysmograph. Furthermore, Wallace (1958) showed that in the range of hydrostatic pressures encountered with the normal use of this instrument the recorded blood flow was not affected. The advantages claimed for it are that because water is incompressible and has a small coefficient of thermal expansion, volume changes are rapidly and accurately reproduced by the recorder. Furthermore, the small coefficient of heat conduction of water permits the maintenance of more stable thermal conditions surrounding the segment of the limb being observed.

Theoretically, the rate of swelling of the whole segment being observed should be measured. In the case of the hand, this requirement can be met since the plethysmograph will accommodate the whole hand and the venous occlusion cuff can be applied close to the apparatus (Fig. A.1). However, in the case of the forearm, only a portion of the total segment can be enclosed (Fig. A.2). It is assumed that the collected blood causes the portion in the plethysmograph to swell in proportion to the rate of inflow without significant displacement of tissue or body fluid from the plethysmograph. As long as the technique is used carefully the assumption is valid (Formel & Doyle, 1957). Grant & Pearson (1938) showed that venous

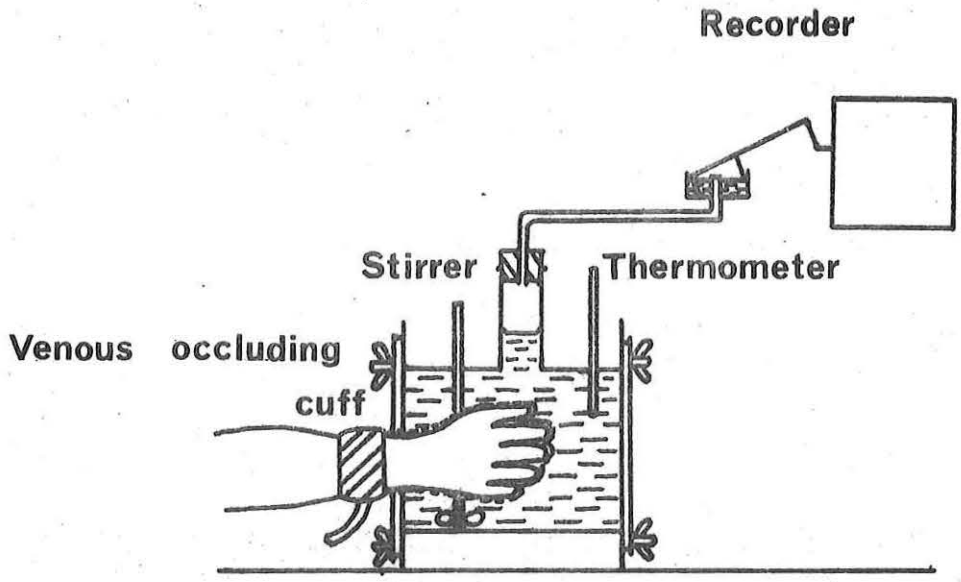


Fig. A.1 Diagrammatic representation of the water-filled plethysmograph used for measurement of blood flow in the hand.

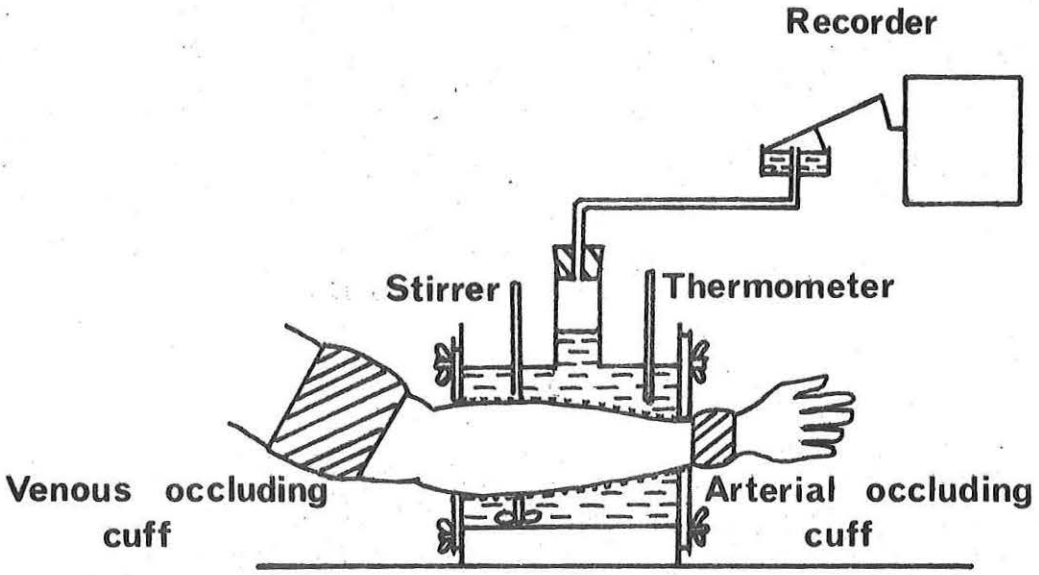


Fig. A.2 Diagrammatic representation of the water-filled plethysmograph used for measurement of blood flow in the forearm.

filling of the enclosed portion of the forearm could be considerably influenced by venous return from the part lying distal to the plethysmograph. They therefore introduced the modification of applying an arterial occlusion cuff at the wrist to eliminate this source of error. Forearm blood flows have been found to be initially disturbed by the arterial occlusion but usually become stable within one minute (Kerslake, 1949). Blood flow in the opposite hand will also be disturbed by a sudden inflation of a wrist cuff, but the disturbance can be avoided by slow and even cuff inflation over ten seconds (Roddie, 1951).

In some of the experiments during the papaverine studies, forearm blood flow was measured using the Whitney strain gauge. This measures the relative change in circumference of the limb at a particular point. In order to relate changes in circumference to changes in limb volume, Whitney (1953) made the following assumptions:

1. When a limb changes in volume, there is no significant change in the dimension measured in the long axis of the limb.
2. The whole of the volume change is absorbed in corresponding changes of the transverse sectional area of the limb.
3. At any particular level of the limb along its axis, the relative change in volume at that level will be equal to the relative change in the transverse sectional area at that level.
4. If the transverse section is truly circular and if the shape

remains unaltered during expansion and contraction, the relative change in area dA/A will be equal to twice the relative change in circumference dC/C of the section for a small change in area.

Geometrical considerations show that this relationship applies to sections of other shapes if the shape remains unaltered by expansion or contraction (Whitney, 1953, Appendix I). Whitney concluded that on theoretical grounds it was feasible to deduce relative changes in volume of a limb, dV/V, at a particular level along its axis from corresponding measurements of the relative changes in circumference, dC/C, of the limb at the same level, i.e. $dV/V = 2 \cdot dC/C$.

Use of the slide caliper

In order to obtain direct readings of blood flow (ml/100 ml/min), the time base (T_x) setting for the slide caliper has to be determined for each flow record. In the case of the water-filled plethysmograph, this time base was calculated using the formula given by Greenfield *et al.* (1963):

$$T_x = \frac{T_{60}}{V \cdot h}$$

where T_x = the distance in cm travelled by the recording paper in x seconds

T_{60} = the distance in cm travelled by the recording paper in 60 seconds

h = the vertical deflection of the recorder writing point for a volume increment of one ml

V = the volume of the limb segment expressed in hundreds of ml.

The formula was modified for determining the time base in the case of Whitney strain gauge flow readings:

$$Tx = \frac{T_{60} \cdot 200}{C \cdot h'}$$

where 200 = the factor required to convert dC/C to dV/V in ml/100 ml/min

h' = the vertical deflection of the recorder writing point per unit stretch of the strain gauge

C = the limb circumference at the point of application of the strain gauge in cm.

Duff's correction

Duff (1952) assumed that, except for the infused drug, conditions on the experimental side were the same as those on the control side. Since flows normally exhibit parallel and approximately equal changes in each limb (Cooper, Cross, Greenfield, Hamilton & Scarborough, 1949; Greenfield & Patterson, 1954b), a correction may be applied to derive an estimate of what the flow on the infused side would have been in the absence of the drug.

Let A = the average of 2 minutes run-in for the test limb blood flow

a = the average of 2 minutes run-in for the control limb blood flow

b = the average of the final 2 minutes of drug infusion for control limb flow

B = the actual average of the final 2 minutes of drug infusion for the test limb flow

E = the estimate of what the test limb flow would have been during the experimental period in the absence of the drug

then A/E = a/b, and E = A.b/a;

therefore the effect of the drug on test limb blood flow = E-B, or expressed as a percentage = (E-B).100/E.

The effect of the I.A. needle or catheter and any local anaesthesia is ignored in this correction and, short of placing a needle in the brachial artery on the control side to infuse saline alone, no correction for such effects can be made.

Duff determined the limits of error for the correction he applied in nine subjects (including four with bilateral sympathectomy of the upper limbs and one with unilateral sympathectomy). In 36 comparisons of actual and estimated blood flow means, the per cent. departure from the expected value of nil was found to have a standard deviation of 12%. This was taken to be a valid expression of the S.D. of per cent. changes in blood flow with which to determine the significance of changes in blood flow. Thus changes in blood flow during an infusion of less than 25% were not regarded as significant. Greenfield & Patterson (1954b) tested, in two subjects, the validity

of applying the correction when determining the effects of experimental procedures on the forearm blood flow. No figures were given, but the variation about the 100% equivalence line of corrected and measured flows shown in a graph was wide (approximately 25%). Changes in F.B.F. should therefore also be regarded as being significant only when they exceed a 25% change as Duff showed for H.B.F.

Histochemistry

The technique for demonstrating the site and density of the sympathetic nerves in the three isolated vessels used in these studies was based on the histochemical method of Falck (1962) as modified for the rabbit ear artery by Waterson & Smale (1967). In principle, this technique consists of freeze drying the tissue, followed by exposure to formaldehyde gas to convert the catecholamines present to isoquinolones. The treated tissue is then embedded in paraffin wax and sections cut and examined by fluorescence microscopy.

After removal from the animal, vessels were placed in Krebs solution at room temperature if histochemical preparation was to be done within half an hour, or kept at 4^{o} C if preparation was delayed longer than this. The first step was to quickly plunge the tissue into an acetone and dry ice mixture to rapidly freeze it before transfer to the pre-cooled freeze drying apparatus (Thermovac, Model F.D./3). The freeze drying continued for 16 to 20 hours at temperatures ranging from -60^{o} C to -40^{o} C and at pressures of 15 to 40

microns of mercury. The exposure to formaldehyde gas then took place in a glass jar containing 5 gm of paraldehyde powder which had been stored over 34% v/v sulphuric acid at a relative humidity of 70% for at least seven days (the paraformaldehyde was changed every two weeks). This standardization of the paraformaldehyde powder for water content (Hamberger, Malmfors & Sachs, 1965) was essential if significant fluorescence due to formaldehyde treatment was to be seen. The glass jar was sealed and placed in an oven at 80° C for one hour, which was sufficient time for the development of the noradrenaline fluophore (Falck & Owman, 1965). Following this phase, the vessels were vacuum infiltrated (water vacuum, using National Appliance Company cabinet) for thirty minutes with paraffin at 65° C. They were then embedded in paraffin wax, and subsequently tissue sections of 7 microns thickness were cut by means of a Leitz model 1212 microtome and mounted in an Entallan and xylol mixture. Tissue sections were examined and photographed through a Leitz Ortholux microscope with a dry dark field condenser. Fluorescence was obtained by means of an HBO 200 W mercury vapour lamp, using a 3 mm Schott BG 12 excitation filter and a 510 millimicron barrier filter. A Leica camera, with microscope and exposure meter attachments, was used to photograph the sections on Kodak Photoflure film at exposures of 5 to 10 seconds and developed in Ilford I.D.2 developer.

Rat Tail Blood Pressure

The method used was similar to that described by Lucas (1971). An appropriate sized cuff is applied to the base of the rat's tail and inflated above systolic blood pressure. The volume of the tail distal to the cuff is measured plethysmographically, and as the cuff pressure is slowly released, tail volume will increase suddenly at about mean arterial pressure. Two modifications of the technique were introduced: (a) a mercury-in-rubber strain gauge (single strand) was used to sense the volume change in the tail instead of a water-filled plethysmograph; and (b) the conscious animal was restrained in a warmed perspex cylinder (Gooden, 1971). Cuff pressure was measured with a Statham pressure transducer (P23 DC), and the outputs of the transducer and the strain gauge were recorded by a Rikadenki pen writing recorder.

Plasma Papaverine Assay

Blood sampling

A 15-20 ml sample of venous blood was taken in a sterile syringe. The sample was then transferred to a cooled tube containing 0.2 ml of oxalic acid (7.5%) and centrifuged at 10,000 r.p.m. for 20 min. Clear plasma (7-10 ml) was pipetted off into a screw capped bottle and stored at -20^{o} C until air freighted to Mead Johnson (Pty Ltd) Laboratories, Sydney. The plasma samples were usually still frozen upon arrival at the laboratories.

Analytical procedure

Five ml of plasma were added to 5 ml of N/5 NaOH solution in a 150 ml separating funnel. To this 25 ml of n-heptane-iso-amyl alcohol was added and shaken for 15 minutes. After removing the aqueous layer, the solvent layer was transferred to a stoppered 50 ml tube for centrifuging at 4,000 r.p.m. for 5 minutes, then 20 ml of the centrifuged solvent layer was transferred to a dry 150 ml separating funnel. After addition of 1 ml N/10 hydrochloric acid, the funnel was shaken for 5 minutes. When the aqueous layer was perfectly clear, this was run into dry matched spectrophotometric cells (Beckman spectrophotometer) and the extinction read at 251 mμ and 266 mμ using N/10 HCl as blank.

At the same time, a standard solution containing 1.8 mcg was run through the same procedure. The papaverine content in the plasma sample was calculated in mcg/ml using the formula:

$$\frac{E\ 251 - E\ 266}{E'251 - E'266} \times 1.8 \times \frac{20}{\text{aliquot solvent used}} \times \frac{25}{20} \times \frac{1}{\text{mls serum used}}$$

where E = extinction of sample

E' = extinction of standard

Recovery percentages determined for 1.6-1.8 mcg papaverine/ml were 95-100%. Plasma levels are quoted without correction for recovery percentages. The lower limit of sensitivity of the assay is approximately 0.05 mcg/ml and accuracy of determinations would fall away sharply below that value.

DRUGS AND SOLUTIONS

Concentrations of l-noradrenaline are expressed in terms of the base; those of clonidine hydrochloride, histamine acid phosphate, phentolamine methanesulphonate, cocaine hydrochloride and methoxamine hydrochloride are expressed in terms of the salts; those of potassium in terms of the ion.

The various drugs used were:

Angiotensin amide (Ciba)

Clonidine hydrochloride (Boehringer Ingelheim)

Cocaine hydrochloride (MacFarlane Smith)

Guanethidine sulphate (Ciba)

Histamine acid phosphate (Koch-Light)

Methoxamine hydrochloride (Burroughs Wellcome)

Noradrenaline bitartrate monohydrate (Koch-Light, animal experiments; Winthrop, human experiments)

Papaverine hydrochloride (solution for injection, Knoll; non-sustained release tablets, Ethnor; sustained release tablets, Mead Johnson)

Phenoxybenzamine hydrochloride (Smith, Kline & French)

Phentolamine methane sulphonate (Regitine, Ciba)

Reserpine (Serpasil, Ciba)

Reserpine placebo (Serpasil placebo, Ciba)

Krebs solution was made up with the following composition in mM:

NaCl 118, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6, $CaCl_2$ 2.5 and $MgCl_2$ 1.0.

STATISTICAL METHODS

1. Mean

Arithmetic: $\bar{x} = \frac{\Sigma x}{n}$

where $\bar{x}$ = mean (arithmetic)

x = individual measurement or item

n = number of individual measurements or items

Geometric: log x is substituted for x and the antilog of the mean is the geometric mean

2. Standard deviation

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

3. Standard error of the mean

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

4. Student unpaired t-Test of Significance

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}} \qquad (n_1 + n_2) - 2 \text{ degrees of freedom}$$

where $\bar{x}_1$ = mean of one series of observations

$\bar{x}_2$ = mean of second series of observations

n_1 = number of items in first series

n_2 = number of items in second series

s = standard deviation of total sample

5. Student paired t-Test of Significance

$$t = \frac{\bar{x}}{S.D.}\sqrt{n-1} \qquad n-1 \text{ degrees of freedom}$$

where $\bar{x}$ = mean of the differences between paired observations

S.D. = the standard deviation of pair differences

n = the number of pairs

Subject	Infused side		Control side		Duff correction A.b/a=E	E-B	% $E-B/_E$
	Run-in A	Final B	Run-in a	Final b			
CLONIDINE 250 ng/min							
D.K.B.	13.5	2.0	19.5	17.6	12.2	10.2	84
P.Mc.	5.5	3.0	3.7	3.3	4.9	1.9	38
J.S.C.	7.9	5.1	12.8	11.6	7.2	2.1	28
P.S.	6.8	1.7	6.8	4.8	4.8	3.1	64
J.O.	2.0	2.2	7.7	11.4	3.0	0.8	25
D.B.	8.0	5.1	6.4	7.7	9.6	4.5	47
Mean	7.3	3.2			7.0		48
± S.E.	1.5	0.6			1.4		9.3
p	A - B >0.1				E - B <0.05		
CLONIDINE 500 ng/min							
D.K.B.	10.2	1.1	12.3	15.4	12.8	11.7	92
P.Mc.	4.6	2.6	3.0	6.6	10.1	7.5	75
J.S.C.	6.3	3.1	10.7	9.5	5.6	2.5	44
B.G.	12.8	1.6	10.0	7.7	9.9	8.3	84
T.R.	5.7	3.8	8.8	9.1	5.9	2.1	38
Mean	7.9	2.4			8.9		67
± S.E.	1.5	0.5			1.4		10.8
p	A - B <0.05				E - B <0.05		

Table A.1(a) Effect of I.A. clonidine (250 and 500 ng/min) on hand blood flow (ml/100 ml/min) in eight subjects. Individual flows on control and infused sides, before (a & A) and after (b & B) clonidine. E, corrected flow on infused side. E-B, effect of drug on injected side (ml/100 ml/min); (E-B)/E, percentage effect of drug. (Student's unpaired t-test.)

CLONIDINE 250 ng/min							
	Infused side		Control side		Duff correction A.b/a=E	E-B	% E-B/E
	Run-in A	Final B	Run-in a	Final b			
G.M.	3.1	1.0	2.4	2.3	3.0	2.0	67
R.S.	2.5	1.4	2.8	2.5	2.2	0.8	34
F.G.	3.1	1.1	2.7	2.9	3.3	2.2	67
J.C.	2.8	1.9	2.0	1.9	2.7	0.8	27
R.Mc.	1.8	1.1	2.2	1.6	1.3	0.2	19
P.F.	3.1	1.5	2.3	2.7	3.6	2.1	59
Mean	2.7	1.3			2.7		46
± S.E.	0.2	0.1			0.3		9
p	A - B <0.01				E - B <0.02		
CLONIDINE 125 ng/min							
G.M.	3.7	2.0	3.1	3.0	3.5	1.5	43
R.S.	2.9	2.5	3.2	3.2	2.9	0.4	17

Table A.1(b) Effect of I.A. clonidine (125 and 250 ng/min) on forearm blood flow (ml/100 ml/min) in six subjects. Notation as given for Table A.1(a).

HAND BLOOD FLOW (ml/100ml/min)								FOREARM BLOOD FLOW (ml/100ml/min)			
250 ng/min				500 ng/min				500 ng/min			
Subject	Run-in A	Final B	Diff. A-B	Subject	Run-in A	Final B	Diff. A-B	Subject	Run-in A	Final B	Diff. A-B
J.S.C.	10.6	10.8	-0.2	J.S.C.	9.4	9.9	-0.5	J.C.	7.3	7.5	-0.2
P.S.	12.3	11.7	0.6	P.Mc.	14.2	14.8	-0.6	R.Mc.	4.5	4.2	0.3
J.O.	21.3	24.8	-3.5	T.R.	9.1	12.4	-3.3	P.F.	9.9	8.6	1.3
Mean	14.7	15.8	-1.0	Mean	10.9	12.4	-1.5	Mean	7.2	6.8	0.3
± S.E.	3.3	4.5	1.3	± S.E.	1.7	1.4	0.9	± S.E.	1.6	1.3	0.4
p			>0.5	p			>0.1	p			>0.4

Table A.2 Effect of adrenergic alpha receptor blockade on the response of hand and forearm blood flow (ml/100 ml/min) to I.A. clonidine (250 and 500 ng/min). Individual flows in eight subjects before (A) and after (B) clonidine in the injected limb. (Student's paired t-test.)

Subject	Control value (units)	Maximum value after clonidine			Minimum value after clonidine		
		Absolute (units)	% of control	Time (min)	Absolute (units)	% of control	Time (min)
S.M.R.	13.4	20	+49	2½	6.9	-49	25
J.A.D.	16.2	32.4	+100	8	10	-38	41
N.C.	5.0	10.9	+118	8	5.3	+6	27
G.G.	6.1	20.5	+236	4	7.7	+26	25

Table A.3 Effect of I.V. clonidine, 150 mcg, on calculated hand vascular resistance in four subjects. Absolute and percentage changes at times when the biphasic effects were maximum are shown.

Subject	I.V. Noradrenaline (mcg/min)	M.A.P.				H.R.				H.V.R.			
		Absolute Rise (mm Hg)		Percentage Rise		Absolute Fall (beats/min)		Percentage Fall		Absolute Rise (Units)		Percentage Rise	
		B	A	B	A	B	A	B	A	B	A	B	A
S.M.R.	5	9	19	11.7	30	-9	-16	14.7	25.4	16	16.5	146	209
	10	14	19	19	28.4	-10	-22	17.2	34.4	20	24.5	383	355
J.A.D.	2.5	6	7	7	10	-11	-15	15.5	20.8	21	11.5	96	114
	5	11	9	13.5	12	-15	-20	21.2	28.6	36.5	23.5	168	171
N.C.	5	12	27	16.5	42	-15	-12	22.4	19	1	5	18	65
	10	29	32	40	47	-16	-18	25	27.2	1.5	8	39	147
G.G.	5	6	21	7.6	29.2	-13	-17	18.9	25	5.5	8	62	82
	10	15	26	18.5	36	-16	-23	22.8	32	5	61	55	790

Table A.4 Effect of I.V. noradrenaline in two doses on mean arterial pressure (M.A.P.), heart rate (H.R.) and calculated hand vascular resistance (H.V.R.) before (B) and after (A) I.V. clonidine, 150 mcg, in 4 subjects.

Subject	I.V. Noradrenaline (mcg/min)	M.A.P. Absolute Rise (mm Hg)		M.A.P. Percentage Rise		H.R. Absolute Fall (beats/min)		H.R. Percentage Fall		H.V.R. Absolute Rise (Units)		H.V.R. Percentage Rise	
		C	T	C	T	C	T	C	T	C	T	C	T
K.D.	1.25		5.5		4.7		-2		2.9		1.1		12
	2.5	5.5	10	4.3	11	-8	-5	8.4	7.1	1.9	3.3	23	37
	5.0	14	21	10	18.5	-14	-7	14.1	10.1	7.2	10.2	130	109
	10.0	20		13.3		-22		23.1		6.2		83	
D.F.A.	1.25	7	20	4.4	14.3	-10	-6	8.5	11.7	0.6	6.6	7	30
	2.5	20	31	12.3	22.7	-14	-12	12.5	21.4	3.8	12.5	44	45
	5.0	25		14.7		-26		24.5		7.1		56	

Table A.5 Effect of I.V. noradrenaline in two or three doses on mean arterial pressure (M.A.P.), heart rate (H.R.) and calculated hand vascular resistance (H.V.R.) before (C) and during (T) clonidine treatment in two patients.

Concentration M	Artery Strips 5 Control		7 Treated		Vein Segments 5 Control		8 Treated	
	Δ1 mm		%		Δ1 mm		%	
	C	T	C	T	C	T	C	T
Noradrenaline								
5.91×10^{-8}	0.017	0.097 ≠	3	11 ∕				
5.91×10^{-7}	0.25	0.47 *	40	51 *	0.23	0.39	14	22
5.91×10^{-6}	0.52	0.78	84	86	0.96	1.38	56	67
5.91×10^{-5}	0.61	0.89	100	99	1.65	2.07	99	100
Methoxamine								
4.04×10^{-7}					0.18	0.1	10	6
4.04×10^{-6}	0.21	0.25	37	38	0.29	0.84	30	46
4.04×10^{-5}	0.51	0.58	90	93	1.35	1.79	99	95
4.04×10^{-4}	0.57	0.62	100	100	1.39	1.88	96	99 *
KCl								
2×10^{-2}					0.2	0.24	15	16
3×10^{-2}					0.46	0.58	37	42
4×10^{-2}	0.029	0.034			0.85	1.10	70	79
5×10^{-2}	0.063	0.085	24	36	0.98	1.27	82	90
6×10^{-2}	0.12	0.13	48	58	1.07	1.41	89	95 ∕
7×10^{-2}	0.17	0.17	67	75	1.16	1.46	96	99 ‡
8×10^{-2}	0.20	0.20	81	87	1.28	1.53	99	100
9×10^{-2}	0.22	0.22	91	95	1.26	1.63	100	99
10^{-1}	0.24	0.22	100	100	1.26	1.62	100	99

Table A.6 Effect of reserpine pretreatment of rats on the absolute (Δ1, mm) and percentage (%) contraction of tail artery strips and portal vein segments to noradrenaline, methoxamine and KCl. C, mean responses in control preparations; T, mean responses in treated preparations. Student's unpaired t-test; ‡, $p<0.001$; ∕, $p<0.01$; ≠, $p<0.02$; *, $p<0.05$.

Concentration M	Artery Strips 3 Control		3 Treated		Vein Segments 5 Control		5 Treated	
	Δ1 mm		%		Δ1 mm		%	
	C	T	C	T	C	T	C	T
Noradrenaline								
5.91×10^{-8}	0.055	0.019	6	5				
5.91×10^{-7}	0.26	0.11	37	40	0.18	0.41	10	18
5.91×10^{-6}	0.56	0.24	83	90	1.09	1.96$^{\neq}$	64	83
5.91×10^{-5}	0.67	0.28	99	100	1.64	2.35$^{\neq}$	99	100
5.91×10^{-4}	0.67	0.28	100	100	1.55	2.09	94	88
Methoxamine								
4.04×10^{-7}					0.015	0.03	1	1
4.04×10^{-6}	0.42	0.12$^{\neq}$	46	40	0.69	1.23*	45	57
4.04×10^{-5}	0.80	0.27	90	88	1.39	2.09$^{\neq}$	92	96
4.04×10^{-4}	0.92	0.30	100	100	1.50	2.17$^{\neq}$	100	99
KCl								
2×10^{-2}					0.03	0.07	3	4
3×10^{-2}					0.2	0.34	16	19
4×10^{-2}	0.03	0.22	6	12	0.69	1.12	51	57
5×10^{-2}	0.15	0.064	35	38	1.02	1.61*	74	82
6×10^{-2}	0.28	0.095	60	61	1.13	1.76*	82	89
7×10^{-2}	0.33	0.12	79	76*	1.24	1.91*	91	96
8×10^{-2}	0.41	0.14	89	85	1.31	1.98*	96	99*
9×10^{-2}	0.43	0.15	96	93	1.34	1.99*	99	100$^{\neq}$
10^{-1}	0.44	0.16	100	100	1.35	1.99*	100	100

Table A.7 Effect of guanethidine pretreatment of rats on the absolute (Δ1, mm) and percentage (%) contraction of tail artery strips and portal vein segments to noradrenaline, methoxamine and KCl. Notation as given for Table A.6.

Concentration M	Artery Strips 6 Control		Artery Strips 6 Treated		Vein Segments 6 Control		Vein Segments 5 Treated	
	Δl mm		%		Δl mm		%	
	C	T	C	T	C	T	C	T
Noradrenaline								
5.91×10^{-8}	0.007	0.024	3	3				
5.91×10^{-7}	0.1	0.21	30	33	0.14	0.29	9	23
5.91×10^{-6}	0.23	0.4	75	76	1.02	1.08	64	78
5.91×10^{-5}	0.30	0.5	100	99	1.45	1.32	95	99
5.91×10^{-4}	0.30	0.55	100	100	1.44	1.27	96	96
Methoxamine								
4.04×10^{-6}	0.12	0.17	47	42	0.25	0.29	22	48
4.04×10^{-5}	0.23	0.30	82	83	1.16	1.07	94	92
4.04×10^{-4}	0.27	0.33	100	100	1.30	1.14	99	97
KCl								
2×10^{-2}					0.04	0.05	4	7
3×10^{-2}					0.30	0.25	32	29
4×10^{-2}	0.007	0.02			0.58	0.59	58	57
5×10^{-2}	0.02	0.05	25	29	0.95	0.81	84	78
6×10^{-2}	0.05	0.10	60	67	1.16	1.04	88	88
7×10^{-2}	0.08	0.12	85	83	1.22	1.11	93	94
8×10^{-2}	0.09	0.14	94	93	1.28	1.15	97	99
9×10^{-2}	0.09	0.17	97	98				
10^{-1}	0.1	0.16	98	99				

Table A.8 Effect of clonidine pretreatment of rats on the absolute (Δl, mm) and percentage (%) contraction of tail artery strips and portal vein segments to noradrenaline, methoxamine and KCl. Notation as given for Table A.6.

Subject	Forearm Blood Flow % Increase		
	Dose mg/min		
	0.25	0.5	1
G.M.		322	
O.L.	80	125	240
N.B.	97	133	255

Subject	Hand Blood Flow % Increase					
	Dose mg/min					
	0.2	0.25	0.4	0.5	1.0	2.0
M.W.	34		48		67	
A.W.		55		48	146	86
D.M.		42		2	87	51

Table A.9 Effect of I.A. papaverine on hand and forearm blood flow (percentage) in six subjects.

Subject and Dose (mg/kg/day)	PLASMA PAPAVERINE CONCENTRATION (mcg/ml)								
	Control	Non-Sustained Release Tablet				Sustained Release Tablet			
		Second Day		Third Day		Second Day		Third Day	
		1 Hour	4 Hours	1 Hour	4 Hours	1 Hour	4 Hours	1 Hour	4 Hours
	A	B	C	D	E	F	G	H	I
S.M.R.	0	0.050	0.089	0.267	0.075	0.045	0.065	0.074	0.155
(8.3)	0	0.105	0.083	0.242	0.071	-	0.058	0.064	0.117
R.L.H.	0.013	0.223	0.047	0.385	0.073	0.027	0.013	0.058	0.085
(9.4)	0	0.219	0.047	-	0.075	0.030	0.022	0.059	0.114
Mean	0.003	0.149	0.067	0.298	0.074	0.034	0.040	0.064	0.118
±									
S.E.	0.003	0.043	0.011	0.044	0.001	0.006	0.013	0.004	0.029
N.S. vs S.		B > F	C > G	D > H	E < I				
p		>0.05	>0.1	<0.01	<0.05				

Table A.10 Duplicate plasma papaverine concentration determinations in two subjects on the second and third days of papaverine administration (150 mg 4 times daily). Samples taken one and four hours after the second dose on each day. B to E, concentrations during non-sustained release tablet intake; F to I, concentrations during sustained release tablet intake. (Student's unpaired t-test.)

Subject	Dose mg/kg/day	Plasma Papaverine Concentration (mcg/ml)		
		Control	Mid-Week	Week
S.M.R.	4.15	0.06 0.06	0.06 0.15	0.10 0.28
R.L.H.	4.7	0.12 0.07	0.08 0.08	0 0.16
D.V.D.	5.1	0.02 0	0.07 0.05	0.11 0.16
J.R.M.	4.3	0.06 0.04	0.05 -	0.13 0.07
Mean ± S.E.	4.56 0.21	0.05 0.013	0.08 0.013	0.13 0.029
p			>0.2	<0.05

Table A.11 Duplicate plasma papaverine concentration determinations in four subjects on the 4th and 7th days of sustained release papaverine administration (150 mg 12 hourly). Samples taken at approximately the same time of the day. (Student's unpaired t-test.)

Subject	Dose mg/kg/day	Plasma Papaverine Concentration (mcg/ml)			
		Control	1 Week	2 Weeks	3 Weeks
S.M.R.	8.3	0.009 0	0.033 0.029	0.060 0.076	0.047 0.048
B.A.G.	10.5	0.008 0	0.210 0.209	0.186 0.201	
D.M.B.	11.8	0.006 0.005	0.507 0.466		
J.N.Mc.	9.5	0.014 0	0.131 0.156	0.230 0.071	
N.W.	10.6	0.039 -	0.154 0.154	0.186 0.201	
B.J.D.	9.8	0.009 0.014	0.162 0.151	0.148 0.136	
Mean of all ± S.E.	10.08 0.48	0.009 0.003	0.197 0.041	0.150 0.019	
Mean (- D.M.B.) ± S.E.	9.74 0.42	0.010 0.004	0.139 0.020	0.150 0.019	

Table A.12 Duplicate plasma papaverine concentration determinations in six subjects during sustained release papaverine intake (150 mg 4 or 5 times daily). Mean (± S.E.) values of all subjects and also those excluding subject D.M.B. are shown. Concentrations at 1 and 2 weeks were significantly greater than control ($p<0.001$) with and without values for D.M.B. There was no significant difference between concentrations at one and two weeks. (Student's unpaired t-test.)

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