CARDIOVASCULAR RESPONSES TO ANGIOTENSIN II AND NORADRENALINE AND THEIR TERMINATION IN PERIPHERAL VASCULAR BEDS

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

MARK JONATHAN SCOTT MILLER

B.Sc.(Hons.)

Department of Physiology

University of Adelaide

South Australia

JUNE, 1980

P R E F A C E

A variety of vasoactive substances are generated within the cardiovascular system.

Such substances may have their actions confined to the vascular bed of origin or they may circulate to have systemic effects.

The magnitude and duration of the resultant cardiovascular responses to such substances will be dictated by the mechanisms of their inactivation.

The processes of inactivation within vascular beds and their influence on the cardiovascular responses to angiotensin II and noradrenaline have been the subject of the studies reported in this thesis.

CONTENTS

ABSTRACT

The cardiovascular effects of angiotensin II and noradrenaline have been studied in a number of peripheral vascular beds in the morphine/chloralose anaesthetized greyhound. The extent to which these vascular beds inactivate blood-borne angiotensin II and noradrenaline has been investigated by comparing the systemic vasoactivity of drug infusions, before and after passage through a particular vascular bed.

Direct administration of angiotensin II in the renal, femoral and splanchnic circulations results in a marked reduction in local blood flow and an increase in local vascular resistance. In addition, intravenous infusion of the same dose of angiotensin also results in a decrease in blood flow and an increase in vascular resistance in renal and splanchnic circulations whereas, femoral blood flow increases passively due to the elevation of systemic blood pressure, with femoral vascular resistance being unaltered. This unique haemodynamic response of the hindquarters is indicative of a redistribution of the cardiac output on intravenous administration of angiotensin II.

Furthermore, only a minor loss of angiotensin II vasoactivity was recorded during passage through the femoral vascular bed, as compared to a uniformly high degree of inactivation in the renal, splanchnic and hepato-portal circulations.

Local administration of the angiotensin II competitive antagonist, Sar^{1}-Ile^{8}-angiotensin II, impaired the ability of the renal and hepato-portal circulations to inactivate infused angiotensin II. The mechanism for this effect is unknown but it appears to be independent of secondary alternations in blood flow. Similar treatment of the femoral

and superior mesenteric vascular beds with Sar^1-Ile^8-angiotensin II, failed to alter their ability to inactivate infused angiotensin II.

The cardiovascular effects of noradrenaline administration were studied in the same peripheral vascular beds at a dose which when administered intravenously, was approximately equipressor to that observed with angiotensin II. The magnitude and nature of these responses of these vascular beds to noradrenaline infusions, were in contrast to those seen previously with angiotensin II. This was particularly apparent in the renal and femoral circulations, both in regard to local blood flow and the degree of noradrenaline inactivation.

In contrast to previous reports, a low degree of noradrenaline inactivation was observed in the renal circulation. Two distinct mechanisms appear to be responsible for this disparity. One mechanism which was elicited by local α-adrenoceptor antagonism, is related to the reduced level of renal blood flow on renal artery infusions of noradrenaline at this dose. The probable mechanism is an impaired ability of the kidney to excrete noradrenaline in the urine, due to a considerable reduction in glomerular filtration rate.

The effect of local β-adrenoceptor antagonism with propranolol was also studied in the renal circulation and this treatment resulted in a significant increase in the degree of noradrenaline inactivation in the kidney. This effect is independent of renal blood flow and glomerular filtration rate changes and combined renal α and β-adrenoceptor antagonism resulted in a summation of their individual effects on noradrenaline inactivation.

The nature and magnitude of these effects of propranolol on the renal

circulation were found to be virtually identical to those seen on renal artery administration of SQ 20881, an inhibitor of angiotensin I converting enzyme, both when administered alone and in conjunction with local α-adrenoceptor antagonism.

The effects of both propranolol and SQ 20881 may be mediated by inhibiting the noradrenaline induced increase in intrarenal levels of angiotensin II, through an inhibition of renin release and angiotensin I conversion, respectively. The mechanism by which angiotensin II influences the fate of infused noradrenaline is not clear from these results, although it appears to be independent of changes in renal blood flow and glomerular filtration rate. The literature indicates that it may involve an inhibition of neuronal uptake.

The intrarenal levels of endogenous angiotensin II appear to have a considerable influence on the kidney's ability to inactivate noradrenaline. The implications of this interaction are far-reaching, particularly in those physiological and pathophysiological states where the intrarenal levels of angiotensin II are elevated. Furthermore, converting enzyme inhibitors are finding increasing clinical use in essential hypertension as well as classical renal hypertension. The demonstration that converting enzyme inhibition may modify catecholamine metabolsim, is an additional aspect which must be considered in understanding the mechanism of their hypotensive action.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and to the best of my knowledge, contains no material previously published to any other person, except where due reference is made in the text. The following papers have been published or communicated to learned societies.

Miller, M. J. S. and Scroop, G. C. (1978). Potentiation of the systemic blood pressure response to renal artery infusions of angiotensin II following local administration of the competitive 1-Sarcosine-8-Isoleucine Angiotensin II.
Proc. Aust. Physiol. Pharmacol. Soc. 9, 75P.

Miller, M. J. S. and Scroop, G. C. (1979). The effects of receptor blockade on the extraction of noradrenaline and angiotensin II in peripheral vascular beds.
Proc. Aust. Physiol. Pharmacol. Soc. 10, 177P.

Miller, M. J. S. and Scroop, G. C. (1980). Disappearance of angiotensin II and noradrenaline from the renal and femoral circulations of the dog. Clin. Sci. 58, 29-35.

Miller, M. J. S. and Scroop, G. C. (1980). The effects of beta-adrenoceptor blockade and angiotensin I converting enzyme inhibition on the renal inactivation of noradrenaline.
Proc. Aust. Physiol. Pharmacol. Soc. 11, 59P.

M. J. S. MILLER

ACKNOWLEDGEMENT

I would particularly like to thank Dr G. C. Scroop for his guidance, encouragement, support and interest throughout the course of my studies.

I would also like to thank Dr D. I. B. Kerr for the opportunity to work in his department and Dr T. S. Miles, Dr M. L. Roberts and P. M. Taylor for their advice and K. James for typing this manuscript.

I am also indebted to my parents and Georgia for their unfailing support throughout these years.

The experimental work described in this thesis was carried out during 1976-1979 in the Department of Human Physiology and Pharmacology, University of Adelaide. During this period, the author was the recipient of a Commonwealth Postgraduate Research Award.

S E C T I O N 1

HISTORICAL INTRODUCTION

HISTORICAL INTRODUCTION

This historical introduction is arranged in three separate sections.

Firstly, a review of the haemodynamic responses of various peripheral vascular beds to intravenous and direct administration of angiotensin II, and the variations in the degree and mechanisms whereby these vascular beds terminate the effects of angiotensin II.

A similar review for noradrenaline is presented in the second section

The final section reviews the interactions between angiotensin II and the sympathetic nervous system and in particular the role of angiotensin II in enhancing noradrenergic neuroeffector transmission.

The general structure of the results presented in this thesis follows a similar pattern.

ANGIOTENSIN II

Angiotensin II acts directly on blood vessels to cause vasoconstriction, a property first described at the time of its discovery as the mediator of the pressor action of renin (Braun-Menendez, Fasciolo, Leloir and Munoz, 1939; Page and Helmer, 1939). In more recent years a variety of additional cardiovascular actions of angiotensin II have been demonstrated, resulting from both central and peripheral sites of action (Peach, 1977; Reid, Morris and Ganong, 1978). In consequence, the cardiovascular response following the intravenous administration of angiotensin II will involve a complex

interplay of these various actions which may well vary from one vascular bed to another. In particular, the increase in total peripheral resistance, which is the principal mechanism of the pressor action of angiotensin II, is an effect which is not shared equally by the various vascular beds (Bunag, 1974).

The pulmonary circulation is relatively insensitive to angiotensin II (Vane, 1969), although initially there was some debate as to whether angiotensin II had a direct effect on pulmonary arteries. Eckert and Rose (1959) substituted the left ventricle with a mechanical constant infusion pump, thereby eliminating the influence of the systemic circulation, and demonstrated that angiotensin II failed to exert a direct effect on the pulmonary circulation. Mandel and Sapirstein (1962) and Barer (1961) found that the increase in perfusion (blood) pressure on intravenous administration of angiotensin II results in a passive increase in pulmonary blood flow.

The splanchnic vascular bed, in contrast to the pulmonary circulation, contributes to the increase in total peripheral resistance on intravenous administration of angiotensin II. Barer (1961) reported a reduced blood flow through the main mesenteric vein during the systemic pressor effect of angiotensin II in the cat. Similar observations of splanchnic blood flow reductions were reported by Gomori, Takacs and Kallay (1962), Krasney (1968) and Kapitola, Kuchel, Schreiberova and Jahod (1968). With a labelled microsphere technique in Rhesus monkeys, Forsyth, Hoffbrand and Melmon (1971) found that mesenteric and hepatic blood flow were reduced on intravenous infusions of angiotensin II to a greater degree than in any other organ.

Renal blood flow is reduced by both angiotensin I and angiotensin II, although angiotensin II is considerably greater in its renal vasoconstrictor

properties (Osborn, Tildesley, Leach and Rigby, 1974). Angiotensin I is not thought to act on the renal vasculature directly but decreases renal blood flow following the intrarenal conversion to angiotensin II (Merrill, Peach and Gilmore, 1973).

The vasoconstrictor action of angiotensin II on the renal circulation is amongst the most effective in the peripheral circulation (Brod, Hejl, Hornych, Jirka, Slechta and Burianova, 1969). Di Salvo and Fell (1970) reported that large doses of renal artery infused angiotensin II may reduce renal blood flow to almost zero, although this effect was not sustained. The local release of vasodilatory substances such as bradykinin and prostaglandins has been implicated in this increase in renal blood flow on continued infusion of angiotensin II (McGiff, Crowshaw, Terragno and Lonigro, 1970; Aiken and Vane, 1973; Malik and McGiff, 1976).

The renal vasoconstrictor action of angiotensin II may, in part, be due to central or peripheral enhancement of neural vasoconstrictor tone (Ferrario, Gildenberg and McCubbin, 1972), although the main effect appears to be independent of renal nerves (Di Salvo and Fell, 1970; Waugh, 1972).

The general vasoconstrictor effects of angiotensin II are reduced by salt depletion, although the kidney appears to be affected to a greater extent than any other vascular bed (Hollenberg, Soloman, Adams, Abrams and Merrill, 1972).

The major intrarenal site of angiotensin II-induced vasoconstriction is thought to be the efferent arteriole, whereas adrenaline and noradrenaline act predominantly on the afferent arteriole (Regoli and Gauther, 1971; Hall, Guyton, Jackson, Coleman, Lohmeier and Trippodo, 1977). A possible role for angiotensin II in the redistribution of intrarenal blood flow is still

debatable (Aukland, 1976).

Skeletal muscle vascular beds present a unique haemodynamic response pattern to angiotensin II. Intra-arterial administration results in a marked reduction in blood flow and an increase in vascular resistance (Scroop, Walsh and Whelan, 1965; Scroop, 1967; Haas, Goldblatt, Lewis and Gipson, 1968, 1969, 1973), whereas vascular resistance is unaltered on intravenous administration, with muscle blood flow increasing passively in response to the elevated systemic blood pressure (Bock, Krecke and Kuhn, 1958; Scroop, Walsh and Whelan, 1965; Scroop, 1967; Kapitola, Kuchel, Schreiberova and Jahoda, 1968; Brod, Hejl, Hornych, Jirka, Slechta and Burianova, 1969; Forsyth, Hoffbrand and Melmon, 1971).

The variation in the haemodynamic responses of different vascular beds to angiotensin II administration may represent variations in receptor populations, vascular architecture and the rate and mechanisms of terminating the effects of angiotensin II. This thesis has investigated certain of these aspects, with particular emphasis on the mechanisms of angiotensin II inactivation in different vascular beds.

There are a number of possible mechanisms by which angiotensin II is removed from the circulation, namely, non-specific binding, tissue sequestration, urinary excretion and metabolism. These pathways are represented schematically in Fig. 1.1 along with the local and systemic sites of angiotensin II generation. While the source of angiotensin II may determine the mechanism of inactivation, the major physiological pathway is thought to be metabolism (Khairallah, Page, Bumpus and Turker, 1966; Ledingham and Leary, 1974; Bailie and Oparil, 1977).

All enzymes with the ability to metabolize angiotensin II are collectively

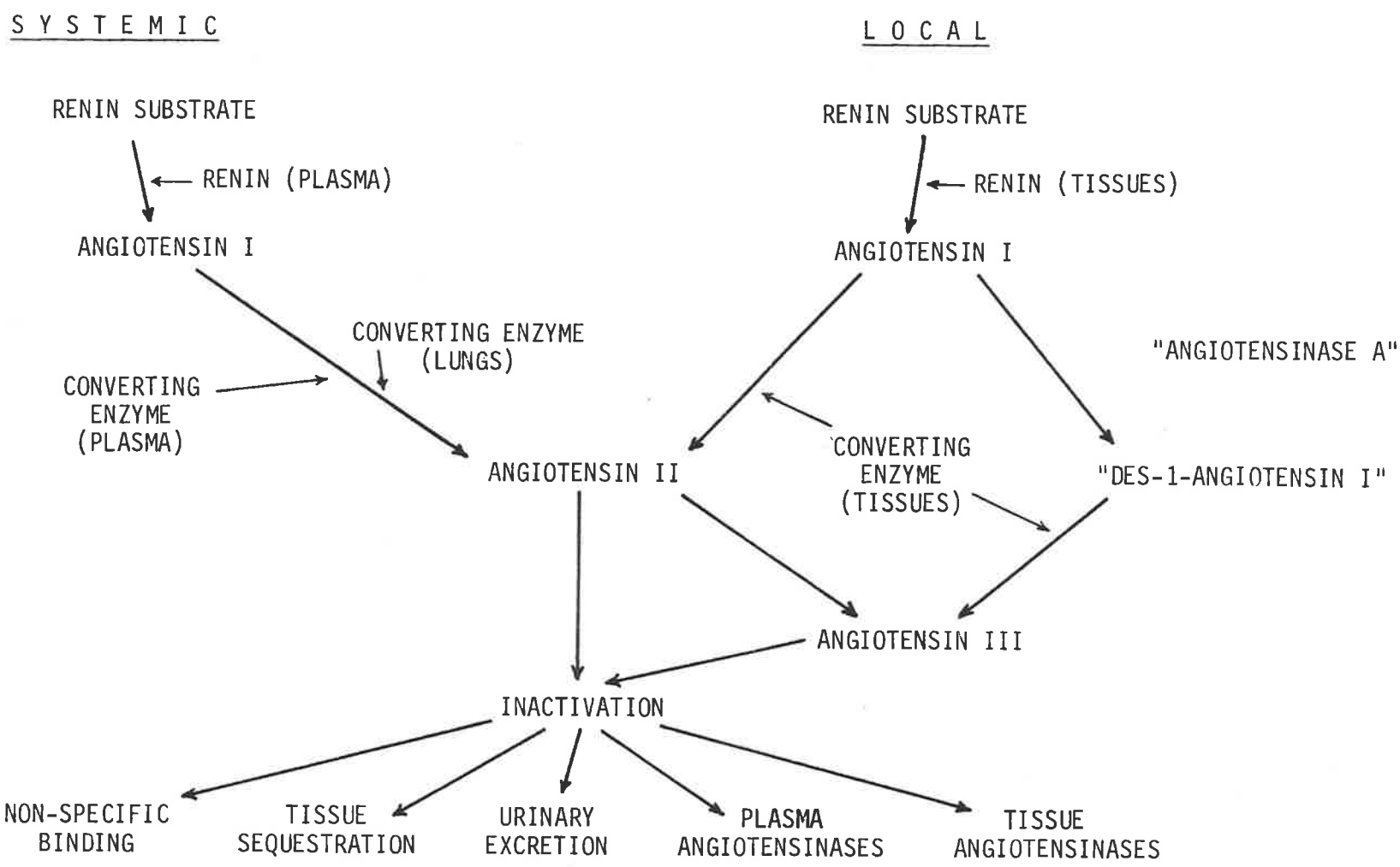


FIGURE 1.1

The generation and inactivation of angiotensin II in peripheral vascular beds.

called "angiotensinases" and these include trypsin, chymotrypsin, pepsin, leucine aminopeptidase and carboxypeptidase. Plasma angiotensinases are now thought to play only a minor role in the *in vivo* inactivation of angiotensin II, with the major site being peripheral vascular beds (Chamberlain, Browse, Gipson and Gleeson, 1964; Hodge, Ng and Vane, 1967; Leary and Ledingham, 1969; Cain, Catt, Coghlan and Blair-West, 1970; Oparil and Bailie, 1973).

While tissue homogenate experiments have revealed that intestinal mucosa, renal cortex and the liver are rich sources of angiotensinase activity (Itskovitz and Miller, 1967; Johnson and Ryan, 1968; Matsunaga, Saito, Kira, Ogino and Takayasu, 1969; Matsunaga, 1971) these studies have failed to clarify the *in vivo* fate of angiotensin II. For example, while homogenized lung tissue was found to destroy angiotensin II with great rapidity (Itskovitz and Miller, 1967), *in vivo* angiotensin II passes through the pulmonary circulation without a significant loss of activity (Hodge, Ng and Vane, 1967; Biron, Meyer and Panisset, 1968; Biron, Campeau and David, 1969; Leary and Ledingham, 1969). This discrepancy probably indicates that tissue homogenization releases intracellular peptidases which normally do not have access to angiotensin II.

The liver was investigated as a major site of angiotensin II inactivation *in vivo* following the reports by Methot, Meyer, Biron, Lorain, Lagrue and Milliez (1964) in the rat and Chamberlain, Browse, Gibson and Gleeson (1964) in dogs and man, where renal hypertension was reversed by a renal-portal venous anastomosis. Biron, Meyer and Panisset (1968) reported a 60-70% inactivation of Asp^1-Val^5-angiotensin II and an 80% inactivation of its β-analogue in both rats and dogs. Similar results were obtained by Hodge, Ng and Vane (1967) and Leary and Ledingham (1969, 1970b).

Hindquarter inactivation of angiotensin II has been studied in a number of species, namely dogs (Hodge, Ng and Vane, 1967; Biron, Meyer and Panisset, 1968; Haas, Goldblatt, Lewis and Gipson, 1968; 1969; 1973), rats (Biron, Meyer and Panisset, 1968; Bakhle, Reynard and Vane, 1969; Biron and Campeau, 1971), sheep (Osborn, Hughes, Purie, Willicombe and Mahler, 1969) and rabbits (Akinkugbe, Brown and Cranston, 1966; Broughton Pipkin, Mott and Roberton, 1971).

Despite the unique haemodynamic response of the femoral circulation to administered angiotensin II, all groups reported an extensive inactivation of 50-70%. This is with the exception of Akinkugbe, Brown and Cranston (1966), who with a similar technique to that used in this thesis, only observed a 15% inactivation of infused angiotensin II.

Haas, Goldblatt, Lewis and Gipson (1973) reported that a loss of angiotensin II activity on passage through the femoral circulation of the dog was due not to metabolism, but to a delayed passage into the general circulation, as the result of a profound, localized vasoconstriction. In previous studies this group had proposed the existence of a cofactor in the blood which protected angiotensin II from destruction (Haas, Goldblatt, Lewis and Gipson, 1968, 1969). This cofactor was later determined to be bradykinin (Haas, Goldblatt, Lewis and Gipson, 1973) which prevented this local vasoconstriction and allowed angiotensin II to pass into the general circulation without loss of activity. These findings indicate that the magnitude of the local haemodynamic effects of angiotensin II on intra-arterial administration have a marked influence on the degree of inactivation within the femoral vascular bed. This effect may also apply to other vascular beds.

The majority of previous reports however, have taken little notice of

the associated haemodynamic responses when studying the fate of administered angiotensin II. This thesis investigates the influence of local blood flow on angiotensin II and noradrenaline inactivation in a number of peripheral vascular beds.

In the kidney, Bailie and Oparil (1977) found a positive relationship between renal blood flow and angiotensin II inactivation, where inactivation increased with reductions in renal blood flow and increases in renal transit time.

A high degree of angiotensin II inactivation in the kidney has been reported in a number of previous studies with a variety of techniques (Hodge, Ng and Vane, 1967; Biron, Meyer and Panisset, 1968; Leary and Ledingham, 1970b; Osborne, Angles d'Auriac, Meyer and Worcel, 1970; Biron and Campeau, 1971; Oparil and Bailie, 1973).

The renal vasculature appears to be the major site of angiotensin II inactivation, with only a minor contribution from glomerular filtration and urinary excretion (Akinkugbe, Brown and Cranston, 1966; Leary and Ledingham, 1970; Bailie, Rector and Seldin, 1971; Bailie and Oparil, 1977).

There has been a growing interest in recent years, in the events associated with the angiotensin II stimulation of membrane-bound receptors. Both angiotensin II receptors and angiotensinases have their greatest specific activity in the microsomal fraction (Dengler and Reichel, 1960). Angiotensinase activity has been described in hepatocyte cell membranes (Lafontaine, Nivez and Ardaillore, 1979), rabbit aorta smooth muscle membrane (Baudoin, Meyer and Worcel, 1971; Peach, 1977) and red blood cell membrane (Khairallah, Bumpus, Page and Smeby, 1963; Itskovitz and Miller, 1967; Moore and Povinelli, 1979). The precise relationships between

angiotensinase activity and receptor function, however, remain to be clarified.

There is strong evidence that a variety of angiotensin receptors exist in a number of tissues including vascular smooth muscle (Caldicott, Taub and Hollenberg, 1977; Caldicott, Taub, Korngold and Hollenberg, 1978; Caldicott and Hollenberg, 1970), between uterine and other smooth muscle types (Meyer, Papadimitriou and Worcel, 1970; Regoli, Park and Rioux, 1974), adrenal cortex (Peach and Chiu, 1974) and the adrenal medulla (Peach, Bumpus and Khairallah, 1971; Peach, 1977).

A number of angiotensin II analogues have been employed to investigate these receptor populations (Regoli, Park and Rioux, 1974) but their influence on the *in vivo* fate of angiotensin II and angiotensin activity is unknown. In this thesis, the influence of receptor function on the inactivation of angiotensin II in a number of peripheral vascular beds, has been examined with the competitive antagonist, Sar^1-Ile^8-angiotensin II.

CATECHOLAMINES

Noradrenaline has been the predominant catecholamine studied in this thesis and, consequently, most of this introduction will be confined to those studies involving the effects of noradrenaline or sympathetic nerve stimulation.

Noradrenaline is a powerful pressor substance which has both general effects on the systemic circulation and entirely local actions within a particular vascular bed. The systemic effects may be mediated by

either circulating noradrenaline or a general vascular sympathetic discharge. The levels of circulating noradrenaline represent the secretions of the adrenal medulla (Driver and Vogt, 1950; Vane, 1969) and other organs with a dense sympathetic innervation such as the hindlimb (Oswald and Branco, 1973) and the kidney (de Leeuw, Falke, Punt and Birkenhäger, 1978).

The mechanisms by which noradrenaline elevates mean arterial pressure are varied, but as is the case with angiotensin II, an increase in total peripheral resistance is involved (Vane, 1969; Starke, 1977). The relative contribution by individual vascular beds to this overall increase in vascular resistance is not uniform and results in a redistribution of the cardiac output (Hoffbrand and Forsyth, 1973).

Vasoconstriction occurs in the mesenteric vasculature to both exogenous noradrenaline and sympathetic nerve stimulation, although in some species, the response to sympathetic nerve stimulation is unusual. In cats, 2-4 minutes after the onset of stimulation, intestinal blood flow returns to normal (Greenway, Lawson and Mellander, 1967; Mellander and Johansson, 1968; Greenway and Stark, 1971). This phenomenon has been labelled "autoregulatory escape". It is thought not to occur in response to exogenous noradrenaline administration or in dogs (Greenway and Oshira, 1972) but this aspect has been investigated in this thesis.

Intravenous and renal artery administration of adrenaline (Barer, 1961) and noradrenaline result in reductions in renal blood flow and increases in renal vascular resistance (Brod, Hejl, Hornych, Jirka, Slechta and Burianova, 1969; Regoli and Gauther, 1971; Hoffbrand and Forsyth, 1973). At doses which failed to exert any systemic action, Hollenberg, Soloman, Adams, Abrams and Merrill (1972) observed a dose-related decrease in renal

blood flow on renal artery infusions of noradrenaline.

These authors also observed a potentiation of the renal vascular response to noradrenaline administration on sodium restriction, in contrast to an attenuation of the angiotensin II response. This finding was confirmed by Strewler, Hinrichs, Guiod and Hollenberg (1972).

The site of action of the catecholamines, adrenaline and noradrenaline, within the renal vasculature, also differs from angiotensin II. Adrenaline and noradrenaline act predominantly on the afferent arteriole, whereas angiotensin II is thought to constrict the efferent arteriole (Regoli and Gauther, 1971; Hall, Guyton, Jackson, Coleman, Lohmeier and Trippodo, 1977).

Variable results have been observed in studies of the skeletal muscle circulatory response to administered noradrenaline. In man, Scroop, Walsh and Whelan (1965) observed a decrease in forearm blood flow on intravenous administration of noradrenaline, whereas Brod, Hornych, Jirka, Slechta and Burianova (1969) described a variable effect. Hoffbrand and Forsyth (1973) found that intravenous infusion of noradrenaline failed to alter skeletal muscle or cardiac vascular resistance, whereas vascular resistance in the brain, skin, gastro-intestinal tract, liver and bone increased, indicating a redistribution of the cardiac output.

These discrepancies may reflect variations in technique, dose and the relative stimulation of α- and β-adrenoceptors populations, as Hoffbrand and Forsyth (1973) found a relatively uniform increase in vascular resistance with no significant cardiac output redistribution, with the pure α-adrenoceptor agonist, methoxamine.

The various mechanisms of terminating the effects of noradrenaline in

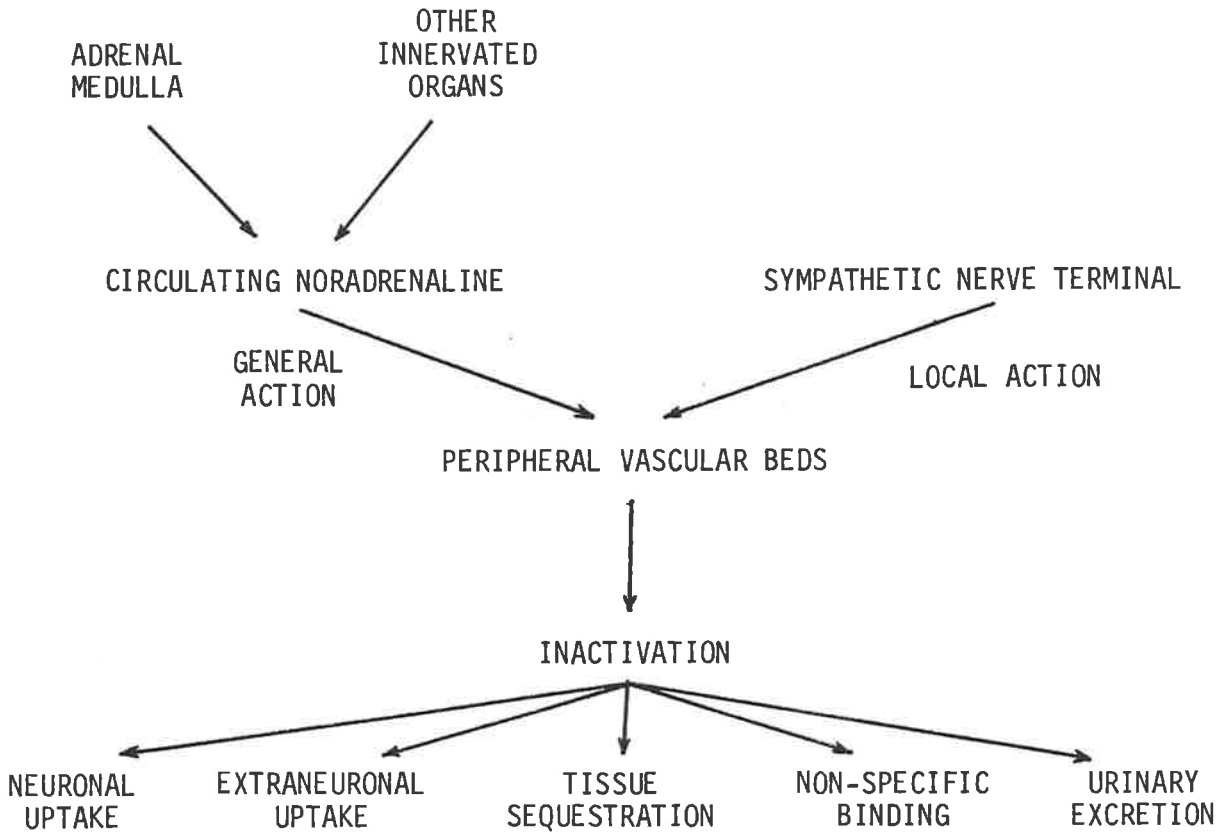


FIGURE 1.2

The generation and inactivation of noradrenaline in peripheral vascular beds.

peripheral vascular beds and the systemic and local sources of noradrenaline are schematically represented in Fig. 1.2. Neuronal and extraneuronal uptake are thought to be the most important processes determining the rate of noradrenaline inactivation *in vivo*, whether it be neurally released or circulating noradrenaline (Iversen, 1967; de la Lande, Frewin and Waterson, 1967; de la Lande and Jellett, 1972). Following these uptake processes, noradrenaline may either be stored or metabolized (Trendelenburg, 1977).

There have been varying reports of the capacity of the hindquarters to inactivate noradrenaline. Vane (1966) and Ginn and Vane (1968) found that 70-95% of intra-arterially infused noradrenaline or adrenaline disappeared during passage through the hindquarters of cats and dogs. Similar results were reported earlier by Celander and Mellander (1955).

These results are contrasted by the distribution studies of Axelrod and colleagues, where only a marginal uptake of labelled catecholamine was observed in mouse skeletal muscle (Axelrod, Weil-Malherbe and Tomchick, 1959; Whitby, Axelrod and Weil-Malherbe, 1961). Furthermore, there is a degree of variation in the results from Vane's laboratory, as in a later study, only 60% of infused noradrenaline and 34% of infused isoprenaline were removed in a single passage through the hindquarters (Gryglewski and Vane, 1970). Isoprenaline, unlike noradrenaline, is only a substrate for extraneuronal uptake (Callingham and Burgen, 1966) which explains the considerably lower inactivation than that observed for noradrenaline.

In contrast to the hindquarters, the pulmonary circulation is considerably less active, with only 20% of infused noradrenaline removed *in vivo* (Ginn and Vane, 1968) and in isolated, blood-perfused lungs (Eiseman, Bryant and Waltuch, 1964). Adrenaline on the other hand passes through the lungs intact (Ginn and Vane, 1968), a result which was

observed many years earlier by Elliot (1905), who concluded that "adrenalin disappears in tissues it excites", an important principle which is consistent with the fate of other vasoactive compounds throughout many fields of physiology.

The splanchnic and renal vascular beds are thought to inactivate noreadrenaline at a comparable rate to that exhibited by the hindquarters (Celander and Mellander, 1955; Gryglewski and Vane, 1970). The major metabolic route of circulating catecholamines in the renal vascular bed involves extraneuronal uptake (Silva, Landsberg and Besarab, 1979).

The kidneys are also capable of inactivating noradrenaline via urinary excretion (Fig. 1.2). The principle mechanism whereby noradrenaline enters the urine is via glomerular filtration (Overy, Pfister and Chidsey, 1967), but recently a tubular secretory mechanism has been found to contribute to the levels of noradrenaline and other catecholamines in the urine (Silva, Landsberg and Besarab, 1979). This finding was previously observed in chickens (Rennick and Yoss, 1962; Quebbeman and Rennick, 1970; Rennick and Quebbeman, 1970) and dogs (Jones, 1968; Rennick, 1968), although a tubular secretory mechanism was not observed by Overy, Pfister and Chidsey (1967) in the dog.

This latter group failed to detect any significant protein binding in their preparation, which may explain why a nett tubular secretion was not evident. Plasma protein binding of catecholamines is extensive. Silva, Landsberg and Besarab (1979) found that only 30% of ^{3}H-catecholamine was freely permeable across cellophane membranes. This result is supported by a number of other studies (Rennick, 1968; Danon and Sapira, 1972; Collier, 1972; May, Sanders and Donabedian, 1974; Powis, 1975).

Chronic renal denervation failed to alter the rate of endogenous noradrenaline excretion (Overy, Pfister and Chidsey, 1967) indicating that the sole source of urinary noradrenaline is the circulation (Fig. 1.2). Hence, reductions in renal blood flow and glomerular filtration rate may result in a decreased renal inactivation of noradrenaline, although the influence of renal haemodynamics on the tubular secretory mechanism is unknown.

Despite the ever increasing understanding of the biochemical mechanisms controlling the concentration of noradrenaline at effector sites, particularly modulation of pre- and post-synaptic receptor activity (for review see Kunos, 1976; Langer, 1977; Starke, 1977; Westfall, 1977), there remains some confusion over the *in vivo* fate of noradrenaline and in particular the influence of local haemodynamics and α- and β-adrenoceptor populations.

Furthermore, the renin-angiotensin system is known to have a number of interactions with the sympathetic nervous system and a summary of these interactions is the subject of this next section of this General Introduction.

INTERACTIONS BETWEEN ANGIOTENSIN II AND THE SYMPATHETIC NERVOUS SYSTEM

Following the availability of a pure synthetic form of angiotensin II, interactions between angiotensin II and the sympathetic nervous system were evident (Zimmerman, 1962; Baum, 1963; McCubbin and Page, 1963; Benelli, Della Bella and Gandini, 1964). The relationships between these two powerful pressor systems have been extensively investigated and reviewed

by several authors (Khairallah, 1972; Reit, 1972; Roth, 1972; Zimmerman, Gomer and Liao, 1972; Severs and Daniels-Severs, 1973; Starke, 1977; Westfall, 1977).

Angiotensin II has been found to enhance the response to nerve stimulation in a wide variety of species and tissues such as cat spleen (Thoenen, Hurliman and Haefely, 1965; Hertting and Suko, 1966); rabbit, rat and cat isolated blood vessels (Panisset and Bourdois, 1968; Hughes and Roth, 1969; Nicholas, 1970; Bell, 1972; Blumberg, Ackerly and Peach, 1975; Malik and Nasjletti, 1976); canine hindquarters and hindpaw (Zimmerman, 1967; Zimmerman and Gisslen, 1968; Kadowitz, Sweet and Brody, 1972); canine kidney (Zimmerman, Gomer and Liao, 1972; Gomer and Zimmerman, 1973); brain (Starke, Endo, Taube and Borowski, 1975) and cat terminal ileum (Turker, 1973).

The mechanism whereby angiotensin II facilitates neurotransmission is unknown. Early investigators thought that the enhanced noradrenergic neuroeffector transmission in the presence of angiotensin II was due to the direct depolarization of the adrenergic nerve terminals (Distler, Liebau and Wolfe, 1965; Gascon and Walaszek, 1968; Kiran and Khairallah, 1969), whereas the majority of present evidence suggests that angiotensin II facilitates the release of noradrenaline per nerve impulse. This effect does not appear to represent an action on vesicular storage (Schumann, 1970). Angiotensin II is also known to stimulate noradrenaline biosynthesis in the nerve terminal (Boadle, Hughes and Roth, 1969; Davila and Khairallah, 1971; Chevillard, Duchene and Alexandre, 1975), although this effect is not responsible for the increased release of noradrenaline.

Furthermore, angiotensin II stimulation of prostaglandin release is not involved, as elevated prostaglandin E concentrations have been

demonstrated to inhibit rather than enhance sympathetic nerve transmission (Starke, 1977).

Angiotensin II also has a post-synaptic action, increasing the responsiveness of the innervated organ to noradrenaline (Thoenen, Hurlimann and Haefely, 1965; Panisset and Bourdois, 1968; Day and Moore, 1976).

The pre-synaptic effects of angiotensin II are thought to be of greater importance however, as a number of investigators have found that angiotensin II potentiated the vasoconstrictor effects of sympathetic nerve stimulation to a far greater degree than the response to exogenous noradrenaline (Benelli, Della Bella and Gandini, 1964; Zimmerman and Gomez, 1965; Zimmerman and Gisslen, 1968; Kadowitz, Sweet and Brody, 1971). Furthermore, Zimmerman and Whitmore (1967) found that angiotensin II significantly potentiated the response to nerve stimulation, at a dose which failed to alter the response to exogenous noradrenaline administration and some workers have failed to demonstrate any action of angiotensin II on the vasoconstrictor effects of exogenous noradrenaline (Hughes and Roth, 1971; Bell, 1972).

These results are contrasted by the recent findings of Jackson and Campbell (1980) who reported a similar degree of potentiation by angiotensin II for nerve stimulation (63%) and exogenous noradrenaline (62%).

Angiotensin II may also be enhancing noradrenergic neuroeffector transmission by inhibiting neuronal uptake. There has been however, a considerable controversy over the dose of angiotensin II required to affect neuronal uptake.

Low concentrations of angiotensin II (2×10^{-11} M) have been reported

to inhibit the accumulation of ^{3}H-noradrenaline in perfused rabbit hearts (Peach, Cline, Davila and Khairallah, 1969; Davila and Khairallah, 1970) and rat hearts (Khairallah, 1972). Studies by other workers with similar angiotensin II concentrations however, failed to support these findings (Hughes and Roth, 1969, 1971; Schumann, 1970; Chevillard and Alexandre, 1970; Starke, 1971).

This effect of angiotensin II does however, occur at very high concentrations of 10^{-5} M and higher (Schumann, 1970; Starke, 1971; Janowsky, Davis, Fann, Freeman, Nixon and Michelakis, 1972). There is evidence that angiotensin II inhibits ^{3}H-noradrenaline uptake centrally (Palaic and Khairallah, 1967) and in a number of other sites *in vivo*, but the physiological importance of these findings has been questioned.

There is no evidence that angiotensin II influences extraneuronal uptake (Salt, 1972).

Recently, subpressor doses of angiotensin II (3×10^{-9} M) were found to exert an inhibitory effect on neuronal uptake whilst not influencing extraneuronal uptake in isolated mesenteric arteries (Jackson and Campbell, 1979).

There is considerable evidence that the pre-synaptic effects of angiotensin II are mediated through a facilitation of adrenergic transmission and not an inhibition of neuronal uptake as the effects of angiotensin II are thought to be unaltered by cocaine (Starke, 1970; Hughes and Roth, 1971) although this has been recently challenged by Jackson and Campbell (1979).

Furthermore, angiotensin II has also been found to potentiate the

effects of tyramine (McCubbin and Page, 1963; Kaneko, Takeda, Nakajima and Ueda, 1966; Day and Owen, 1969; Blumberg, Ackerly and Peach, 1975). Starke (1971) and Chevillard and Alexandre (1972) failed to demonstrate a potentiation of the effects of tyramine. However, as the response was not attenuated, they propose that this remains evidence for a facilitation of neural transmission.

Angiotensin II also results in an enhanced release of dopamine-β-hydroxylase from rabbit atria. This effect is quite independent of an uptake blocking action and is indicative of increased exocytotic release (Blumberg, Ackerly and Peach, 1975; Ackerly, Blumberg and Peach, 1976).

In the majority of previous investigations, angiotensin II has been administered exogenously and as a result, there is some difficulty in interpreting the effects of endogenous angiotensin II formation, particularly when considering the local concentration of angiotensin II needed to inhibit neuronal uptake. There have only been a limited number of *in vivo* studies investigating the effects of endogenous angiotensin II (Malik and Nasjelleti, 1976).

Ichikawa, Johnson, Fowler, Payne, Kurz and Keitzer (1978) have recently reported that the vascular responses to noradrenaline were potentiated in the early stages of renovascular hypertension in rabbits, before there was an elevation in systemic blood pressure. The possibility that angiotensin II may be mediating this effect, however, was not confirmed by Collis and Vanhoutte (1978).

Collis and Alps (1975, 1976) noted that the noradrenaline supersensitivity of tissues from renal/salt hypertensive rats, which have a low plasma renin activity, was not due to endogenous angiotensin II as it was unaffected by

Sar^{1}-Ile^{8}-angiotensin II. The observed supersensitivity could only be explained, in part, by the dietary salt-loading.

Intrarenally generated angiotensin II was thought to contribute to the systemic pressor response (Seliq, Anderson and Korner, 1979) and increase in renal vascular resistance (Bomzon and Rosendorff, 1975) to renal artery infusions of noradrenaline, through studies with local angiotensin I converting enzyme inhibition. These authors however, failed to account for a possible inhibitory effect on neuronal uptake by angiotensin II, which would also explain the attenuated responses following renal converting enzyme inhibition.

SECTION 2

GENERAL METHODS

GENERAL METHODS

CHOICE OF EXPERIMENTAL ANIMAL

The experimental animals in all studies were ex-racing greyhounds. Their pedigreed breeding in conjunction with the excellent husbandry they receive in preparation for racing, make them essentially similar to laboratory-bred dogs. In addition, they are of a quiet disposition which greatly assists handling and induction of anaesthesia. Their relative lack of subcutaneous fat facilitates all surgical procedures.

Greyhounds have a robust cardiovascular system with large vessels and high flows and a considerable degree of cardiac vagal tone, all of which make it comparable to that of man and particularly suitable for circulatory studies.

ANAESTHETIC

Following premedication with intravenous morphine sulphate (approximately 2 mg/kg), anaesthesia was induced by intravenous alpha-chloralose (120-140 mg/kg). The chloralose was prepared as a 5% solution in the following manner. Four grammes of sodium tetraborate were dissolved in 80 ml of normal saline. To this solution 4 g of α-chloralose were added and stirred over a Bunsen burner. To prevent the formation of the β-isomer, the temperature of the solution was kept below $45^{o}C$. This solution was then filtered, cooled and stored overnight.

The combination of morphine and chloralose was chosen as the anaesthetic

agent because of its suitability for cardiovascular investigations. It has a minimal effect on the responses to exogenous catecholamines (Cox, 1972) and is not associated with the pronouced tachycardia seen when sodium pentobarbitone is used as the principal anaesthetic agent (Shabetai, Fowler and Hurlburt, 1963).

Supplementary anaesthesia was provided when required, with small doses of intravenous sodium pentobarbitone (10-30 mg). This need was determined from the corneal reflex. In general, supplementary anaesthesia was not required in the first few hours of experimentation and each dog, on average, received at the most 150 mg throughout the entire experimental period.

Following induction of anaesthesia, all dogs were intubated and artifically respired throughout the experiment. The respirator employed was a Harvard Model 613 positive pressure respirator, operating at 12 "breaths" per minute, at a tidal volume calculated from body weight (Radford-Kleinman nomogram: Harvard Apparatus). With this procedure the respiratory depressant effects of morphine were overcome and blood pO_2, pCO_2 and pH have been shown to be more stable throughout (Brown, 1976).

METHOD OF INTRAVASCULAR CATHETERIZATION

Catheters

Catheters were of polyethylene (Dural Plastics, Australia 2158) and two sizes of tubing were used. Catheters inserted in the femoral artery, femoral vein and hepato-portal vein had an internal diameter of 0.86 mm

and an external diameter of 1.27 mm (Dural SP61). Catheters of small dimensions (internal diameter 0.58 mm, external diameter 0.96 mm, Dural SP45) were chosen for the renal and mesenteric arteries to ensure that blood flow was not obstructed in these smaller vessels.

The total length of all catheters was 100 cm giving internal volumes (infusion "dead-space") of 0.45 ml (SP61) and 0.26 ml (SP45). With an infusion pump speed of 1 ml/min this represents a delay in drug delivery of approximately 30 and 15 seconds, respectively.

All catheters were filled with heparinized saline (20 units Heparin/ml saline) prior to insertion.

Method of insertion

For arterial catheterization, the flow through each vessel was interrupted with two ligatures and the artery punctured with a needle the external diameter of which was smaller than that of the catheter (femoral artery - Yale, 19-gauge disposable; renal and superior mesenteric artery - Yale, 23-gauge disposable).

Essentially the same procedure was used for venous catheterizations although flow obstructing ligatures were not required.

Catheters inserted into the femoral and renal arteries had their tips directed centrally to promote mixing of the infused drug with flowing blood. For anatomical reasons this was not possible with superior mesenteric artery catheters and their tips were directed peripherally.

The length of catheter inserted varied. Catheters inserted into

the femoral artery for monitoring blood pressure and into the femoral vein for drug infusion were passed 20 cm centrally. Drug infusion catheters in the renal, femoral and superior mesenteric arteries and the hepato-portal vein were inserted approximately 1.5 cm.

To avoid accidental withdrawal of these catheters a small square of adhesive tape was attached to the tubing approximately 2 cm from the catheter tip. A ligature was then passed through the tape and tied around the artery in a manner which did not interfere with blood flow.

CARDIOVASCULAR VARIABLES

Arterial blood pressure

Arterial blood pressure was measured through a saline-filled catheter previously inserted into a femoral artery through a needle puncture. Polyethylene tubing (internal diameter 0.86 mm, external diameter 1.27 mm, Dural Plastics SP61, length 100 cm) on a 1 cm length of 19 gauge needle (Yale, disposable) gave optimum damping (Geddes, 1977) with the Statham P23Gc transducer and Grass recorder used for these experiments.

Calibration of the pressure recording system was checked before each experiment with a mercury manometer, over the pressure range of 0 to 200 mm Hg. Blood pressure was recorded on a Model 7 Grass Polygraph and the changes expressed as their integrals, these having been calculated by planimetric measurement of the areas contained by these responses.

Blood flow recordings

Arterial blood flow was recorded continuously by applying electromagnetic flow probes directly to the vessels under study. Their output was monitored on both Carolina Medical Electronics and E.M.I. Type 28 squarewave electromagnetic flowmeters and displayed as a hard copy on a Grass Polygraph.

Various electromagnetic flow-probes were used to ensure adequate contact between the artery under study and the flow-probe. Zero blood flow measurements were obtained when necessary by mechanically occluding the artery with a loose ligature placed distal to the probe.

All blood flow responses are expressed as their integrals, these having been calculated by planimetric measurement of the areas contained by the responses.

Vascular resistance

Vascular resistance was calculated at minute intervals from the simultaneous levels of mean arterial pressure and blood flow. Changes in vascular resistance are expressed as a percentage, calculated in the following manner. The average value of vascular resistance during the 4 min immediately prior to the drug infusion was subtracted from the average vascular resistance calculated in the last 4 min of the infusion period. This difference was then expressed as a percentage of the pre-infusion value.

Radioimmunoassay of plasma renin activity

This is a competitive binding assay technique where endogenously

generated angiotensin I competes with a known amount of labelled angiotensin I for specific angiotensin I antibody binding sites.

The method used in this thesis is that of Johnston, Hutchinson and Mendelson (1970). The materials used were; phosphosaline-casein buffer (pH 7.4), standards - angiotensin I (Sigma) diluted with assay buffer over the 0.10 ng/ml-4.00 ng/ml range, ^{125}I-angiotensin I and a specific antibody raised in rabbits against an angiotensin I - B.S.A. conjugate.

Radioimmunoassay of plasma levels of angiotensin II

This is also a competitive binding assay technique where endogenous angiotensin II competes for specific angiotensin II antibody binding sites with a known amount of ^{125}I-angiotensin II. The degradative effects of plasma angiotensinases were inhibited by ethylenediamine tetracetic acid (E.D.T.A.) and dimercaprol at blood concentrations of 0.01 M each.

The method of extracting angiotensin II from plasma in this study is as follows. Plasma (2-3 ml) was extracted on to 500 mg Dowex 50 W cation exchange resin and the tubes were shaken gently for 1 hr at 4^{o}C. Angiotensin II was eluted from the Dowex resin with 2 ml of ammonia/ methanol (9:1 v/v) and vigorous shaking.

The eluate was dried down at 40^{o}C under nitrogen and resuspended in phosphosaline-caesin buffer (pH 7.4). The average extraction efficiency was 76.2 ± 1.0% (mean ± s.e.m.).

Following this extraction procedure, the assay technique was the same as that used for estimating plasma renin activity. The assay materials used were; phosphosaline-casein buffer (pH 7.4), standards - angiotensin II

(Sigma) diluted with assay buffer over 1.10 ng/ml-4.00 ng/ml range, ^{125}I-angiotensin II. The specific antibody was less than 0.1% cross-reactive with angiotensin I and was raised in rabbits against an angiotensin II - B.S.A. conjugate.

EXPRESSION OF RESULTS

Attenuation of the systemic pressor response as an index of drug inactivation

In most cardiovascular studies vasoactivity of a drug under study is assessed during infusion into a peripheral vein. With this route of administration the drug reaches the general circulation after first passing through the pulmonary vascular bed and the heart.

The ability of the drug to raise blood pressure will therefore depend upon the administered dose, its effect on the pulmonary circulation, the degree of inactivation in the pulmonary vascular bed and its effect on the heart and the vascular beds of the systemic circulation. These effects may result from either direct stimulation of cardiac and vascular receptors or indirectly through the release of additional vaso-active hormones and activation of the autonomic nervous system.

If the same dose of drug is administered into a peripheral artery rather than a peripheral vein, the only added factor to those described above will be the influence of events within this vascular bed. A change in the pressor response on intra-arterial administration will imply either additional inactivation of the drug in that vascular bed or local activation of neurohumoral factors having systemic effects.

If, for example the response to a given dose of drug is smaller on intra-arterial administration, this implies further inactivation of the drug during passage through that vascular bed. A larger response on intra-arterial administration indicates that either a hormone with systemic effects has been released or that a major component of the autonomic nervous system has been activated. If there is no difference in the pressor response with these two routes of administration, the most likely explanation is that the vascular bed under study does not inactivate this drug.

It should be noted that any effect of the drug on a given vascular bed will not by itself change systemic blood pressure. This homeostatic response reflects the excellent buffering systems of the circulation. This is exemplified by the fact that blood pressure is unaltered during sphygmomanometry even though the blood flow in one arm has been interrupted.

Therefore, if blood pressure does change on intra-arterial administration, this indicates that this dose of drug is sufficient to reach the systemic circulation, in which case the local effects will then contribute to the overall response.

In the studies reported in this thesis all drugs, with the exception of isoprenaline, were pressor. Arterial infusions were given into a number of peripheral vascular beds and compared to the standard response obtained on intravenous infusion.

The systemic pressor responses were recorded as their integral, obtained by planimetric measurement of the area contained by the response. The degree of inactivation of the drug under study was quantified in the following manner; the integrated pressor response on infusion into the vascular bed under study was subtracted from the pressor response observed

on intravenous infusion of the same dose. This difference was then expressed as a percentage of the standard intravenous response.

This index of inactivation was employed for several reasons. Firstly, it can be regarded as more representative of physiological events than the various radioimmunoassay and organ superfusion techniques. Radioimmunoassay methods suffer from problems of a loss of specific activity and uniform labelling (Ryan, 1974) and furthermore, the blood concentrations of a drug measured in this manner may not necessarily be indicative of pressor activity. Superfusion techniques have problems with specificity and selectivity and the release of endogenous excitatory substances (Turker, Yamamoto, Bumpus and Khairallah, 1971; Ryan, 1974).

The attenuated pressor technique also allows a concurrent detailed study of the haemodynamic responses of each vascular bed to direct and intravenous infusions of each vasoactive substance. This is particularly important in view of the results of Haas, Goldblatt, Lewis and Gipson (1973) who demonstrated that femoral haemodynamics have a marked influence on the inactivation of angiotensin II and noradrenaline in the femoral vascular bed.

Statistical analysis

Results have generally been given as the mean ± the standard error of the means (s.e.m.) when the number of experiments (n) have been greater or equal to 3. If parametric statistics were applicable, as determined by the F-test, then data was analysed by a Student's t-test. Either a paired or unpaired t-test was used, depending which was more appropriate for the particular evaluation. Similarly, if the nature of the change was not predicted then the test was two-tailed, but one-tailed if a shift

in a particular direction was included in the working hypothesis. Any other more specific tests used are documented in the appropriate place. A significance level of $p<0.05$ was used as the probability that an observed difference was not due to chance.

SECTION 3

THE INFLUENCE OF ROUTE OF ADMINISTRATION ON THE CARDIOVASCULAR RESPONSE TO ANGIOTENSIN II

INTRODUCTION

This section investigates the *in vivo* inactivation of angiotensin II in a number of peripheral vascular beds in conjunction with a haemodynamic study of the responses of these vascular beds to direct and intravenous infusions of angiotensin II.

The peripheral vascular beds examined were the renal, femoral, superior mesenteric and hepato-portal circulations, all of which have previously been found to be highly active in removing angiotensin II from the circulation (Hodge, Ng and Vane, 1967; Biron and Campeau, 1971; Broughton Pipkin, Mott and Roberton, 1971; Broughton Pipkin, 1972; Haas, Goldblatt, Lewis and Gipson, 1968, 1973). The exceptional study was that of Akinkugbe, Brown and Cranston (1966), who only observed a minor degree of angiotensin II inactivation in the femoral circulation of the rabbit.

Despite published evidence of a fairly uniform activity in destroying angiotensin II, these vascular beds vary markedly in their haemodynamic responses to administered angiotensin II.

The femoral and other skeletal muscle vascular beds have a unique haemodynamic response pattern to angiotensin II. Direct intra-arterial administration results in a dose-dependent decrease in muscle blood flow. However, on intravenous administration there is an increase in blood flow (Scroop, Walsh and Whelan, 1965; Forsyth, Hoffbrand and Melmon, 1971). This increase is passive and secondary to the elevated systemic blood pressure as calculated muscle vascular resistance is unaltered.

In contrast, renal, mesenteric and hepatic blood flows are all reduced during both intra-arterial and intravenous infusions of angiotensin II

These latter circulations therefore contribute to the overall rise in peripheral vascular resistance involved in the increase in mean arterial pressure while muscle vascular beds do not. In consequence, raised circulating levels of angiotensin II result in a redistribution of the cardiac output form the renal and splanchnic circulations to the skeletal musculature (Krasney, 1968; Di Salvo and Fell, 1970; Forsyth, Hoffbrand and Melmon, 1971). It seems possible therefore, that the hindquarters with their unique circulatory response, may inactivate angiotensin II in a manner different from that of the renal and splanchnic circulations.

The pulmonary circulation is not responsive to either intra-arterial or intravenous administration of angiotensin II and concurrently there is no loss of angiotensin II activity during passage through the pulmonary vascular bed (Biron, Meyer and Panisset, 1968).

The majority of previous studies have not accounted for the influence of local haemodynamics on the *in vivo* fate of angiotensin II, with the exception of Haas, Goldblatt, Lewis and Gipson (1973). In their study, femoral blood flow was found to be intimately associated with angiotensin II inactivation in the hindquarters. Consequently, this section deals with the *in vivo* inactivation of angiotensin II in peripheral vascular beds and the influence of local haemodynamics on this activity.

METHODS

The experiments were performed on 19 ex-racing greyhounds, weighing 26-34 kg. The techniques for anaesthesia induction and maintenance are discussed in detail in Section 2.

The cardiovascular variables monitored were mean arterial pressure, and local blood flow with vascular resistance calculated subsequently. Details of the techniques used for recording these variables appear in Section 2.

Angiotensin II inactivation in all peripheral vascular beds studied was examined with the attenuated systemic pressor response technique (detailed in Section 2). For comparative purposes, angiotensin II inactivation was also studied in the renal and femoral vascular beds by radioimmunoassay of angiotensin II in the arterial and venous blood from these circulations.

With the exception of those dogs where the influence of dose on angiotensin II inactivation was examined, all angiotensin II infusions were made at 500 ng/min (0.5 nmol/min) for 5 min.

The drugs used were, angiotensin II (Val^5-Hypertensin II, Asp-β-amide, Hypertensin, Ciba), alpha-chloralose ($C_8H_{11}O_6Cl_3$, B.D.H.), morphine sulphate (D.B.L.). All drugs were dissolved in physiological saline (sodium chloride 154 mmol/l) and infusions were administered with a constant speed syringe-infusion pump at 1 ml/min. At least 10 min was allowed to elapse between consecutive infusions of angiotensin II, with each infusion being preceded by a control infusion of saline. In all experiments contained within this section, the dose of administered angiotensin II was 500 ng/min for 5 min (0.5 nmol/min).

Statistical significance unless otherwise stated was examined by a Student's t-test and the null hypothesis was rejected with p values for less than 0.05.

RESULTS

RENAL AND FEMORAL VASCULAR BEDS

CARDIOVASCULAR RESPONSES TO INTRAVENOUS, RENAL ARTERY AND FEMORAL ARTERY INFUSIONS OF ANGIOTENSIN II

GENERAL

Mean arterial pressure

The changes in mean arterial pressure in response to intravenous, renal artery and femoral artery infusions of angiotensin II in a single experiment are found in Fig. 3.1. Mean arterial pressure was elevated with all three routes of infusion and the pooled data from this dog and a further 11 dogs, in which similar results were obtained, are recorded in Table 3.1.

Intravenous infusions of angiotensin II resulted in the greatest increase in mean arterial pressure (184.0 ± 21.9 mm Hg x min) with a significantly smaller pressor response on femoral artery administration (136.7 ± 17.1 mm Hg x min, $p<0.05$).

The smallest pressor response was observed on renal artery infusion (30.1 ± 5.4 mm Hg x min) bring significantly less than both the intravenous ($p<0.001$) and the femoral artery responses ($p<0.02$).

Renal blood flow and renal vascular resistance

Renal blood flow was reduced with all three routes of administration

FIG. 3.1. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]).

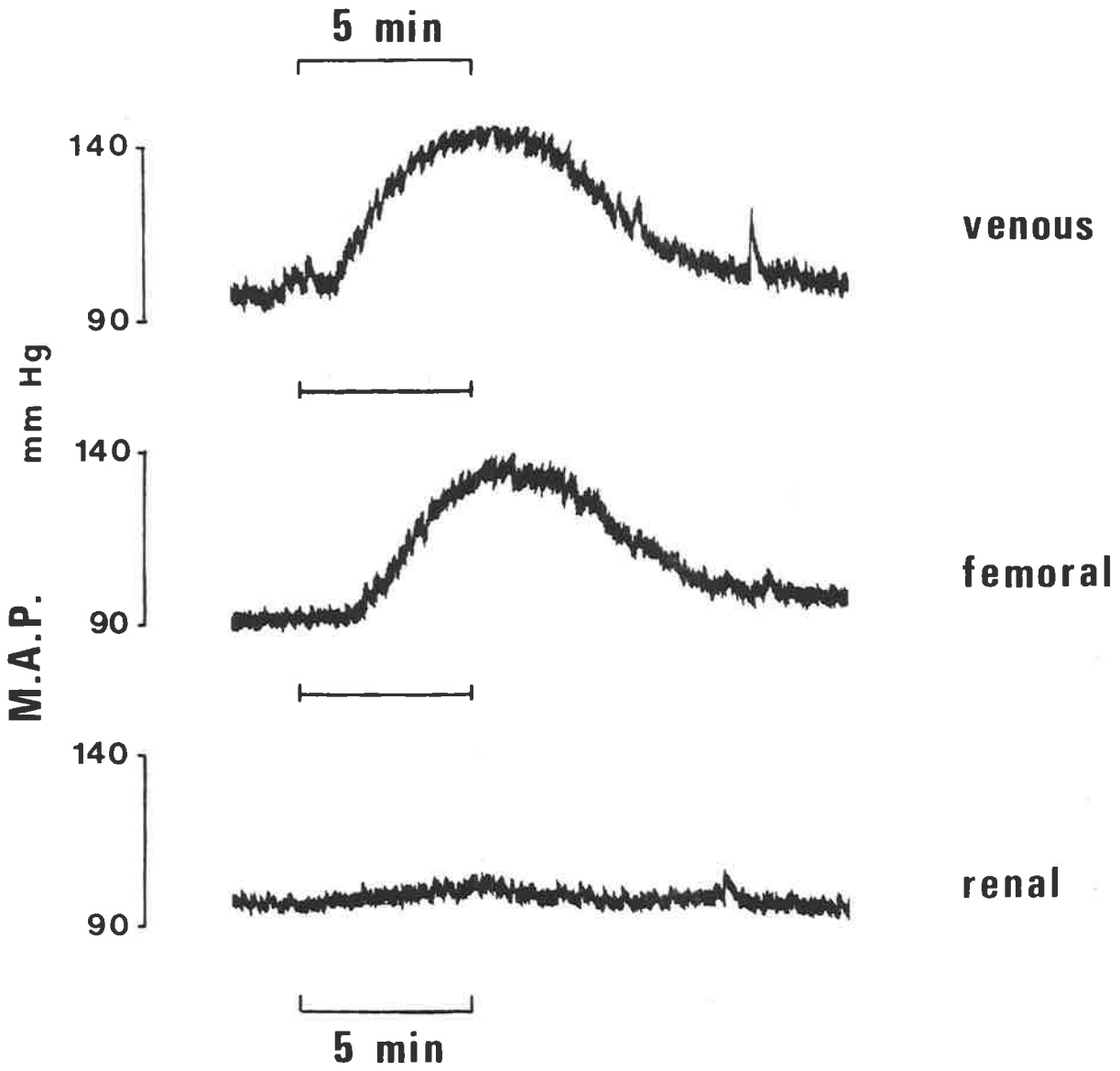
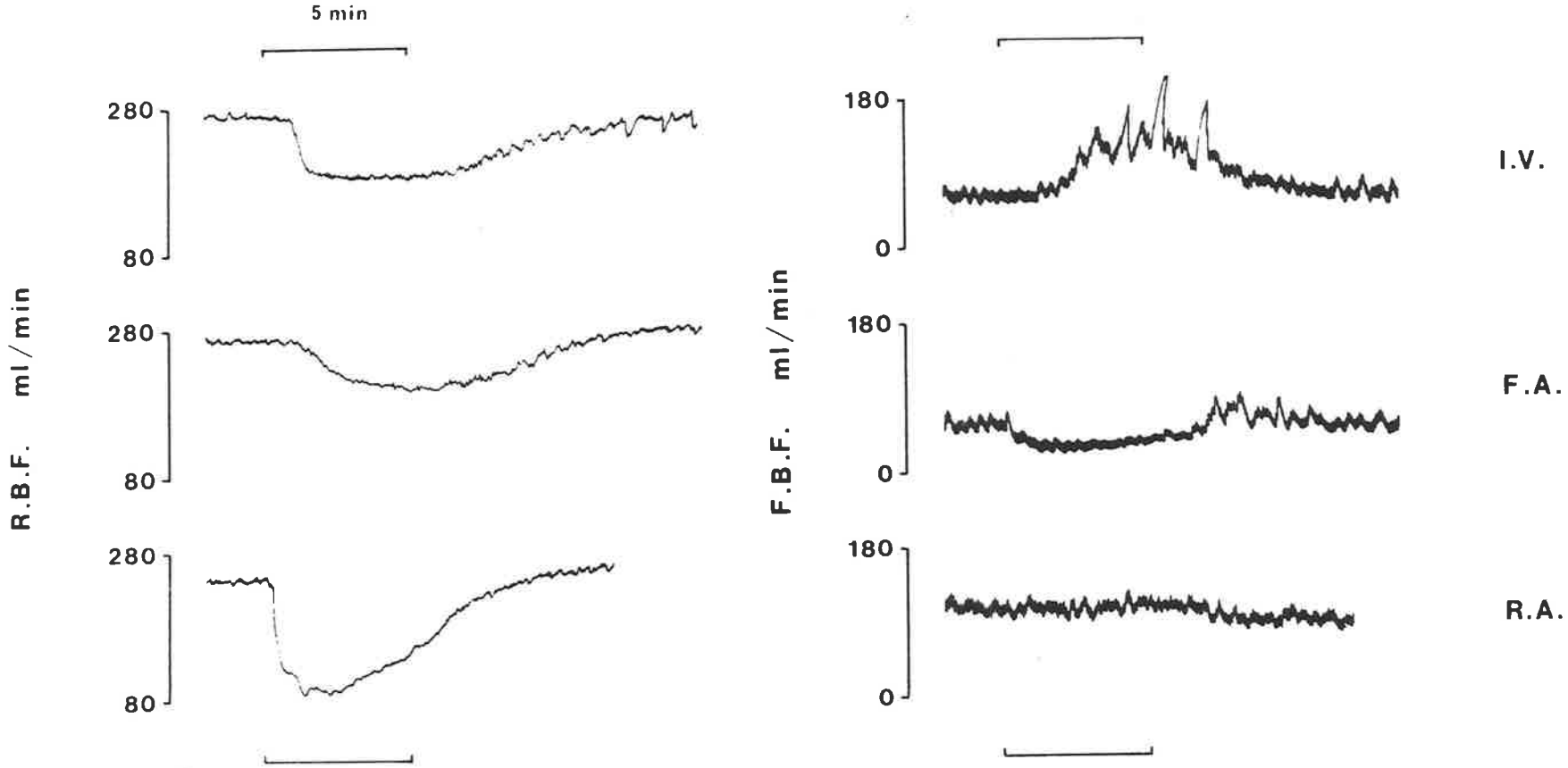

ANGIOTENSIN II
5 min
140
90
mm Hg
M.A.P.
venous
140
90
femoral
140
90
renal
5 min

FIG. 3.2. The changes in renal blood flow (R.B.F., ml/min) and femoral blood flow (F.B.F., ml/min) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]).

ANGIOTENSIN II



as illustrated in the results from a single experiment (Fig. 3.2). The greatest reduction in renal blood flow was seen on renal artery infusion.

Similar results were observed in all 12 dogs studied and the pooled results appear in Table 3.1. The fall in renal blood flow on renal artery infusions (-651.7 ± 39.8 ml, n=12) was significantly greater than on either intravenous (-286.4 ± 59.6 ml, n=12, $p<0.005$) or femoral artery infusions (-235.3 ± 33.8 ml, n=11, $p<0.005$). There was no significant difference between the renal blood flow responses with these latter two routes of administration.

Renal vascular resistance was calculated from the minute to minute levels of mean arterial pressure and renal blood flow and the pooled results for this series of dogs are found in Table 3.2. Renal vascular resistance was increased with all three infusion routes. The increase in renal vascular resistance on renal artery infusion (95.8 ± 16.1%, n=7) was significantly greater than the response to either intravenous infusion (48.2 ± 12.5%, n=7) or femoral artery infusion (24.9 ± 10.2%, n=7, $p<0.01$). The intravenous response was significantly greater than that obtained to femoral artery infusion ($p<0.05$).

Femoral blood flow and femoral vascular resistance

In contrast, to the responses of the renal vascular bed to infusions of angiotensin II, femoral blood flow was only decreased on femoral artery infusions (-261.1 ± 64.8 ml, n=7). This is demonstrated in Fig. 3.2, which contains the results from a single experiment. A similar pattern of femoral blood flow responses was observed in each dog and the pooled results appear in Table 3.1.

Femoral blood flow increased on both intravenous (243.7 ± 39.5 ml, n=7) and renal artery infusions (22.6 ± 10.6 ml, n=7). The response to intravenous infusions was significantly greater ($p<0.05$) than that to renal artery infusions (Table 3.1).

Femoral vascular resistance was substantially elevated on direct infusion (79.0 ± 16.3%, n=7), whereas intravenous and renal artery infusions were virtually without effect (-1.4 ± 4.2%, 0.1 ± 2.3%, n=7, respectively, Table 3.2).

CALCULATED ANGIOTENSIN II INACTIVATION

The degree of angiotensin II inactivation during passage through the renal and femoral vascular beds was estimated by two methods. Firstly, the attenuated pressor response technique and secondly by comparison of the angiotensin II levels measured by radioimmunoassay in arterial and venous blood. Details of the methodology of each of these techniques appear in Section 2.

Attenuated pressor response technique

The mean values from 12 dogs for the percentage inactivation of angiotensin II in the renal and femoral vascular beds are found in Table 3.1 and represented in Fig. 3.3. A considerable disparity between the renal and femoral vascular beds was observed with the mean renal percentage angiotensin II inactivation being 82.0 ± 3.2% (mean ± s.e.m., n=12) and the femoral inactivation of angiotensin II, 27.2 ± 4.9% (mean ± s.e.m., n=12). This difference is significant ($p<0.001$).

FIG. 3.3. The calculated percentage inactivation of angiotensin II in 12 dogs in the femoral (F.A.) and renal (R.A.) circulations as determined by the attenuated systemic pressor response method.

ANGIOTENSIN II

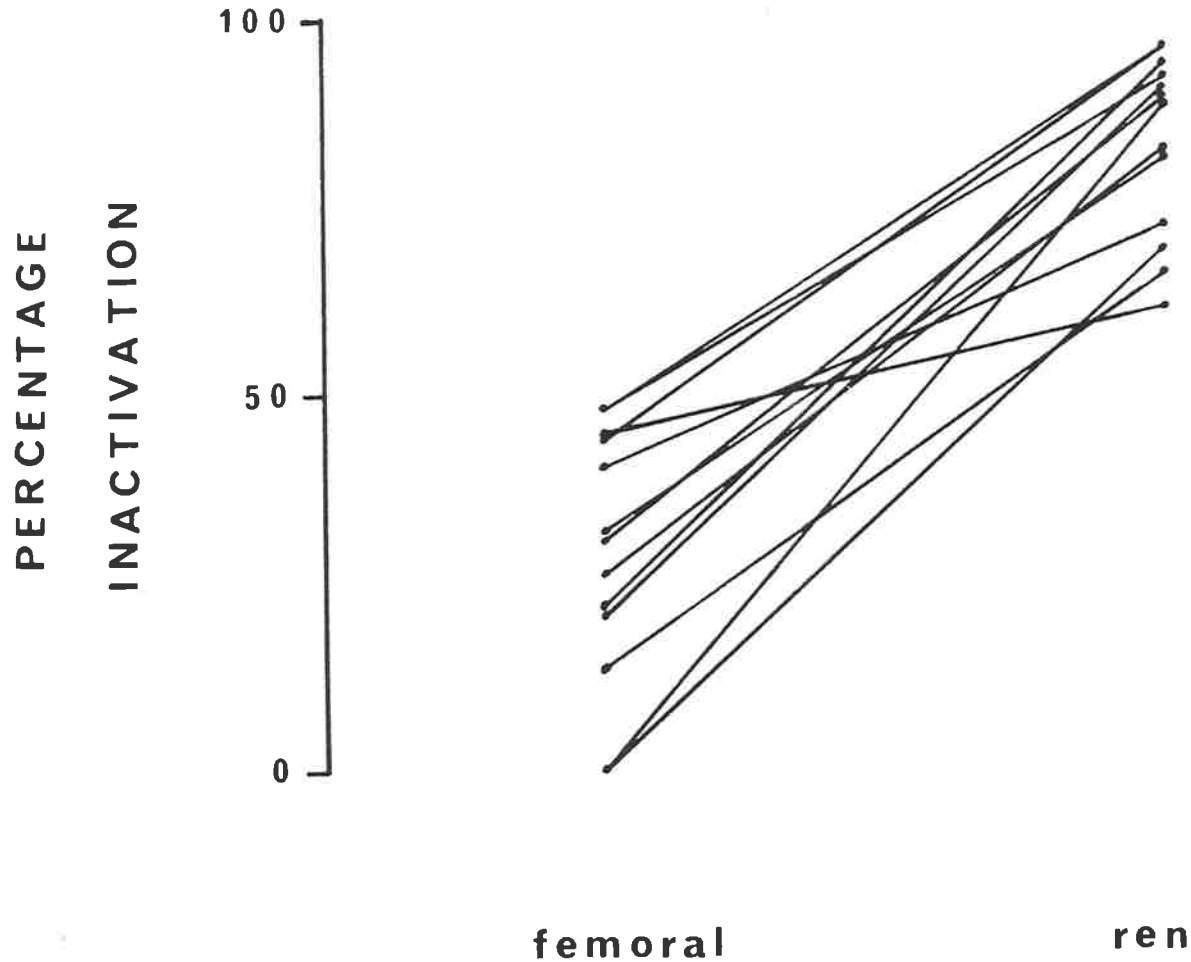
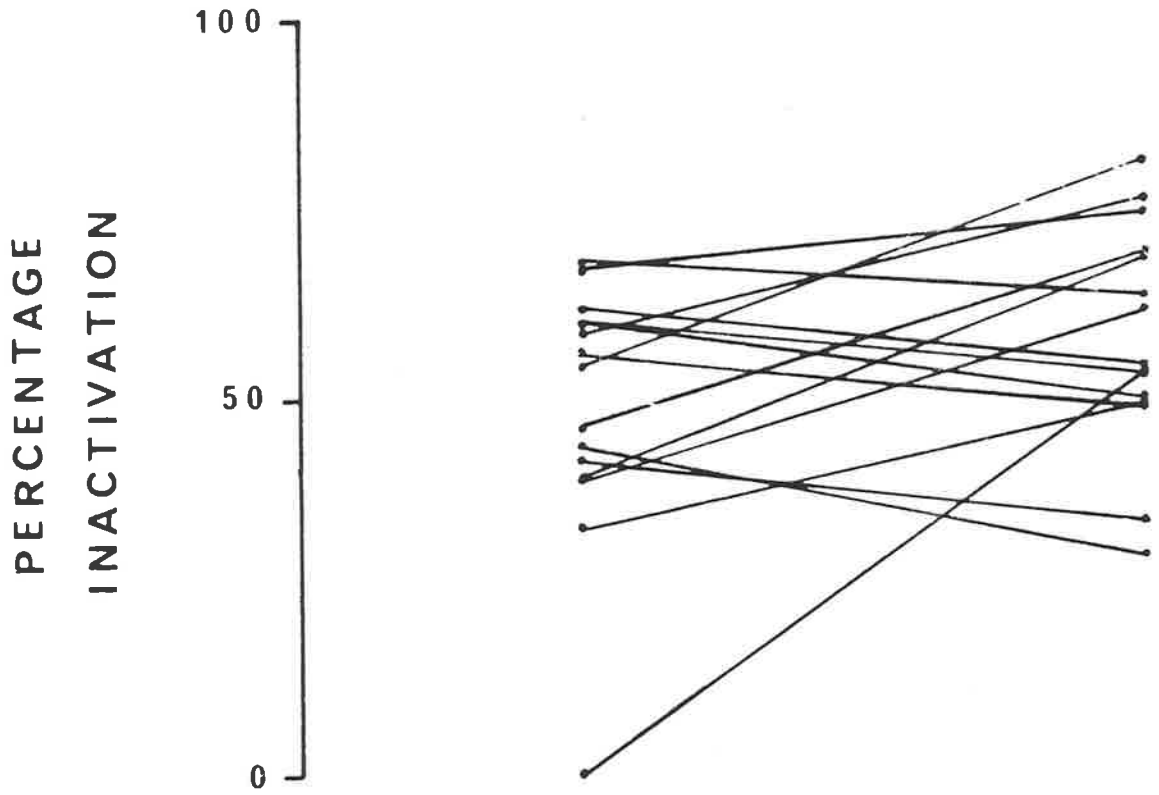


FIG. 3.4. The calculated percentage, inactivation of angiotensin II in the femoral and renal circulations as determined by radioimmunoassay. The results plotted are those for 5 dogs, with three estimations being made in each dog during a 15 min intravenous infusion of angiotensin II (500 ng/min [0.5 nmol/min]).

ANGIOTENSIN II



Estimation of angiotensin II inactivation by radioimmunoassay

In this group of 5 dogs, angiotensin II was administered by a 15 min intravenous infusion (500 ng/min; 0.5 nmol/min). Abdominal aortic, left renal vein and inferior vena caval blood samples were withdrawn at 5 min intervals and assayed for angiotensin II. The individual and mean results of plasma angiotensin II concentration and the calculated degree of angiotensin II inactivation in the renal and femoral vascular beds are found in Table 3.3.

In contrast to the findings with the attenuated pressor response technique, these vascular beds are not significantly different in their ability to inactivate angiotensin II, renal 55.0 ± 4.7% (mean ± s.e.m., n=15), femoral 47.6 ± 4.6% (mean ± s.e.m., n=15, $0.30<p<0.20$). These results are represented in Fig. 3.4.

THE INFLUENCE OF DOSE ON THE DEGREE OF RENAL INACTIVATION OF ANGIOTENSIN II

GENERAL

Mean arterial pressure and renal blood flow

Three doses of angiotensin II were studied (250, 500 and 750 ng/min for 5 min) and the pooled pressor responses for 7 dogs appear in Table 3.4. A dose-dependent increase in mean arterial pressure was observed on both intravenous and renal artery infusions. At each dose-level, the intravenous response was substantially greater than the response to renal artery infusion ($p<0.01$).

The changes in renal blood flow were also dose-dependent, with the greatest response being observed on renal artery infusion at each dose level. The pooled results for 7 dogs appear in Table 3.4.

CALCULATED ANGIOTENSIN II INACTIVATION

Angiotensin II inactivation in the renal circulation was calculated in this group of dogs with the attenuated systemic pressor response technique. The degree of angiotensin II inactivation was unaltered over the dose range studied (250 ng/min, 80.6 ± 6.0%; 500 ng/min, 78.4 ± 4.1%; 750 ng/min, 75.4 ± 4.8%) and was comparable to that seen in previous groups of dogs, Table 3.4.

A relationship between renal blood flow and the ability of the kidney to inactivate angiotensin II was examined by correlating the degree of renal blood flow reduction on renal artery infusion with the observed degree of angiotensin II inactivation, with each dose. No relationship was observed ($r=0.0151$, $n=21$, Fig. 3.5).

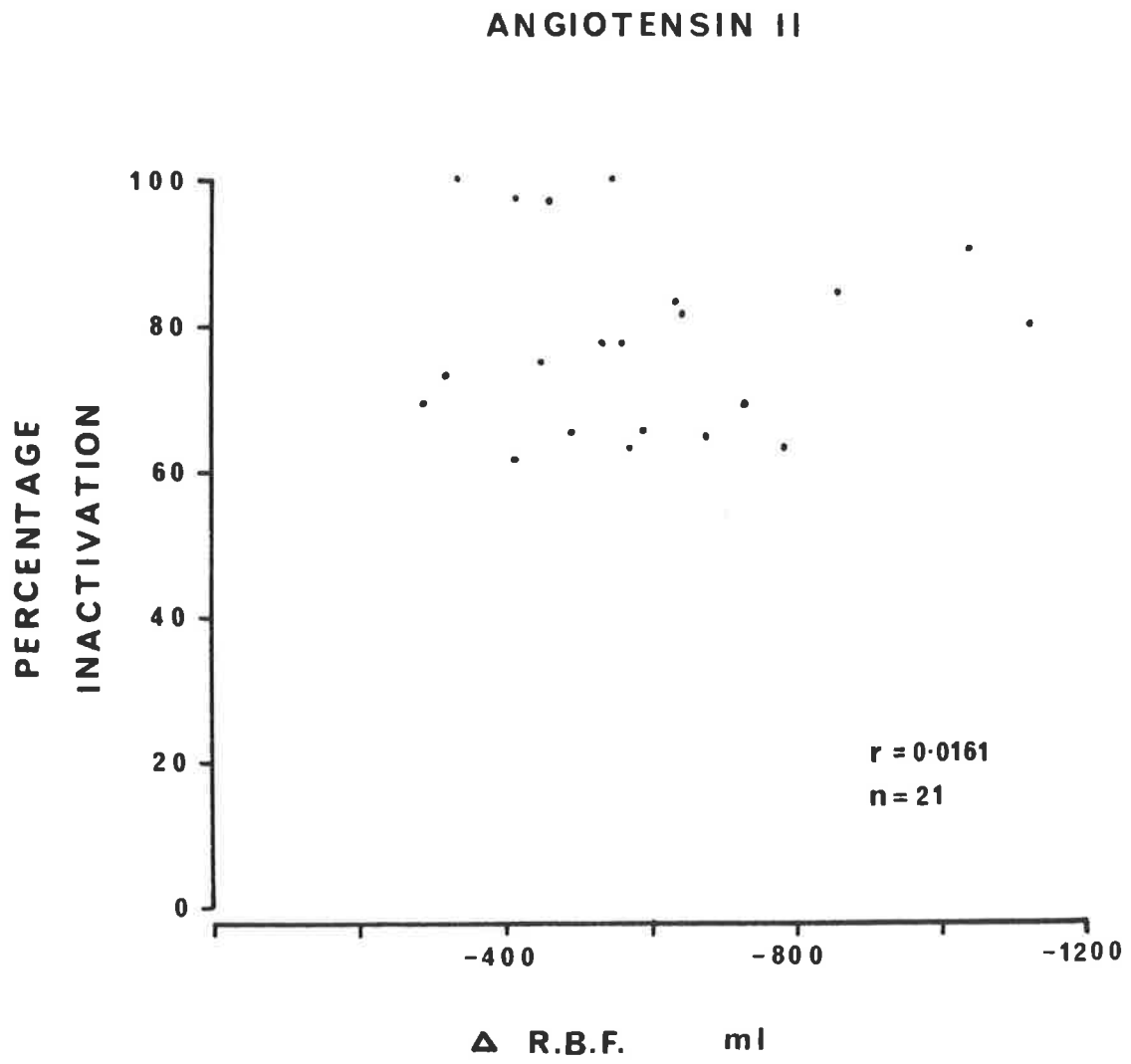


FIG. 3.5. The relationship between the calculated percentage inactivation of angiotensin II in the renal circulation and the integrated fall in renal blood flow (R.B.F., ml/min) on 5 min renal artery infusions of angiotensin II (250, 500 and 750 ng/min).

SUPERIOR MESENTERIC HEPATO-PORTAL VASCULAR BEDS

CARDIOVASCULAR RESPONSES TO INTRAVENOUS, SUPERIOR MESENTERIC ARTERY AND HEPATO-PORTAL VEIN INFUSIONS OF ANGIOTENSIN II

GENERAL

Mean arterial pressure

The mean arterial pressure responses to intravenous, superior mesenteric artery and hepato-portal vein infusions of angiotensin II in a single experiment are found in Fig. 3.6. All three routes of infusion resulted in an elevation of mean arterial pressure, with the greatest increase occurring on intravenous infusion.

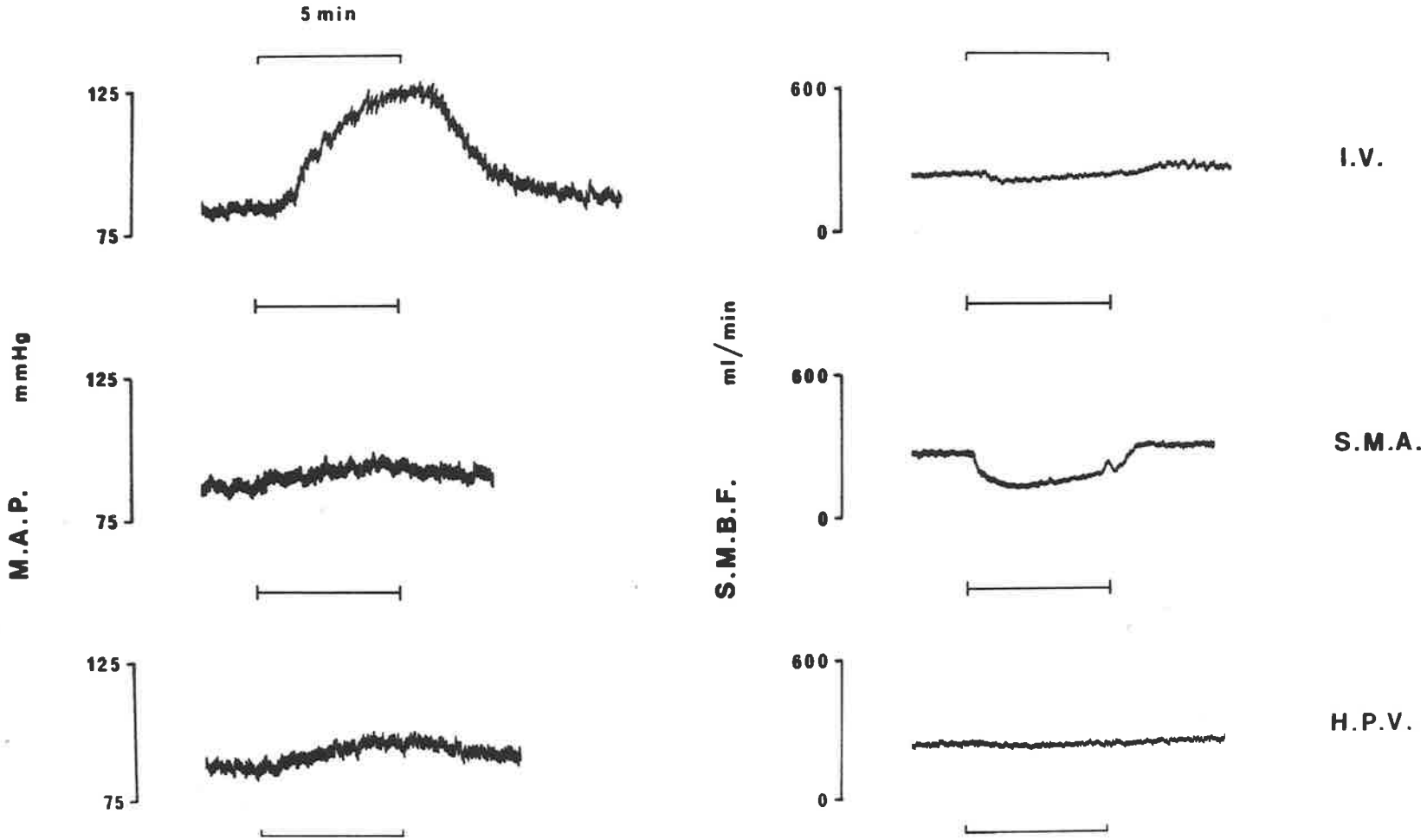
Similar results were obtained in all 7 dogs studied and the pooled data are included in Table 3.5. The pressor responses to superior mesenteric artery and hepato-portal vein infusions were not significantly different (34.3 ± 4.2, 26.0 ± 3.3 mm Hg x min, respectively), although both were considerably less than the intravenous response (183.9 ± 22.7 mm Hg x min, $p<0.001$).

Superior mesenteric blood flow

Superior mesenteric blood flow was reduced on all three routes of infusion and, as expected, the greatest reduction was associated with superior mesenteric artery infusions. The results from a single experiment appear in Fig. 3.6 and the pooled results for 7 dogs are recorded in Table 3.5.

FIG. 3.6. The changes in mean arterial pressure (M.A.P., mm Hg) and superior mesenteric blood flow (S.M.B.F., ml/min) in a single experiment, in response to 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]).

ANGIOTENSIN II



There is no significant difference between the superior mesenteric blood flow responses to intravenous and hepato-portal vein infusions (-280.2 ± 105.1, -69.7 ± 11.6 ml, respectively) although both are significantly less than the superior mesenteric artery response ($p<0.025$).

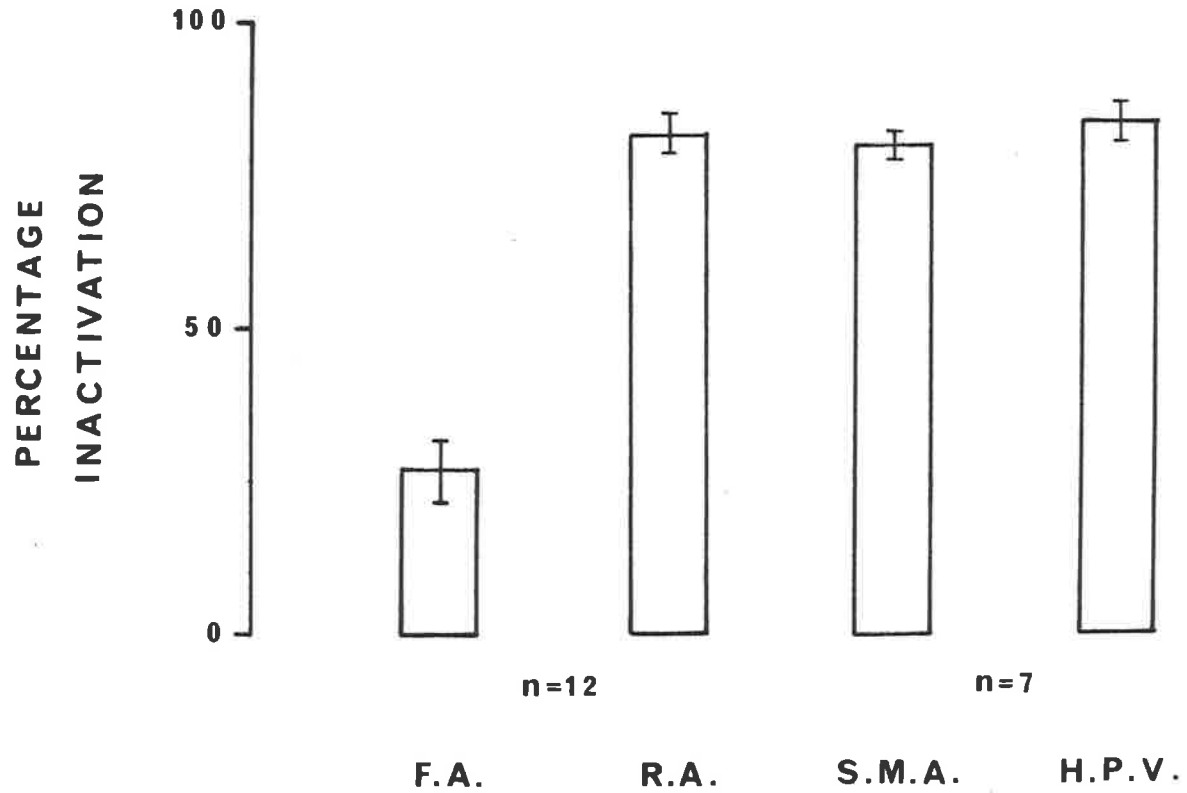
CALCULATED ANGIOTENSIN II INACTIVATION

The degree of angiotensin II inactivation in each vascular bed was calculated from the loss of systemic pressor activity on passage through superior mesenteric artery and hepato-portal vein circulations as outlined in Section 2.

Angiotensin II inactivation in each vascular bed was extensive and of a similar degree, the respective inactivation percentages being, superior mesenteric artery 80.6 ± 2.3% and hepato-portal vein 85.3 ± 1.9%.

The degree of angiotensin II inactivation in the superior mesenteric and hepato-portal circulations is comparable to that observed in the renal circulation (Fig. 3.7), although significantly greater than that in the femoral circulation ($p<0.001$).

FIG. 3.7. The mean calculated percentage inactivation of angiotensin II in the renal (R.A.), femoral (F.A.), superior mesenteric (S.M.A.) and hepato-portal (H.P.V.) circulations, as determined by the attenuated systemic pressor response technique.


ANGIOTENSIN II
PERCENTAGE INACTIVATION
100
50
0
n=12
n=7
F.A.
R.A.
S.M.A.
H.P.V.

DISCUSSION

From these studies it is apparent that peripheral vascular beds vary both in their ability to inactivate infused angiotensin II and in the pattern of their haemodynamic responses. The renal, femoral and superior mesenteric vascular beds all exhibit blood flow reductions and increases in vascular resistance on direct angiotensin II administration.

However, the nature of the haemodynamic response of the femoral vascular bed to systemic administration was markedly different from the other peripheral circulations studied. Intravenous infusion of angiotensin II was associated with a pressure-related increase in blood flow with femoral vascular resistance being unaltered. The renal and superior mesenteric vascular beds, in contrast, exhibited reductions in blood flow and increases in vascular resistance.

The responses of the femoral vascular bed confirm previous observations in other skeletal muscle circulations (Scroop, Walsh and Whelan, 1965; Kapitola, Kuchel, Schreiberova and Jahoda, 1968; Forsyth, Hoffbrand and Melmon, 1971). The femoral artery in the greyhound supplied not only hindquarter skeletal muscle but also the circulations of skin and bone. However, the femoral blood flow response pattern is taken to represent principally the response of the skeletal muscle vasculature.

Unlike the renal and mesenteric vascular beds, the femoral circulation is not involved in the elevation of total peripheral resistance in response to increased levels of circulating angiotensin II. However, vascular angiotensin II receptors do exist in the femoral vasculature as demonstrated by the marked elevation in femoral vascular resistance on direct infusion.

The mechanism whereby a vasoconstriction fails to occur in the femoral vasculature during intravenous infusion is unknown. The blood concentration of angiotensin II should be sufficient, as decreases in superior mesenteric and renal blood flow are observed.

The elevation in mean arterial pressure on intravenous infusion at this dose is known to partially involve a central component (Scroop and Lowe, 1969) and therefore, this increase in femoral blood flow on intravenous infusion may well involve a reflex which acts to prevent an increase in femoral vascular resistance.

Elevated circulating levels of angiotensin II result therefore in a redistribution of the cardiac output from the renal and mesenteric circulations to the skeletal musculature, in addition to an increased total peripheral resistance and mean arterial pressure.

The nature of the superior mesenteric blood flow reduction to direct infusion of angiotensin II is similar to the response of the femoral vascular bed and contrasts with the renal circulation. The degree of superior mesenteric and femoral blood flow reduction is constant throughout the infusion period and returns to the control level immediately after the infusion has been completed.

Renal blood flow on the other hand, steadily increased towards the control value despite continued infusion of angiotensin II. This effect has been attributed to the intrarenal generation of vasodilatory substances such as bradykinin and prostaglandin E (McGiff, Crowshaw, Terragno and Lonigro, 1970; Aiken and Vane, 1973; Malik and McGiff, 1976).

The majority of previous reports, with the exception of Akinkugbe,

Brown and Cranston (1966), have indicated that the hindquarters are as active in removing angiotensin II from the circulation as the renal and splanchnic vascular beds (Hodge, Ng and Vane, 1967; Biron, Meyer and Panisset, 1968; Broughton Pipkin, Mott and Robertson, 1971; Haas, Goldblatt, Lewis and Gipson, 1968, 1969, 1973).

This finding has not been confirmed in the present study, where the hindquarters have been found to be significantly less active. This lack of agreement may be due to the method of angiotensin II administration. In the majority of previous studies angiotensin II has been administered by bolus injection with the peak response being monitored. In the present study, the integrated response to 5 min infusions of angiotensin II has been examined.

Haas and his colleagues (1973) attributed a high degree of angiotensin II inactivation in the femoral vascular bed to a slow leakage of injected angiotensin II from the hindquarter into the general circulation as a consequence of a severe local vasoconstriction. When this severe vasoconstriction was prevented by concomitant administration of bradykinin, angiotensin II passed through the hindquarter without loss of vasoactivity.

In the present experiments this delayed transit of angiotensin II across the femoral vasculature is demonstrated by the delayed onset of the mean arterial pressure response. The false positive results described by Haas, Goldblatt, Lewis and Gipson (1973) were avoided in the present experiments both by administering angiotensin II as a five minute infusion, thereby achieving a constant release of angiotensin into the general circulation from the hindquarter and by monitoring the integrated mean arterial pressure responses and not the peak pressor response, thereby measuring the total vasoactivity of infused angiotensin II.

It is interesting to note that the only other study in agreement with the present results (Akinkugbe, Brown and Cranston, 1966) used a similar method in rabbits, involving 5 min infusions of angiotensin II and a planimetric measurement of the area contained by the blood pressure response. The present findings therefore do not appear to be peculiar to the greyhound.

In the present study, the degree of renal blood flow reduction on renal artery infusion of angiotensin II, was independent of the observed degree of angiotensin II inactivation, as was the dose of administered angiotensin II. They may indicate that the vascular compartment is the site of this inactivation and not the renal tubules.

The pulmonary circulation, like the femoral, is not involved in the elevation in total peripheral resistance in response to increased circulating levels of angiotensin II (Barer, 1961) and, in addition, it is not a site of angiotensin II inactivation *in vitro* (Biron, Meyer and Panisset, 1968). Thus there appears to be a substantial relationship between haemodynamic responsiveness and angiotensin II inactivation.

A radioimmunoassay technique was also used to investigate angiotensin II inactivation in the renal and femoral vascular bed and failed to confirm the results obtained with the attenuated pressor technique.

This variance may be due to a lack of antigenic specificity, although the antisera used was less than 0.1% cross-reactive with the decapeptide angiotensin I. However, metabolic fragments may have competed with intact angiotensin II for the specific binding sites. This effect has been demonstrated in sheep (Cain, Catt and Coghlan, 1969). A high blood concentration of angiotensin II metabolic fragments at the time of sampling is conceivable, as the major route of inactivation is thought to

to be metabolism (Bailie and Oparil, 1977).

The lack of agreement between these two techniques may also reflect a fundamental difference between the index of inactivation used. The pressor technique examines angiotensin II activity in terms of its vasoactivity, whereas radioimmunoassay examines its concentration in the blood. These two may not be equatable.

Furthermore, angiotensin II was administered intravenously in the radioimmunoassay experiments, whereas the attenuated pressor technique was examined on arterial infusion. This difference in results may therefore be due to variations in route of administration, blood concentration and haemodynamics.

SUMMARY

1. On all routes of administration the doses of angiotensin II studied elevated mean arterial pressure. The pressor response to interavenous infusions was consistently greater than to either renal, fomoral and superior mesenteric artery and hepato-portal vein infusions.

2. The increase in mean arterial pressure on femoral artery infusions of angiotensin II was significantly greater than renal and superior mesenteric artery and hepato-portal vein infusions of the same dose.

3. Direct infusion of angiotensin II reduced local blood flow in the renal, femoral and superior mesenteric vascular beds although on intravenous infusion, femoral blood flow increased whilst femoral vascular resistance was unaltered. In contrast, renal and superior mesenteric blood flow was reduced on intravenous administration of angiotensin II.

4. A high degree of angiotensin II inactivation was observed in the renal, splanchnic and hepato-portal circulations but only a minor loss of angiotensin II vasoactivity was observed during passage through the femoral vascular bed.

5. While the loss of vasoactivity of angiotensin II in the renal circulation was significantly greater than that seen in the hindquarters, the arterio/venous differences in plasma angiotensin II concentration were of a similar magnitude in these two vascular beds.

6. Angiotensin II inactivation in the renal circulation is independent of both renal blood flow and of dose in the range studied.

SECTION 4

THE INFLUENCE OF ROUTE OF ADMINISTRATION ON THE CARDIOVASCULAR RESPONSE TO CATECHOLAMINES

INTRODUCTION

The experiments reported in this section investigate *in vivo* fate of catecholamines in a number of peripheral vascular beds. Noradrenaline has been the prime catecholamine studied, whilst for comparative purposes adrenaline and isoprenaline have been included in some of the studies.

Variable haemodynamic responses have been reported in peripheral vascular beds, to both intravenous and intra-arterial administration of adrenaline and noradrenaline (Barer, 1961; Hoffbrand and Forsyth, 1973), in part reflecting the differing populations of α- and β-adrenoceptors in the peripheral circulation. Nevertheless, both adrenaline and noradrenaline elevate mean arterial pressure at least in part through an increase in total peripheral resistance. Hoffbrand and Forsyth (1973) observed a redistribution of the cardiac output from the splanchnic, renal and cutaneous circulations to those of the heart and skeletal musculature. The present study investigates further the relative contribution of each peripheral vascular bed studied to this increase in total peripheral vascular resistance.

Despite the variable haemodynamic responses of peripheral vascular beds to noradrenaline and adrenaline their inactivation in these vascular beds is thought to be uniformly high (Ginn and Vane, 1968; Vane, 1969; Gryglewski and Vane, 1970), predominantly through the effects of neuronal and extraneuronal uptake (Iversen, 1967). Isoprenaline inactivation, however, is slightly smaller in magnitude (Gryglewski and Vane, 1970) as it is only a substrate for extraneuronal uptake (Callingham and Burger, 1966). Isoprenaline is not a naturally occurring catecholamine and, unlike adrenaline and noradrenaline, is a pure β-adrenoceptor agonist.

Haas, Goldblatt, Lewis and Gipson (1973) found that both noradrenaline and angiotensin II inactivation could be inhibited in the femoral vascular bed when the local vasoconstrictor response was blocked by a concomitant administration of bradykinin, indicating that local haemodynamics may greatly influence estimations of noradrenaline inactivation in the hind-quarters. The influence of blood flow changes on the rate of inactivation of noradrenaline has been closely examined in a number of vascular beds in this section.

METHODS

The experiments were performed in 13 ex-racing greyhounds weighing 26-33 kg. The techniques used for inducing and maintaining anaesthesia and monitoring cardiovascular variables are discussed in detail in Section 2.

Mean arterial pressure and blood flow in the vascular bed under study were monitored during intravenous and direct infusion into the vascular bed of noradrenaline, adrenaline and isoprenaline. Catecholamine inactivation was calculated by the attenuated systemic pressor response method.

The drugs used were alpha-chloralose ($C_8H_{11}O_6Cl_3$, B.D.H.), morphine sulphate (D.B.L.), l-noradrenaline bitartrate monohydrate (Levophed, Winthrop), adrenaline (USV, Knoll), isoprenaline hydrochloride (Isuprel, Winthrop). All drugs were dissolved in physiological saline (sodium chloride 154 mmol/) and 5 mg l-ascorbic acid (Sigma) was added to noradrenaline solutions to prevent oxidation (final concentration 1×10^{-4} mol/l).

All infusions were made on a constant speed syringe infusion pump at 1 ml/min. At least 10 min was allowed to elapse between consecutive catecholamine infusions, with each infusion being preceded by a control infusion of saline.

With the exception of those experiments where the influence of dose on cardiovascular responses and mechanisms of inactivation was investigated the doses of catecholamines infused through all routes were as follows: noradrenaline 10 µg/min (59.1 nmol/min); adrenaline 10 µg/min (54.6 nmol/min); isoprenaline 1.0 µg/min (4.7 nmol/min).

RESULTS

SECTION 4.1 NORADRENALINE

CARDIOVASCULAR RESPONSES TO INTRAVENOUS, RENAL ARTERY AND FEMORAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

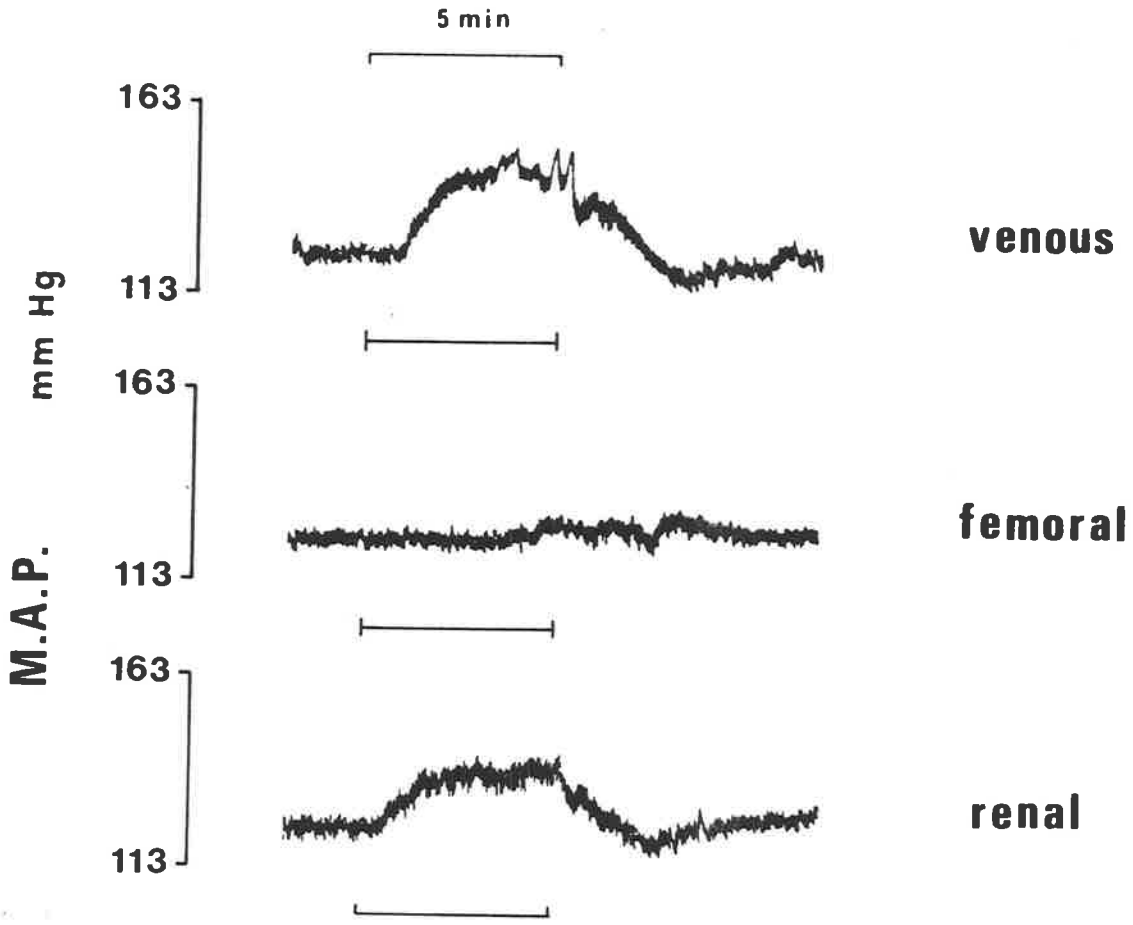
The mean arterial pressure responses to intravenous, renal artery and femoral artery infusions of noradrenaline (10 μg/min; 59.1 nmol/min) in a single experiment are found in Fig. 4.1.1. Mean arterial pressure was elevated on all three routes of administration although the magnitude of this pressor response varied substantially with route of administration.

Similar results were observed in each of the 7 dogs studied and the pooled data appear in Table 4.1.1. Intravenous infusions of noradrenaline resulted in the greatest increase in mean arterial pressure (160.8 ± 80.1 mm Hg x min). The magnitude of the renal artery pressor response (105.7 ± 48.2 mm Hg x min) was only slightly less than the intravenous response and the difference was not significant. The pressor response to femoral artery infusions of noradrenaline was significantly less than both the intravenous and renal artery responses (6.1 ± 6.1 mm Hg x min, $p<0.05$).

Renal blood flow and renal vascular resistance

The renal blood flow response to intravenous, renal artery and femoral

FIG. 4.1.1. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]).


NORADRENALINE
5 min
163
113
mm Hg
M.A.P.
venous
163
113
femoral
163
113
renal

artery infusions of noradrenaline in a single experiment are found in Fig. 4.1.2. Renal artery administration resulted in a substantial fall in renal blood flow, an effect which persisted for some time after the infusion period. The pooled renal blood flow responses for this group of 7 dogs appear in Table 4.1.1.

The fall in renal blood flow on renal artery infusion of noradrenaline (-1275.6 ± 324.6 ml) was significantly greater than the response to either intravenous (-160.7 ± 21.7 ml, $p<0.01$) or femoral artery infusion (-9.4 ± 9.4 ml, $p<0.005$). The latter route of administration reduced renal blood flow in only one of seven dogs studied (Table 4.1.1).

Similar results were obtained with calculated renal vascular resistance. A substantial increase in renal vascular resistance occurred on renal artery infusion (284.1 ± 76.9%) with a significantly smaller response associated with intravenous infusion (38.7 ± 15.7%, $p<0.025$). Renal vascular resistance was virtually unaltered on femoral artery infusion of noradrenaline (-0.8 ± 1.6%, Table 4.1.2).

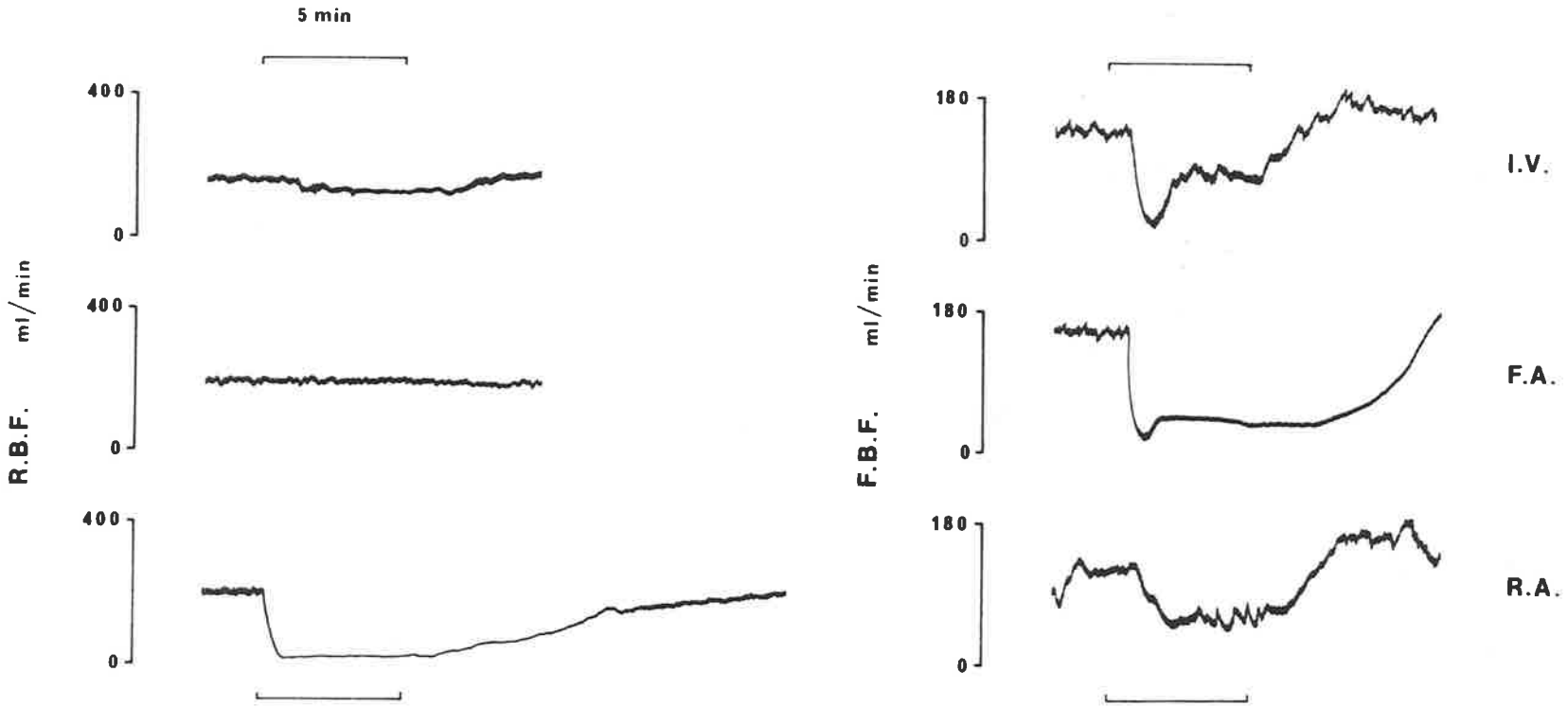
Femoral blood flow and femoral vascular resistance

Femoral blood flow was reduced with all three routes of noradrenaline infusion and the results from a single experiment appear in Fig. 4.1.2. As expected, the greatest reduction in femoral blood flow occurred on femoral artery infusion and this response was followed by a period of marked hyperaemia.

Similar results were found in each of the 7 dogs studied and the pooled results appear in Table 4.1.1. The reduction in femoral blood flow was significantly greater on femoral artery infusion (-1057.3 ± 273.6 ml) than

FIG. 4.1.2. The changes in renal blood flow (R.B.F., ml/min) and femoral blood flow (F.B.F., ml/min) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]).

NORADRENALINE



on either intravenous (-67.7 ± 49.4 ml, $p<0.01$) or renal artery infusions (-181.3 ± 112.1 ml, $p<0.01$). There was no significant difference between these latter two routes of administration.

Similarly, the increase in femoral vascular resistance was significantly greater on femoral artery infusion (318.5 ± 80.2%) than on either intravenous (66.7 ± 13.8%, $p<0.05$) or renal artery infusions (42.2 ± 12.0%, $p<0.025$). The renal artery response was significantly less than the intravenous response ($p<0.005$).

CALCULATED NORADRENALINE INACTIVATION

In this group of 7 dogs, noradrenaline inactivation was determined by the loss of systemic vasoactivity after passage through the renal or femoral vascular beds using the attenuated systemic pressor response technique. The mean results appear in Table 4.1.1.

An extensive degree of noradrenaline inactivation was observed in the hindquarters, 99.0 ± 1.0%, which was significantly greater than the results obtained in the renal vascular bed, 28.9 ± 6.8% ($p<0.001$).

These results are in direct contrast to those obtained with angiotensin II.

THE INFLUENCE OF DOSE AND RENAL HAEMODYNAMICS ON THE RENAL INACTIVATION OF NORADRENALINE

GENERAL

Mean arterial pressure and renal blood flow

Noradrenaline was infused through the intravenous and renal artery routes of administration for 5 min, at three dose levels (2.5, 5.0 and 10.0 μg/min; 14.2, 28.5 and 59.1 nmol/min). Mean arterial pressure was elevated in a dose-related manner with each route of infusion (Table 4.1.3).

With each dose, the intravenous pressor response was greater than the renal artery response. This difference was significant for the 2.5 and 5.0 μg/min doses ($p<0.001$, $p<0.005$, respectively) but not for the 10.0 μg/min dose.

Renal blood flow was reduced on each route of administration and with each dose (Table 4.1.3). Renal artery infusion resulted in a significantly greater reduction in renal blood flow than on intravenous infusion for each dose studied, 2.5 μg/min ($p<0.05$), 5.0 μg/min ($p<0.005$) and 10.0 μg/min ($p<0.001$).

The renal blood flow response to renal artery infusion of noradrenaline at 2.5 μg/min is significantly smaller than the response to either the 5.0 μg/min or 10.0 μg/min doses ($p<0.02$). These latter two doses were not significantly different in their renal blood flow responses on direct infusion.

CALCULATED NORADRENALINE INACTIVATION

The inactivation of noradrenaline in the renal circulation and the influence of infused dose and renal haemodynamics was investigated by the attenuated systemic pressor response technique.

Renal inactivation of infused noradrenaline decreased with increasing dose, (2.5 μg/min, 73.2 ± 7.3%; 5.0 μg/min, 43.2 ± 10.2%; 10.0 μg/min, 21.1 ± 3.7%; n=7, Table 4.1.3), with the calculated percentage inactivation for the 2.5 and 10.0 μg/min doses being significantly different ($p<0.01$). The influence of concurrent renal blood flow alterations are demonstrated in Fig. 4.1.3.

The relationship between percentage inactivation of renal artery infused noradrenaline and fall in renal blood flow associated with direct infusion of noradrenaline was examined and a significant negative correlation was obtained ($p<0.05$). These results are also in sharp contrast to the study with angiotensin II which was discussed in Section 3.

CARDIOVASCULAR RESPONSES TO INTRAVENOUS, SUPERIOR MESENTERIC ARTERY AND HEPATO-PORTAL VEIN INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

Intravenous, superior mesenteric artery and hepato-portal vein infusions

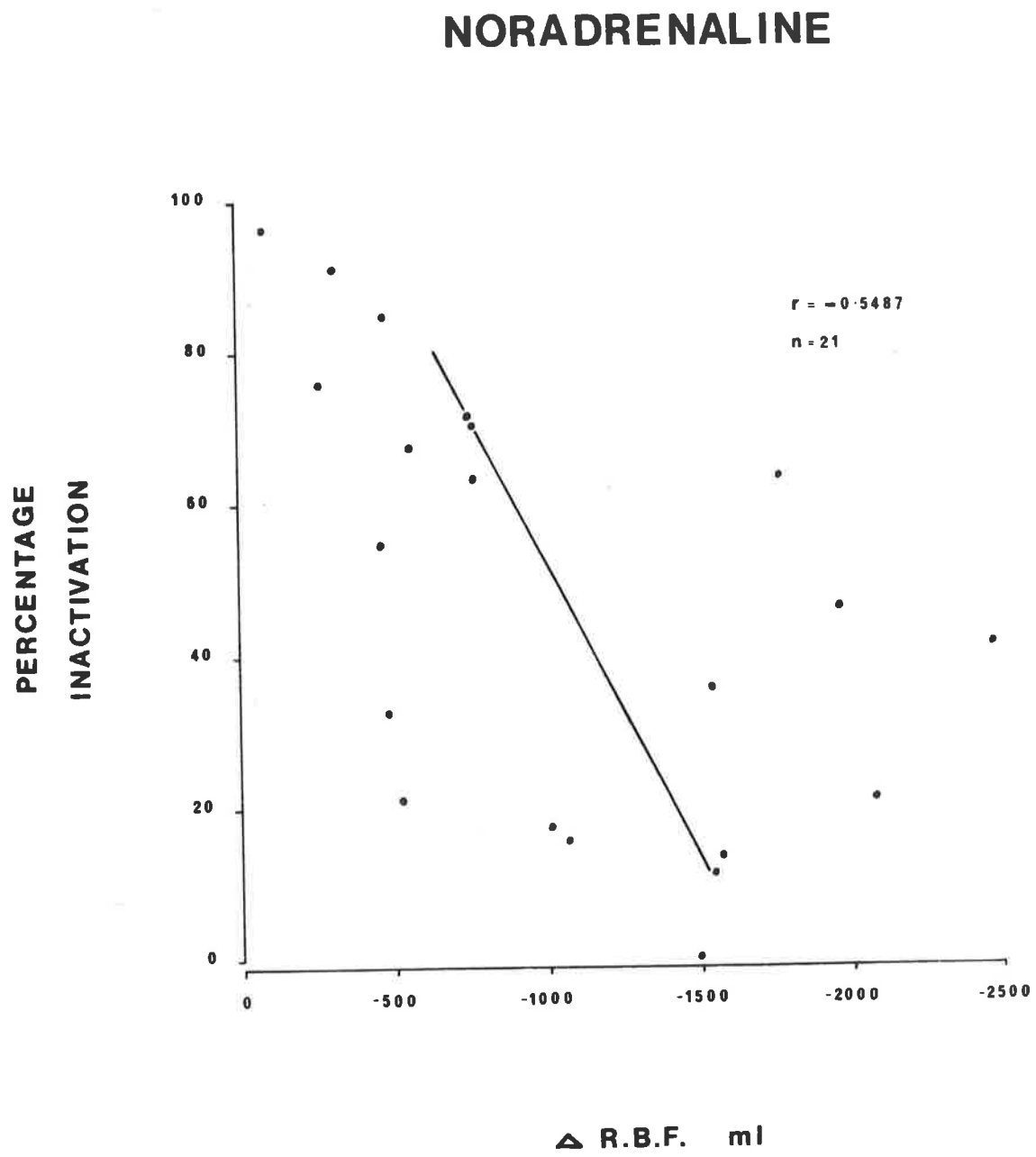


FIG. 4.1.3. The relationship between the calculated percentage inactivation of noradrenaline in renal circulation and the integrated fall in renal blood flow (R.B.F., ml/min) on 5 min renal artery infusions of noradrenaline (2.5, 5.0 and 10.0 μg/min [14.2, 28.5 and 59.1 nmol/min]).

of noradrenaline resulted in an elevation of mean arterial pressure as demonstrated in Fig. 4.1.4, from a single experiment. Similar pressor responses were observed on superior mesenteric artery and hepato-portal vein infusions and these were considerably less than the intravenous response.

Consistent results were obtained in the other 5 dogs studied and the mean results appear in Table 4.1.4. The pressor response to superior mesenteric artery infusions (38.2 ± 7.2 mm Hg x min) and hepato-portal vein infusions (52.1 ± 9.2 mm Hg x min) were both significantly less than the response to intravenous infusions (157.3 ± 25.0 mm Hg; $p<0.005$, $p<0.001$, respectively). The mean pressor response to superior mesenteric artery infusions was significantly less than that to hepato-portal vein infusions ($p<0.05$).

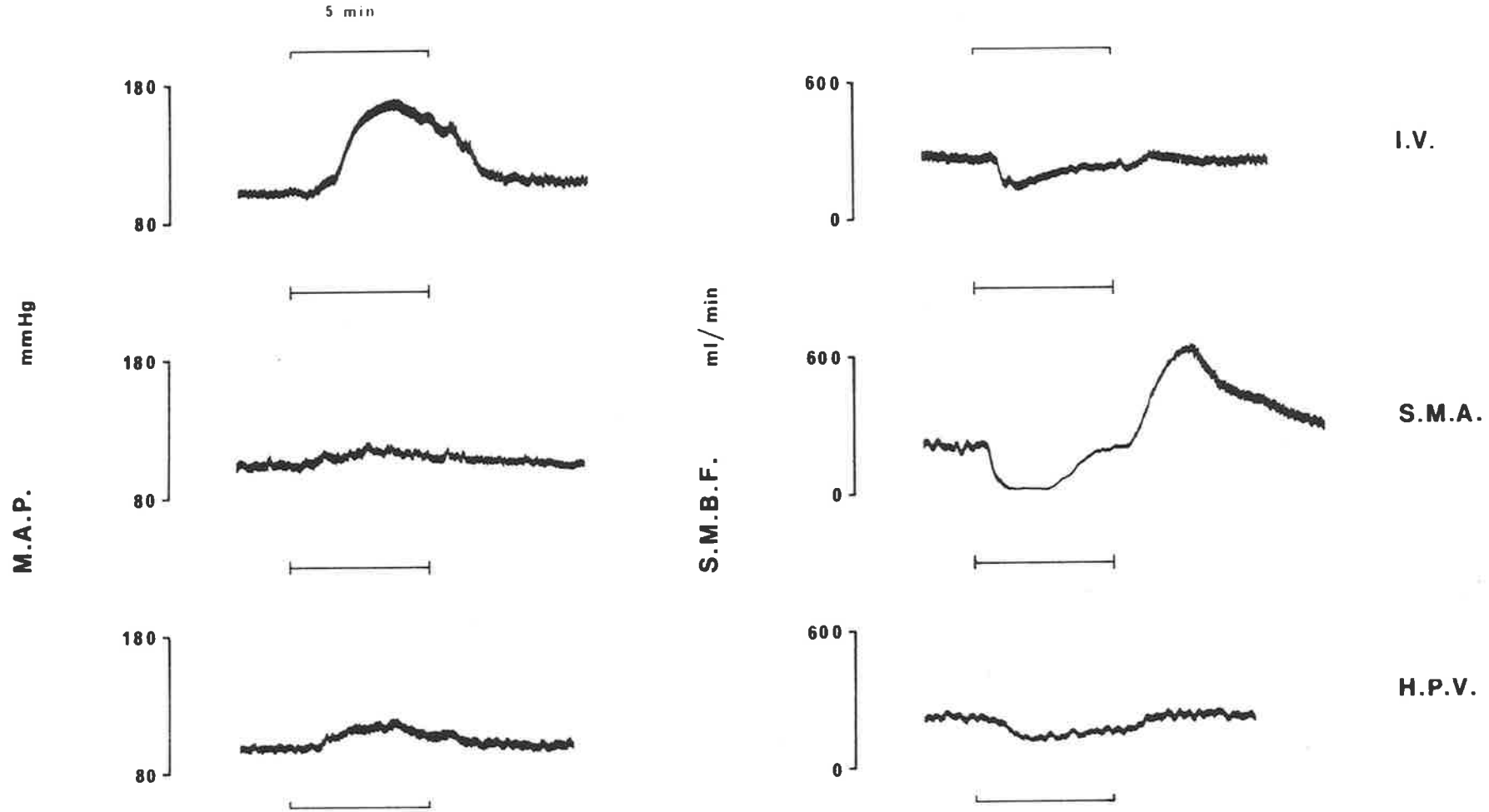
Superior mesenteric blood flow

Superior mesenteric blood flow was reduced on all three routes of administration and, as expected, the greatest reduction was associated with superior mesenteric artery infusion.

However, the nature of this blood flow response contrasted with that observed in the renal and femoral vascular beds in that the degree of superior mesenteric blood flow reduction was not constant during the infusion period but rather after an initial sharp fall, steadily increased to the pre-infusion value, despite the continued infusion of noradrenaline (Fig. 4.1.4). In this experiment, by the completion of the infusion period, superior mesenteric blood flow had returned to its pre-infusion value and this was followed by a period of marked hyperaemia. A similar, although substantially smaller effect was observed on intravenous infusion

FIG. 4.1.4. The changes in mean arterial pressure (M.A.P., mm Hg) and superior mesenteric blood flow (S.M.B.F., ml/min) in a single experiment, to 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]).

NORADRENALINE



in all 6 dogs studied with this protocol and the mean integrated blood flow responses appear in Table 4.1.4.

The greatest reduction in superior mesenteric blood flow occurred on direct infusion (-809.7 ± 226.1 ml), with significantly smaller responses observed on intravenous (-216.7 ± 196.8 ml, $p<0.01$) and hepato-portal vein infusions (-97.5 ± 95.1 ml, $p<0.01$).

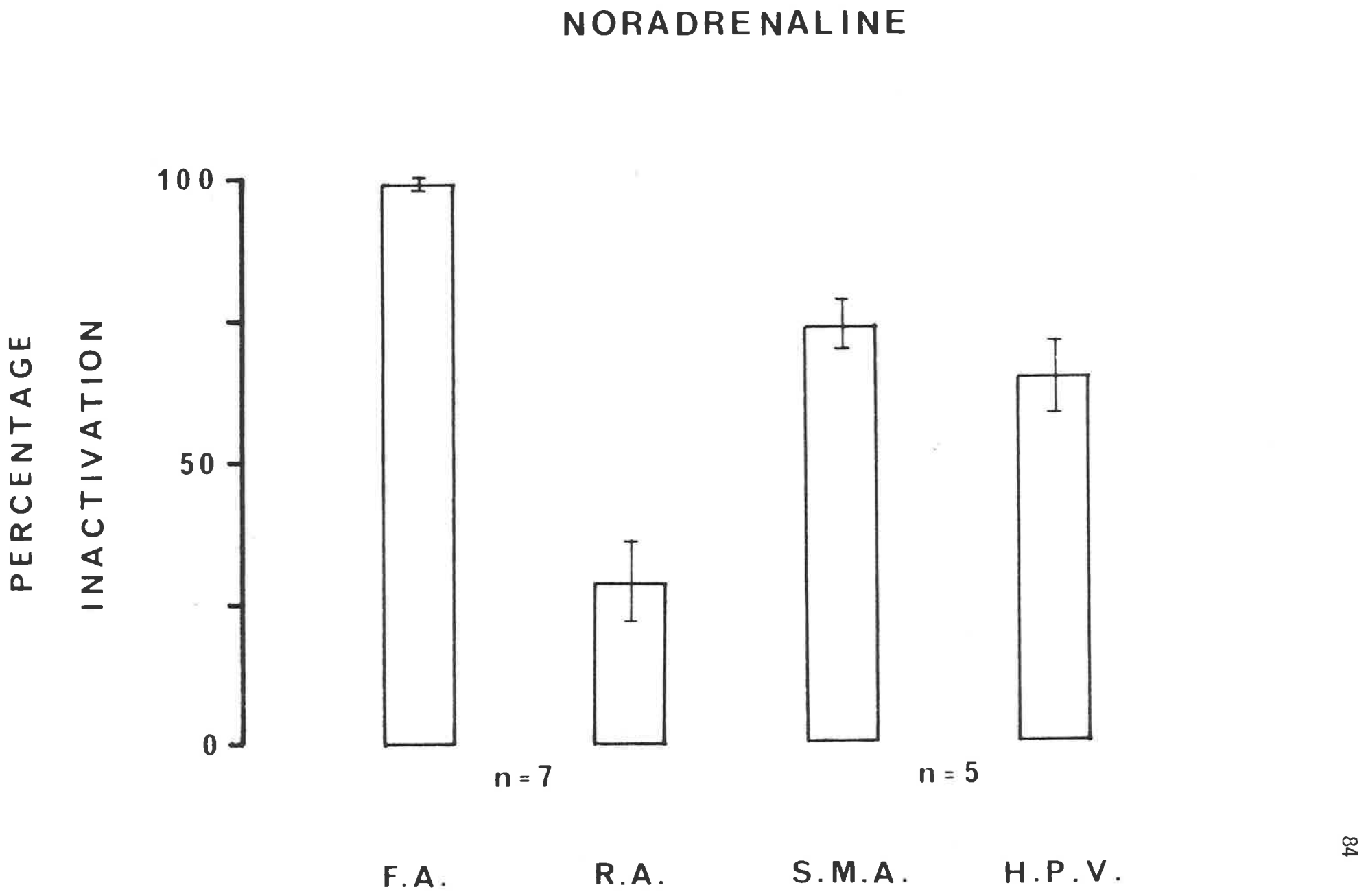
CALCULATED NORADRENALINE INACTIVATION

The loss of systemic pressor activity of noradrenaline during passage through the splanchnic and hepato-portal venous circulations was used as the index of noradrenaline inactivation, as outlined in Section 2.

The degree of noradrenaline inactivation on superior mesenteric artery and hepato-portal vein infusions was extensive, namely 73.3 ± 4.4%; 62.5 ± 9.0%, respectively (n=6, Table 4.1.4). These values are not significantly different. They are, however, significantly less than the degree of noradrenaline inactivation in the femoral vascular bed ($p<0.005$). Nevertheless, noradrenaline inactivation in the renal circulation is significantly less than both the splanchnic ($p<0.001$) and the hepato-portal circulations ($p<0.02$).

This substantial variation in noradrenaline inactivation in the peripheral vascular beds studied is represented in Fig. 4.1.5.

FIG. 4.1.5. The mean calculated percentage inactivation of infused noradrenlaine (10 μg/min [59.1 nmol/min] for 5 min) in the renal (R.A.). femoral (F.A.), superior mesenteric (S.M.A.) and hepato-portal (H.P.V.) circulations.


NORADRENALINE
PERCENTAGE INACTIVATION
100
50
0
n = 7
n = 5
F.A.
R.A.
S.M.A.
H.P.V.

SECTION 4.2 ADRENALINE

CARDIOVASCULAR RESPONSES TO INTRAVENOUS RENAL ARTERY AND FEMORAL ARTERY INFUSIONS OF ADRENALINE

GENERAL

Mean arterial pressure

The mean arterial pressure responses to intravenous, renal artery and femoral artery infusions of adrenaline in a single experiment are found in Fig. 4.2.1. Mean arterial pressure was elevated on all three routes of infusion although only a minor pressor response was observed on femoral artery infusions. Similar results were obtained in the four other dogs studied with this protocol and the pooled results appear in Table 4.2.1. The pressor response on femoral artery infusion (8.4 ± 2.0 mm Hg x min) was significantly less than both the intravenous (149.6 ± 27.3 mm Hg x min, $p<0.02$) and renal artery responses (100.4 ± 20.3 mm Hg x min, $p<0.01$). The intravenous pressor response was greater than the pressor response to renal artery infusion, although this difference was not significant.

Renal blood flow

The nature of the renal blood flow responses to adrenaline infusions via these three routes of administration was similar to that observed with noradrenaline. Renal artery infusions of adrenaline resulted in a dramatic reduction of renal blood flow (-1784.8 ± 379.0 ml) which was prolonged in duration (Fig. 4.2.2). Significantly smaller reductions in renal blood flow were observed on intravenous infusions (-175.6 ± 46.9 ml, $p<0.02$) while femoral artery infusions were without effect (Table 4.2.1).

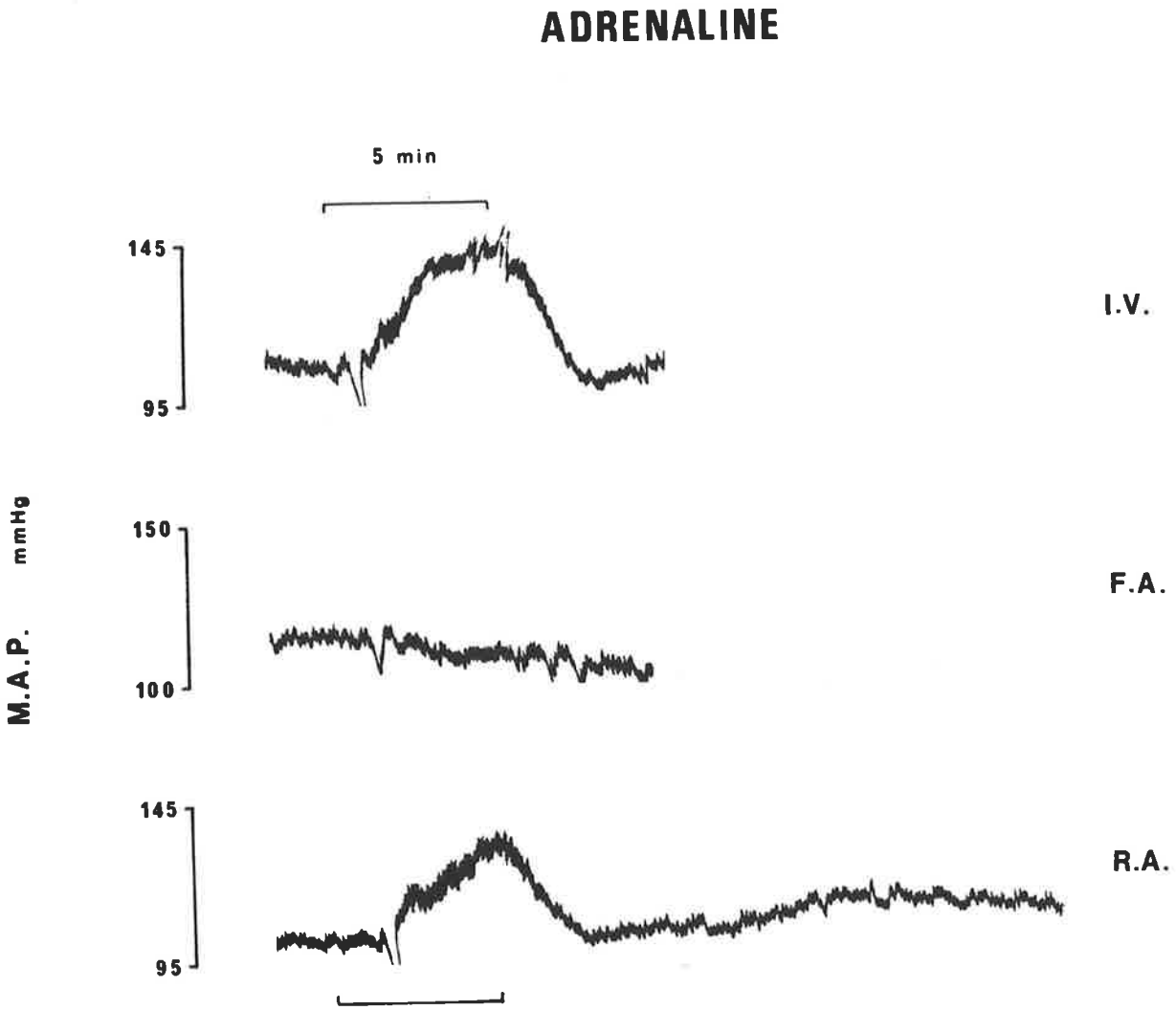
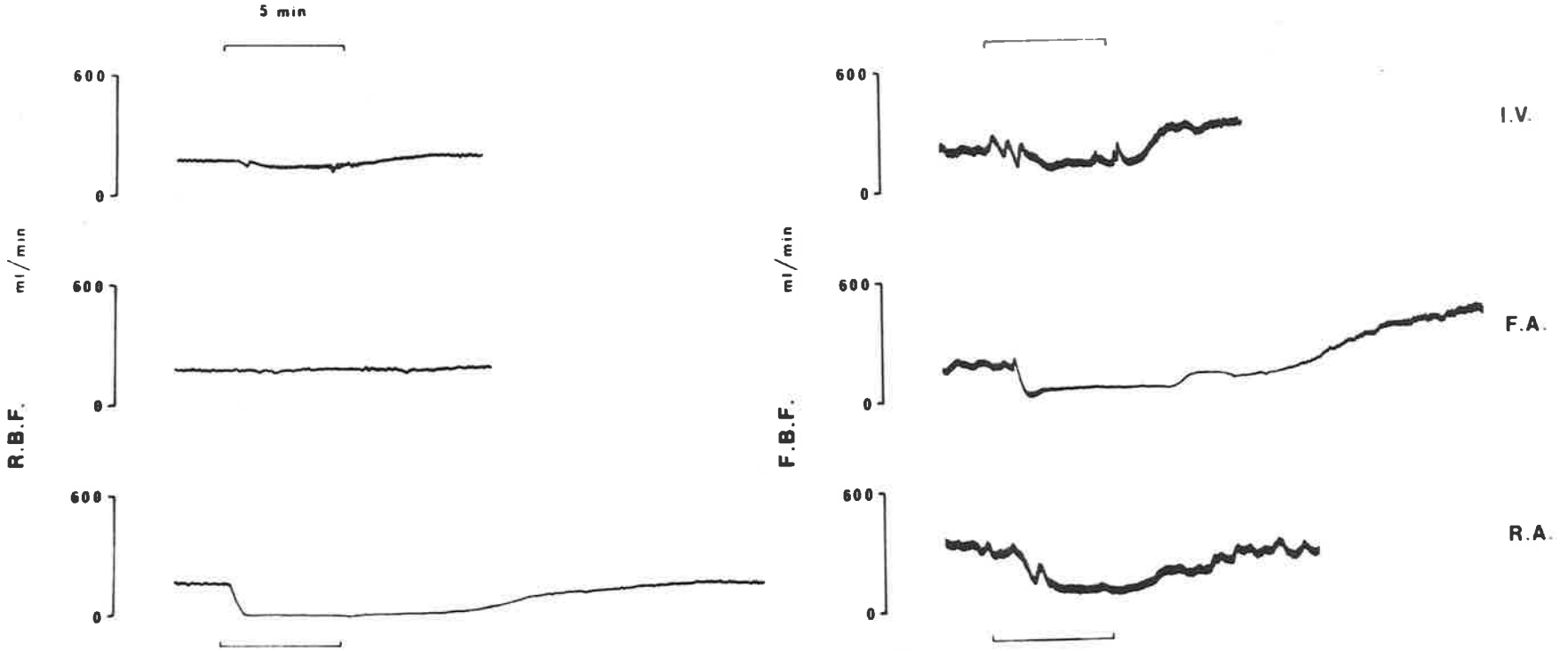


FIG. 4.2.1. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of adrenaline (10 µg/min [54.6 nmol/min]).

FIG. 4.2.2. The changes in renal blood flow (R.B.F., ml/min) and femoral blood flow (F.B.F., ml/min) in a single experiment in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of adrenaline (10 μg/min [54.6 nmol/min]).

ADRENALINE



Femoral blood flow

The femoral blood flow responses to intravenous, femoral artery and renal artery infusions of adrenaline are also contained in Fig. 4.2.2. Femoral blood flow was reduced and all three routes of infusion with a considerably greater response observed on femoral artery infusion. This response was followed by a period of marked hyperaemia. Similar effects were seen in the 4 other dogs studied and the pooled results are found in Table 4.2.1.

The substantial fall in femoral blood flow on direct infusion of adrenaline (-1109.7 ± 45.4 ml) was significantly greater than the intravenous response (-323.7 ± 182.9 ml, $p<0.05$).

CALCULATED ADRENALINE INACTIVATION

The index of adrenaline inactivation in the renal and femoral vascular beds was the loss of systemic pressor activity on renal artery and femoral artery infusions when compared to the intravenous response. The calculated values of adrenaline inactivation in these two vascular beds are found in Table 4.2.1.

Adrenaline was almost completely removed in the femoral circulation 93.7 ± 1.4% (n=5) whereas a considerably lower degree of inactivation was observed on renal artery infusion (29.2 ± 9.2%, n=5, $p<0.005$). This disparity between the femoral and renal vascular beds is similar to that seen with noradrenaline (Table 4.1.1).

SECTION 4.3 ISOPRENALINE

CARDIOVASCULAR RESPONSES TO INTRAVENOUS, SUPERIOR MESENTERIC ARTERY AND HEPATO-PORTAL VEIN INFUSIONS OF ISOPRENALINE

GENERAL

Mean arterial paressure

The mean arterial pressure responses to intravenous, superior mesenteric artery and hepato-portal vein infusions of isoprenaline in a single experiment are found in Fig. 4.3.1. All three routes of infusion resulted in a reduction in mean arterial pressure with the greatest response being on intravenous infusion.

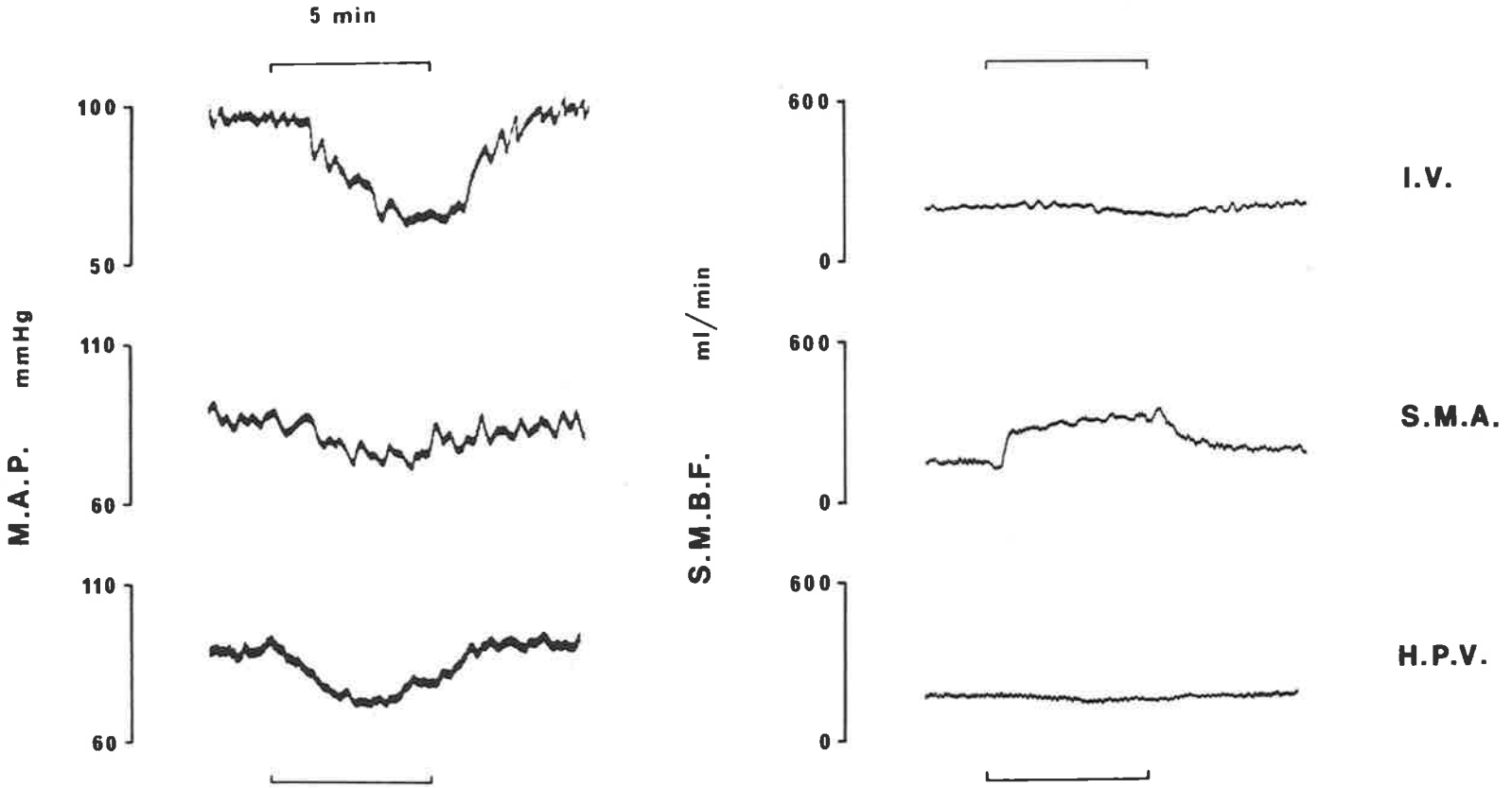
The pattern of depressor responses in each of the 5 dogs studied was similar and the mean results appear in Table 4.3.1. The reduction in mean arterial pressure associated with intravenous infusion (-86.4 ± 20.8 mm Hg x min) was significantly greater than that observed on either superior mesenteric artery (-21.8 ± 5.4 mm Hg x min, $p<0.02$) or hepato-portal vein infusions (-30.8 ± 11.5 mm Hg x min, $p<0.02$). The mean arterial pressure responses to these latter two routes of infusion were not significantly different.

Superior mesenteric blood flow

The superior mesenteric blood flow responses to these three routes of isoprenaline infusions in a single experiment are found in Fig. 4.3.1. Direct infusion of isoprenaline resulted in a substantial increase in superior mesenteric blood flow. Considerably smaller responses were seen on intravenous and hepato-portal vein infusions.

FIG. 4.3.1. The changes in mean arterial pressure (M.A.P., mm Hg) and superior mesenteric blood flow (S.M.B.F., ml/min) in a single experiment in response to 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of isoprenaline (1.0 μg/min [4.7 nmol/min]).

ISOPRENALINE



The pooled data for 5 dogs indicate that the intravenous and hepato-portal venous responses are subject to considerable variation (Table 4.3.1). However, the increase in superior mesenteric blood flow on superior mesenteric artery infusions of isoprenaline (1278.0 ± 233.6 ml) was considerably greater than on either intravenous (52.8 ± 76.1 ml) or hepato-portal vein infusions (-6.8 ± 25.3 ml, $p<0.01$).

CALCULATED ISOPRENALINE INACTIVATION

Isoprenaline inactivation was examined by studying the loss of systemic vasoactivity on superior mesenteric artery or hepato-portal vein infusions, when compared to the intravenous response, although in contrast to previous experiments these responses were depressor and not pressor. The mean results for 5 dogs appear in Table 4.3.1.

Isoprenaline inactivation was greater on superior mesenteric artery infusion than on hepato-portal vein infusions (73.3 ± 4.7%, 64.8 ± 7.0%, respectively, n=5). However, this difference was not significant. The levels of isoprenaline inactivation are lower than the results obtained for noradrenaline with the same routes of administration.

DISCUSSION

It is apparent from these findings that peripheral vascular beds vary in their handling of administered catecholamines. Although intra-arterial administration of noradrenaline in the renal, femoral and superior mesenteric arteries resulted in a reduction in local blood flow, the nature of this reduction varied markedly between vascular beds.

The decrease in renal blood flow on renal artery infusions of noradrenaline was of long duration, with the pre-infusion blood flow level not being restored until approximately 10 min after completion of the infusion. The mechanism for this prolonged renal vasoconstriction may involve the intrarenal renin-angiotensin system. Bomzon and Rosendorff (1975) observed an attenuated increase in renal vascular resistance to renal artery infusions of noradrenaline following renal angiotensin I-converting enzyme inhibition. This may reflect a direct vasoconstrictor effect on the renal vasculature by locally generated angiotensin II or possibly an inhibition of neuronal uptake by increased intrarenal levels of angiotensin II, thereby increasing the duration of noradrenaline's vasoconstrictor activity in the renal vasculature. The concentration of angiotensin II required to inhibit neuronal uptake however, is thought to be high (Starke, 1977). The involvement of the intrarenal renin-angiotensin system in the response of the renal vasculature to infusions of noradrenaline are considered, in detail, in Section 7.

Femoral blood flow was dramatically reduced on direct infusion of noradrenaline, although the duration of this reduction was shorter than the renal response. This reduction was followed by an extensive hyperaemia in the post-infusion period. Adrenaline produced similar blood flow changes in the renal and femoral circulations to those observed with noradrenaline.

The haemodynamic response pattern of the superior mesenteric vasculature to noradrenaline infusions was quite different from that in both the renal and femoral circulations. Superior mesenteric artery infusion resulted in an initial dramatic reduction in local blood flow, which was followed by a progressive increase in blood flow, with restoration of the resting level occurring by the conclusion of the infusion period. An extensive post-infusion hyperaemia follows.

A similar haemodynamic response pattern in the mesenteric vasculature has been observed on sympathetic nerve stimulation (Greenway and Stark, 1971). After a period of 1-2 minutes of continued stimulation, splanchnic blood flow failed to be maintained at a reduced level and steadily increased to the resting value.

This effect has been labelled "autoregulatory escape" and appears to be confined to the splanchnic circulation. The mechanisms involved in this response are not well understood and are subject to species variation. Autoregulatory escape has previously only been described on neural stimulation and not to administration of exogenous noradrenaline. In addition, it is thought not to occur in dogs (Greenway and Oshira, 1972). These results however, indicate that autoregulatory escape occurs in greyhounds and to infusions of exogenous noradrenaline.

Certainly these haemodynamic results contrast with those observed in the renal and femoral circulations and the superior mesenteric artery response to an equipressor dose of angiotensin II, discussed in Section 3.

The haemodynamic response of the mesenteric vasculature to isoprenaline infusions was also examined. Isoprenaline is a pure β-agonist and results in an increase in superior mesenteric blood flow on both direct and

intravenous infusions.

Intravenous administration of noradrenaline results in an increase in mean arterial pressure, which is partially mediated by an increase in total peripheral resistance. Hoffbrand and Forsyth (1972) observed a redistribution of the cardiac output with intravenous infusions of noradrenaline. Vascular resistance was increased in the brain, skin, gastrointestinal tract, bone and kidneys although there was no significant alteration in cardiac or skeletal muscle vascular resistance.

In the present study, intravenous infusion of noradrenaline resulted in an increase in mean arterial pressure and decreased blood flow in the renal, femoral and superior mesenteric circulations. Therefore, unlike previous studies, this route of noradrenaline administration did not appear to be associated with a redistribution of cardiac output to the skeletal musculature.

These results contrast with those observed with angiotensin II, where there is an obvious redistribution of blood flow from the renal and mesenteric circulations to the hindquarters.

In recent years, there has been an increasing understanding of the many complex mechanisms regulating sympathetic nerve transmission and the effects of noradrenaline in a number of tissues (Westfall, 1977; Starke, 1977). The majority of these studies have been performed *in vitro*, whereas this thesis has attempted to clarify the *in vivo* fate of circulating catecholamines.

A high degree of noradrenaline inactivation is thought to occur in all densely, sympathetically innervated organs (Celander and Mellander, 1955;

Gryglewski and Vane, 1970). This result has not been confirmed in the present experiments, where a low degree of noradrenaline inactivation was observed in the kidney.

Renal artery infusions of noradrenaline are associated with a marked reduction in renal blood flow and consequently glomerular filtration rate. As noradrenaline is known to be excreted in the urine, this low degree of inactivation of infused noradrenaline may be due to a reduced capacity of the kidney to excrete noradrenaline. Support for this view comes from the findings of Overy, Pfister and Chidsey (1967) who noted the source of urinary noradrenaline was the circulation and not intrarenal neuronally released noradrenaline.

This hypothesis is further supported by the experiments in which the effects of dose on the renal inactivation of noradrenaline were examined. Renal noradrenaline inactivation was inversely related to the concurrent fall in renal blood flow, in that greater reductions in renal blood flow were associated with a reduction in noradrenaline inactivation. With greater renal blood flow reductions, the ability of the kidney to excrete noradrenaline in the urine is impaired. With smaller doses of infused noradrenaline which resulted in only minor decreases in renal blood flow, the degree of noradrenaline inactivation was comparable to that in the other vascular beds studied.

Intrarenal generation of angiotensin II may also contribute to this low degree of inactivation, due to its inhibitory action on neuronal uptake (Jackson and Campbell, 1979). Both of these possibilities are considered in detail, in Sections 6 and 7.

Noradrenaline infused via the femoral artery was almost completely

inactivated during passage through the hindquarters. A slightly lower but still extensive degree of inactivation (85-90%) has been reported previously (Celander and Mellander, 1955; Regoli and Vane, 1964; Ginn and Vane, 1968). A later study from Vane's laboratory however, only observed a 60% degree of noradrenaline inactivation in the hindquarters (Gryglewski and Vane, 1970).

In this study, adrenaline inactivation was almost identical to that observed with noradrenaline in the renal and femoral circulations. This is in addition to their similar haemodynamic response pattern.

Noradrenaline and isoprenaline inactivation were examined in the splanchnic and hepato-portal circulations. The degree of isoprenaline inactivation was lower than that seen with noradrenaline in each vascular bed. This difference may be explained in terms of uptake specificities. Isoprenaline, unlike noradrenaline, is only a substrate for extraneuronal uptake (Callingham and Burger, 1966), whereas noradrenaline is removed by both neuronal and extraneuronal uptake processes.

In summary, intra-arterial administration of noradrenaline resulted in an array of haemodynamic responses in the vascular beds studied, although in each case blood flow was reduced. In contrast to the results obtained with angiotensin II, all these vascular beds contributed to the overall increase in peripheral vascular resistance on intravenous infusions of noradrenaline.

Furthermore, the degree of noradrenaline inactivation in the renal and femoral vascular beds was quite different from that observed with an equipressor dose of angiotensin II, although similar results were observed in the splanchnic circulation.

The remaining sections of this thesis investigate these differences between angiotensin II and noradrenaline in the renal and femoral circulations and possible mechanisms regulating the *in vivo* inactivation of noradrenaline and angiotensin II in the peripheral circulation.

SUMMARY

1. Mean arterial pressure was elevated on all routes of administration at the dose of noradrenaline studied. The response on intravenous infusions was consistently greater than on all other routes of administration. Femoral artery infusion of noradrenaline resulted in only a marginal increase in blood pressure.

2. Renal, femoral and superior mesenteric blood flow were reduced on both intravenous and direct infusion of noradrenaline. The nature of the blood flow reduction on direct infusion varied markedly between these three vascular beds.

3. "Autoregulatory escape" of blood flow was observed in the superior mesenteric vascular bed to direct infusions of noradrenaline.

4. There was a considerable variation in the degree of noradrenaline inactivation in the peripheral vascular beds studied with a significantly lower degree of inactivation being observed in the renal circulation.

5. The renal inactivation of noradrenaline was dose and renal blood flow dependent with inactivation decreasing on increasing dose and greater reductions in renal blood flow.

6. The haemodynamic response patterns to intravenous renal and femoral artery infusions of adrenaline were similar to those seen with the same dose of noradrenaline, as was the degree of adrenaline inactivation in the renal and femoral circulations.

7. Intravenous, superior mesenteric artery and hepato-portal vein infusions of isoprenaline resulted in a decrease in mean arterial pressure and an increase in superior mesenteric blood flow. Isoprenaline inactivation in the superior mesenteric and hepato-portal circulations was extensive but less than that seen with noradrenaline.

SECTION 5

MECHANISMS OF ANGIOTENSIN II INACTIVATION IN PERIPHERAL VASCULAR BEDS

INTRODUCTION

The peripheral vascular beds examined in Section 3, demonstrated a marked variation in their haemodynamic responses to intravenous and intra-arterial administration of angiotensin II. Furthermore, these vascular beds were not equiactive in their ability to terminate the effects of infused angiotensin II.

The possible pathways by which peripheral vascular beds inactivate angiotensin II were outlined in Fig. 1.1. Metabolism is generally thought to be the most important physiological mechanism (Bumpus, Smeby, Page and Khairallah, 1964; Leary and Ledingham, 1974; Bailie and Oparil, 1977). Angiotensinases are found in both plasma and tissues although plasma angiotensinases are considerably less important in the *in vivo* fate of angiotensin II.

Urinary excretion does not appear to be a major route of angiotensin II inactivation despite its small molecular weight and reports that the proximal tubule rapidly hydrolyzes microperfused angiotensin II (Pullman, Oparil and Carone, 1975). Furthermore, a number of studies have demonstrated that ureteric ligation fails to alter the degree of angiotensin II inactivation in the kidney (Akinkugbe, Brown and Cranston, 1966; Leary and Ledingham, 1970b).

The major site of angiotensin II degradation in the renal circulation is thought to be the vascular compartment, as reductions in renal blood flow and increases in renal transit time increase the renal inactivation of angiotensin II (Bailie and Oparil, 1977). Similarly, the hindquarter inactivation of angiotensin II is increased on delayed transit of administered angiotensin II across the femoral vascular bed (Haas, Goldblatt, Lewis and Gipson, 1973).

This influence of local haemodynamics on angiotensin II inactivation has been investigated in this section through local receptor-antagonism with Sar^1-Ile^8-angiotensin II. Direct infusions of angiotensin II during receptor-blockade are not accompanied by reductions in local blood flow and mean transit time is consequently unchanged.

Furthermore, with this technique, the influence of angiotensin II receptor function on angiotensinase activity may be examined. Farruggia, Sachs and Palaic (1979) reported that altered membrane-bound angiotensinase activity affects agonist-receptor activation and conversely, therefore, altered receptor function may influence angiotensinase activity.

METHODS

The experimental animal was the ex-racing greyhound (26-34 kg) and the methods of anaesthesia induction and maintenance, drug administration, measurement of cardiovascular variables and estimation of angiotensin II are outlined in the General Methods.

Zero blood flow measurements were achieved with a mechanical occluder, consisting of a loose ligature and a 7 cm length of polyethylene tubing placed on the artery proximally to the intra-arterial infusion catheter. This occluding device was also used to partially occlude the renal artery in those experiments requiring controlled reductions in renal blood flow to a predetermined level.

All angiotensin II infusions were made at 500 ng/min (0.5 nmol/min) for 5 min. In those experiments investigating the role of receptor function and local haemodynamics, the angiotensin II competitive antagonist Sar^1-Ile^8-angiotensin II was administered for 5 min, immediately prior to the angiotensin II infusion. The dose of Sar^1-Ile^8-angiotensin II administered into each vascular bed was chosen such that its antagonistic actions were confined to the infused vacular bed, whilst achieving adequate local receptor-blockade. The femoral vascular bed required a smaller dose than the other vascular beds studied.

The index of adequate receptor-blockade was the inhibition of the local vasoconstrictor response to direct infusions of angiotensin II, as determined from the degree of attenuation of the local blood flow response. Since this was not possible in the hepato-portal circulation, an identical dose to that administered via the superior mesenteric artery was chosen.

Only minimal agonistic effects on local blood flow were observed on Sar^1-Ile^8-angiotensin II administration. This agonistic action was transient and local blood flow was fully restored before the conclusion of the antagonist infusion and the commencement of the angiotensin II infusion.

The following drugs were used: angiotensin II (Val^5-hypertensin II-Asp-β-amide, Hypertensin, Ciba), alpha-chloralose ($C_8H_{11}O_6Cl_3$, B.D.H.), morphine sulphate (D.B.L.), Sar^1-Ile^8-angiotensin II (Beckman). All drugs were dissolved in physiological saline (sodium chloride 154 mmol/l) and all infusions were administered with a constant speed, motor-driven, syringe infusion pump at 1 ml/min.

Results are usually expressed as the mean, with the standard error of the mean (s.e.m.) used as the index of dispersion. Unless otherwise stated, the data were analysed with a Student's t-test and the null hypothesis was rejected when a p value of less than 0.05 was achieved.

RESULTS

THE EFFECT OF SAR^1-ILE^8-ANGIOTENSIN II AND RENAL HAEMODYNAMICS ON THE CARDIOVASCULAR RESPONSES TO INTRAVENOUS AND RENAL ARTERY INFUSIONS OF ANGIOTENSIN II

GENERAL

The effects of the angiotensin II competitive antagonist, Sar^1-Ile^8-angiotensin II, on the cardiovascular responses to renal artery infusions of angiotensin II were studied in two series of dogs.

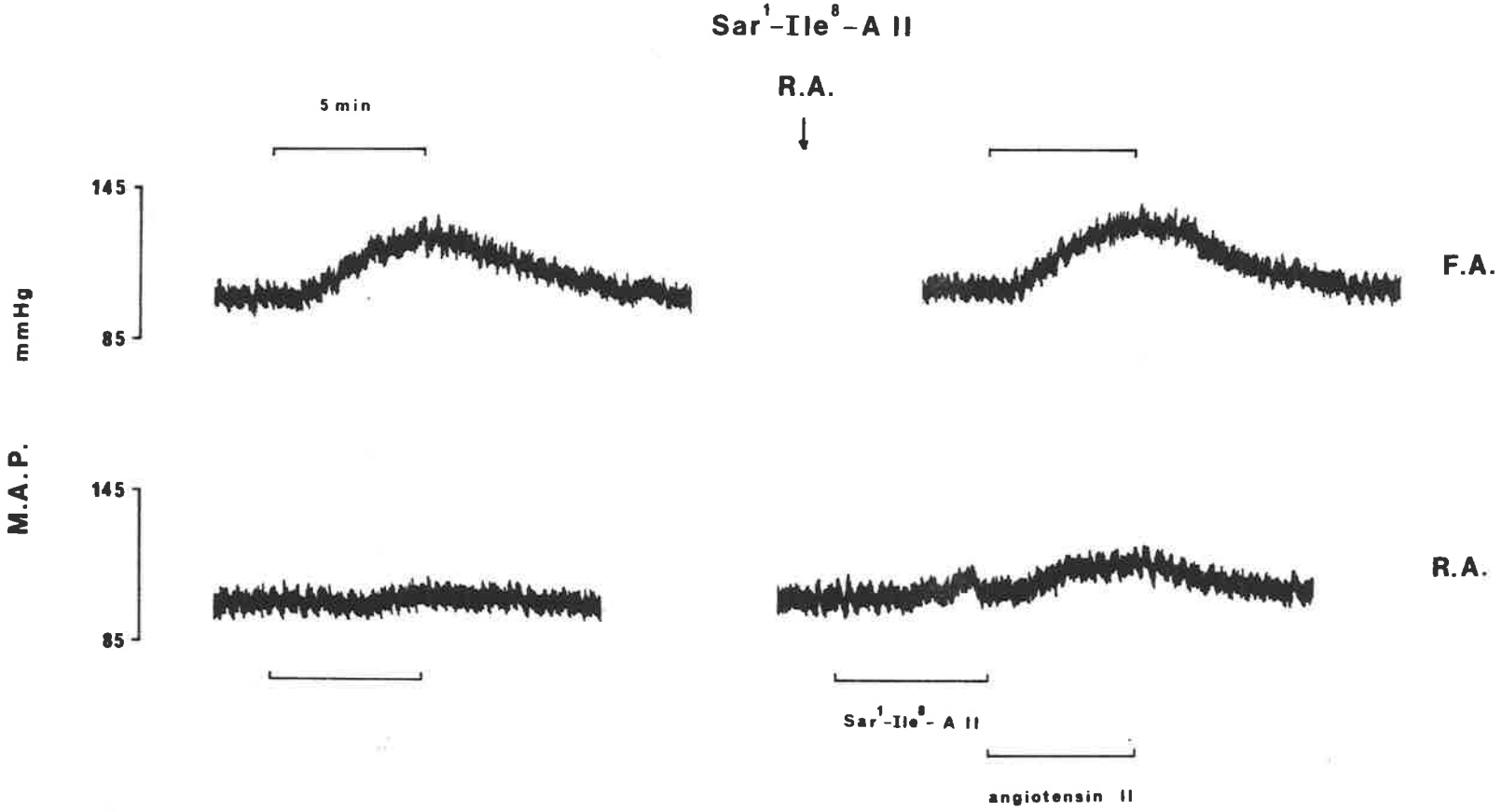
In the first group of 8 dogs the mean arterial pressure, renal blood flow and femoral blood flow responses to renal artery and femoral artery infusions of angiotensin II were monitored before and after renal artery administration of Sar^1-Ile^8-angiotensin II (3.3 μg/min [3.5 nmol/min] for 5 min).

Following renal Sar^1-Ile^8-angiotensin II administration, the increase in mean arterial pressure in response to renal artery infusions of angiotensin II was potentiated by approximately three-fold, as demonstrated in Fig. 5.1, from a single experiment.

Similar effects were observed in the 7 other dogs studied and the pooled results appear in Table 5.1. Renal artery administration of the antagonist resulted in a significant increase in the systemic pressor response to renal artery infusions of angiotensin II (before 23.4 ± 5.7 mm Hg x min, after 78.4 ± 10.7 mm Hg x min, $p<0.005$). The systemic pressor response to femoral artery infusions of angiotensin II was however, not significantly altered by the renal artery infusion of the antagonist (before 123.5 ± 14.1 mm Hg x min, after 126.1 ± 15.4 mm Hg x min).

FIG. 5.1. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min femoral artery (F.A.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]), before and after renal artery administration of Sar^1-Ile^8-angiotensin II (3.3 μg/min [3.5 nmol/min] for 5 min).

ANGIOTENSIN II



Adequate renal angiotensin II receptor antagonism was evident by the dramatically attenuated renal blood flow (before -549.0 ± 79.2 ml, after -164.0 ± 24.7 ml) and renal vascular resistance responses (before 122.4 ± 28.0%, after 26.1 ± 5.6%) to renal artery infusions of angiotensin II ($p<0.005$ respectively, Table 5.1).

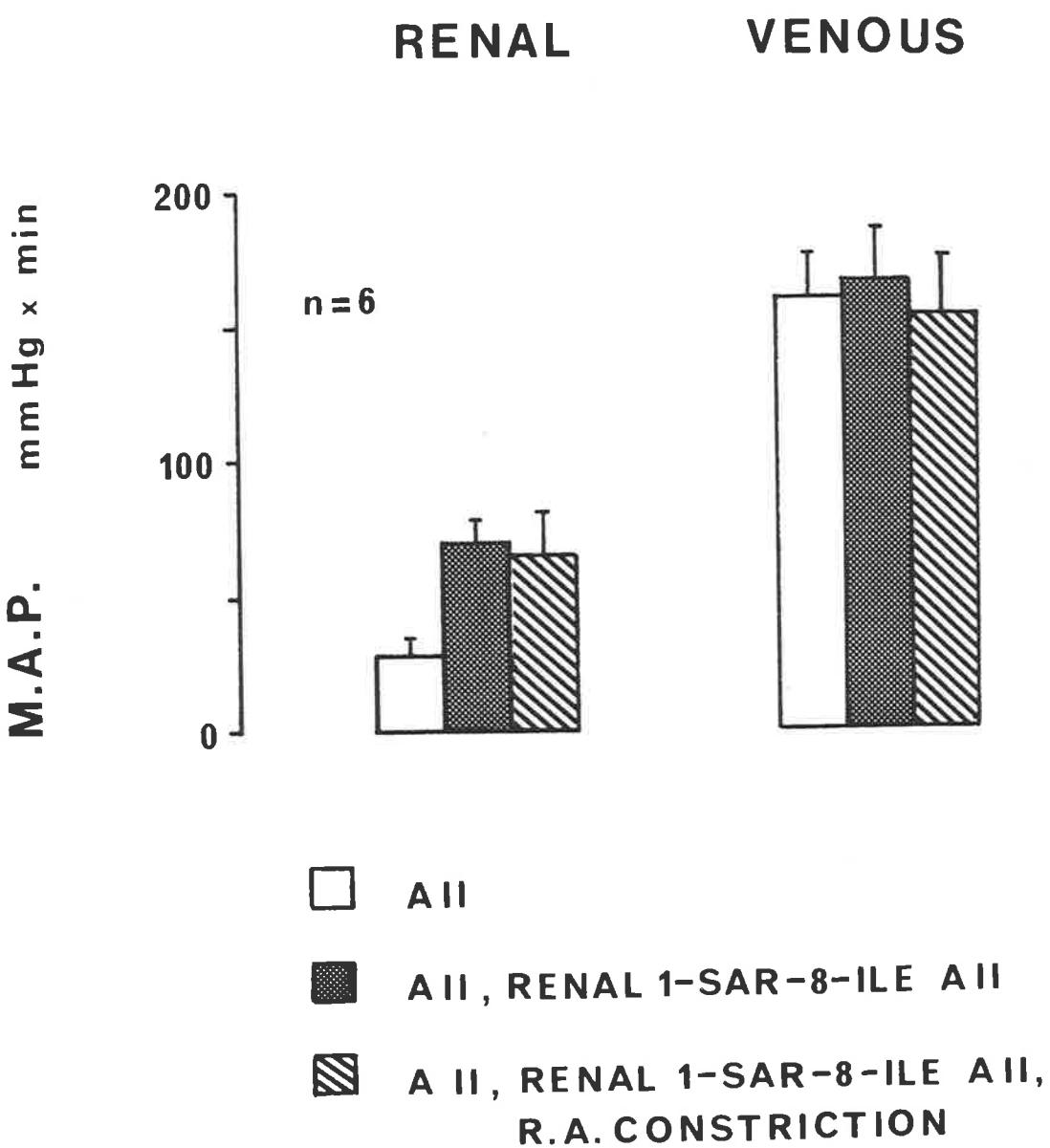
The femoral blood flow and femoral vascular resistance responses to femoral artery infusions of angiotensin II were unaltered by renal artery administration of Sar^1-Ile^8-angiotensin II (femoral blood flow before -175.0 ± 40.1 ml, after -162.6 ± 39.6 ml; femoral vascular resistance before 64.4 ± 17.1%, after 70.0 ± 27.4%, Table 5.1).

These results do not indicate whether the effects of the antagonist were mediated by inhibiting angiotensin II receptor function in the renal vasculature or through the subsequent altered renal blood flow response to renal artery infusions of angiotensin II. The relative contribution of these actions to this effect of Sar^1-Ile^8-angiotensin II was examined in a further group of 7 dogs, in which mechanical constriction of the renal artery was employed to reproduce the control renal blood flow response to angiotensin II in the presence of Sar^1-Ile^8-angiotensin II. This treatment did not modify the effect of the antagonist in potentiating the pressor response to renal artery infusions of angiotensin II (control 28.4 ± 5.6 mm Hg x min, after Sar^1-Ile^8-angiotensin II 68.6 ± 7.4 mm Hg x min, after Sar^1-Ile^8 angiotensin and renal artery constriction 65.1 ± 14.8 mm Hg x min, Fig. 5.2 and Table 5.2).

CALCULATED ANGIOTENSIN II INACTIVATION

The degree of angiotensin II inactivation in the renal circulation

FIG. 5.2. The increases in mean arterial pressure (M.A.P., mm Hg x min) in response to 5 min intravenous (I.V.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]) before and after renal artery administration of Sar^1-Ile^8-angiotensin II (3.3 μg/min [3.5 nmol/min] for 5 min) alone and in conjunction with a renal artery constriction. The height of each column represents the mean response from 7 dogs and the vertical lines, one standard error of the mean.


RENAL
VENOUS
M.A.P.
mm Hg x min
200
100
0
n=6
A II
A II, RENAL 1-SAR-8-ILE A II
A II, RENAL 1-SAR-8-ILE A II,
R.A. CONSTRICTION

was calculated by comparing the systemic pressor response to renal artery infusion of angiotensin II with the response to intravenous infusion and expressed as a percentage as outlined in Section 2. The results used to make this calculation were those obtained in the second group of dogs discussed above. Renal artery administration of Sar^1-Ile^8-angiotensin II resulted in a substantial reduction in the kidney's ability to inactivate infused angiotensin II (before 79.2 ± 5.2%, after 53.4 ± 5.5%, $p<0.005$). When the control level of renal blood flow during renal artery infusions of angiotensin II was artifically reproduced in the presence of the antagonist, there was no further modification of this impaired ability of the kidney to inactivate infused angiotensin II (52.3 ± 11.7%, Table 5.2).

THE EFFECT OF SAR^1-ILE^8-ANGIOTENSIN II ON THE CARDIOVASCULAR RESPONSES TO FEMORAL ARTERY INFUSIONS OF ANGIOTENSIN II

GENERAL

Mean arterial pressure

The effects of femoral artery infusion of Sar^1-Ile^8-angiotensin II (2.5 μg/min [2.7 nmol/min] for 5 min) on the systemic pressor responses to intravenous, femoral artery and renal artery infusions of angiotensin II were studied in a group of 5 dogs.

Following femoral artery administration of the antagonist there was a slight decrease in the systemic pressor response to femoral artery infusions

of angiotensin II, as seen in Fig. 5.3 from a single experiment. A similar result was observed in the four other dogs studied and the pooled results appear in Table 5.3 (before 164.4 ± 35.0 mm Hg x min, after 131.0 ± 18.1 mm Hg x min).

Femoral aftery infusion of Sar^1-Ile^8-angiotensin II did not significantly alter the pressor responses to angiotensin II infusions on any of the other routes examined (intravenous before 224.6 ± 37.9 mm Hg x min, after 214.0 ± 36.2 mm Hg x min; renal artery before 53.6 ± 6.0 mm Hg x min, after 70.0 ± 10.0 mm Hg x min).

Blood flow and vascular resistance

Following femoral artery infusion of Sar^1-Ile^8-angiotensin II, the substantial fall in femoral blood flow associated with direct angiotensin II infusions was converted to anincrease in blood flow (before -381.8 ± 59.5 ml, after 54.4 ± 18.8 ml). This effect is demonstrated in Fig. 5.3 from a single experiment and pooled results for all 5 dogs studied appear in Table 5.3.

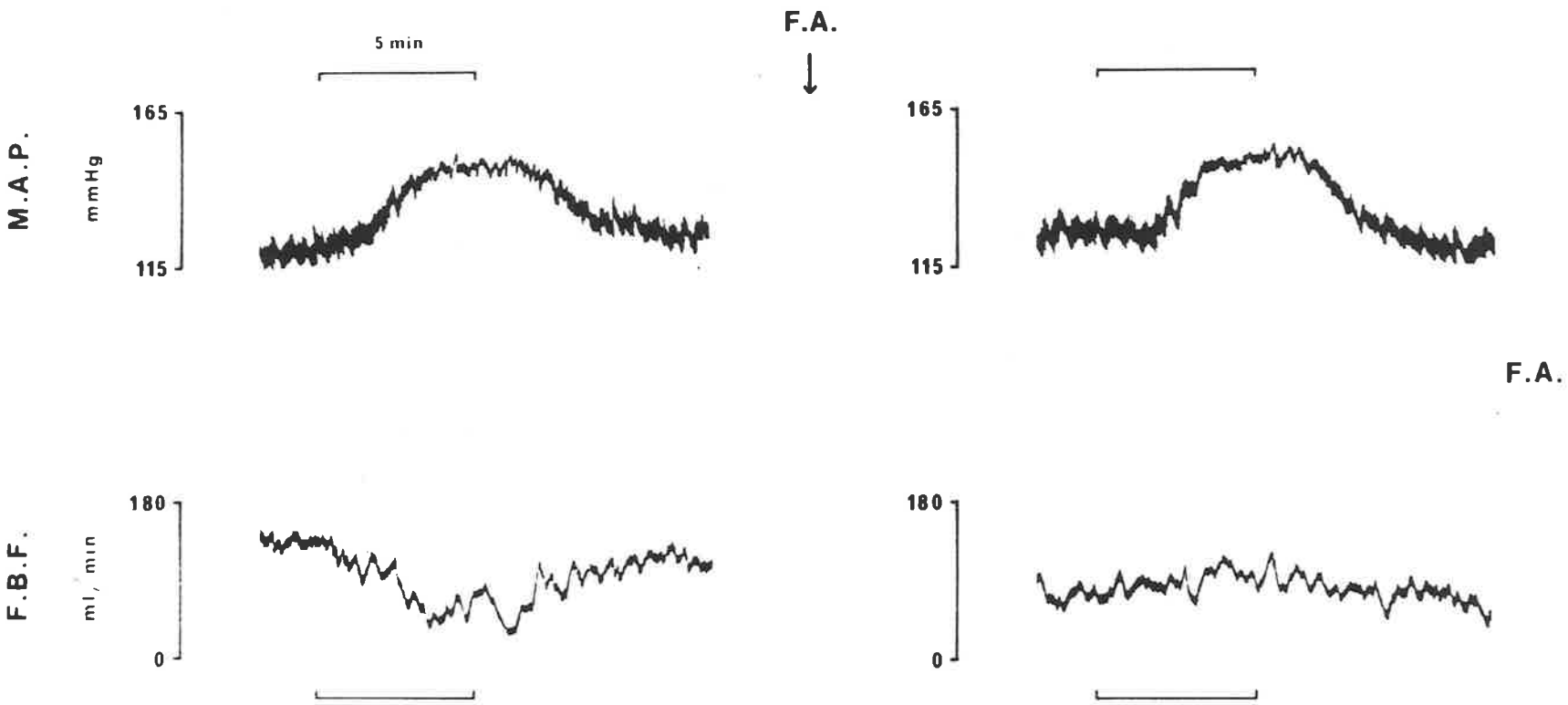
Adequate angiotensin II receptor-antagonism in the femoral vascular bed was also demonstrated by the attenuated percentage increase in femoral vascular resistance on femoral artery infusions of angiotensin II (before 124.0 ± 22.6%, after 8.9 ± 7.8%, $p<0.02$, Table 5.4), following femoral artery infusion of Sar^1-Ile^8-angiotensin II.

CALCULATED ANGIOTENSIN II INACTIVATION

The degree of angiotensin II inactivation was calculated by the

FIG. 5.3. The changes in mean arterial pressure (M.A.P., mm Hg) and femoral blood flow (F.B.F., ml/min) in a single experiment in response to 5 min femoral artery infusions of angiotensin II (500 ng/min [0.5 nmol/min]) before and after femoral artery administration of Sar^1-Ile^8-angiotensin II (2.5 μg/min [2.7 nmol/min] for 5 min).

ANGIOTENSIN II



attenuated systemic pressor response technique. Following local Sar^1-Ile^8-angiotensin II administration, there was a slight increase in the hindquarter inactivation of infused angiotensin II, although this effect was not significant (before 27.6 ± 4.8%, after 36.3 ± 6.0%, Table 5.3).

Angiotensin II inactivation in the renal vascular bed was slightly decreased following femoral artery infusion of the antagonist, Table 5.3, however this effect was not significant (before 74.8 ± 6.0%, after 65.5 ± 4.5%).

THE EFFECT OF SAR^1-ILE^8-ANGIOTENSIN II ON THE CARDIOVASCULAR RESPONSES TO INTRAVENOUS, SUPERIOR MESENTERIC ARTERY AND HEPATO-PORTAL VEIN INFUSIONS OF ANGIOTENSIN II

GENERAL

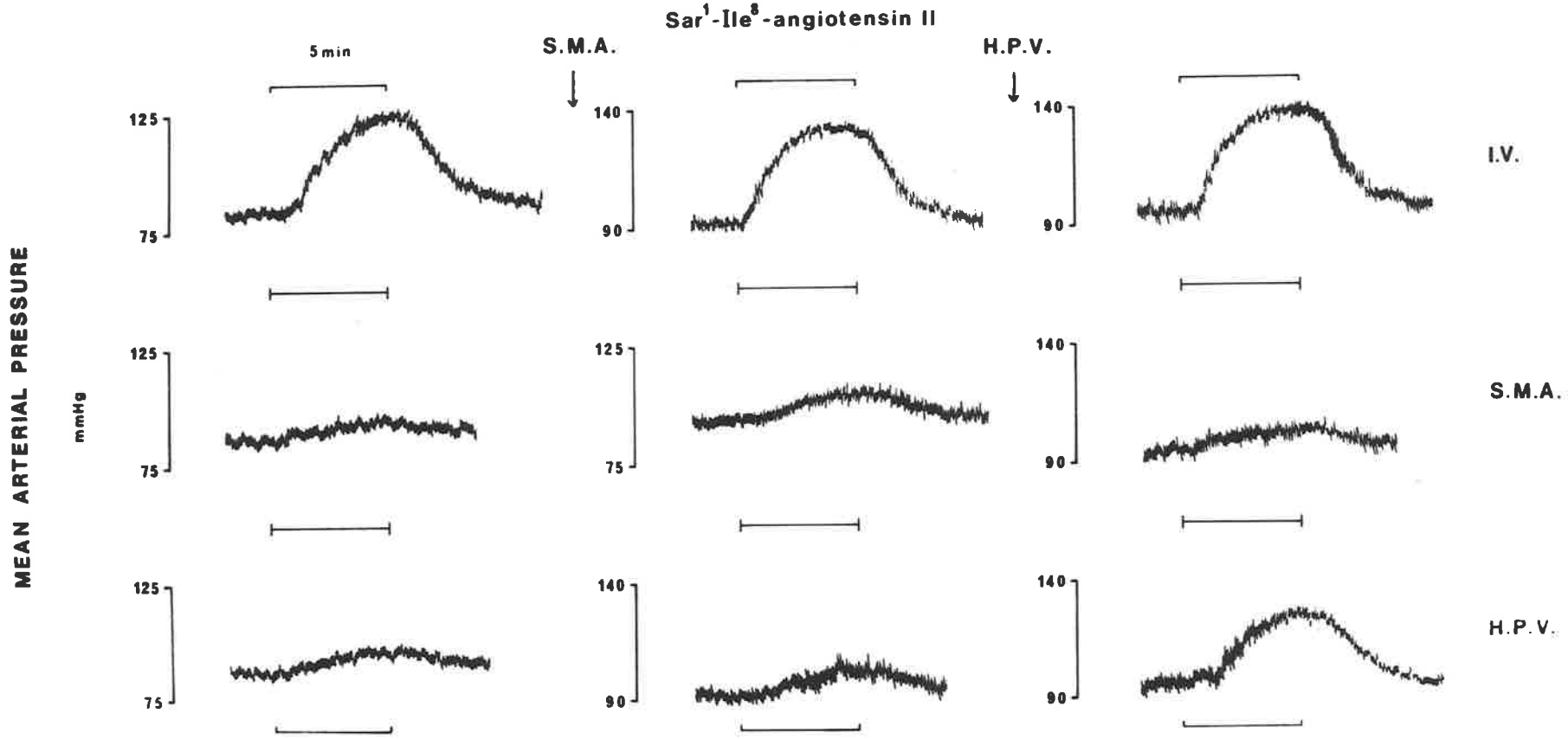
Mean arterial pressure

The mean arterial pressure responses to intravenous, superior mesenteric artery and hepato-portal vein infusions of angiotensin II, before and after superior mesenteric artery and hepato-portal vein infusions Sar^1-Ile^8-angiotensin II (5.0 µg/min [5.3 nmol/min] for 5 min) in a single experiment appear in Fig. 5.4.

Before administration of the antagonist the intravenous pressor response is considerably greater than the response to either superior mesenteric artery or hepato-portal vein infusions of angiotensin II. This is indicative of the high degree of angiotensin II inactivation in

FIG. 5.4. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]) before and after administration of Sar^1-Ile^8-angiotensin II (5.0 μg/min [5.3 nmol/min] for 5 min) initially via the superior mesenteric artery and subsequently, the hepato-portal vein.

ANGIOTENSIN II



these vascular beds.

These pressor responses were unaltered after antagonist administration via the superior mesenteric artery. However, following a further antagonist infusion, in this case via the hepato-portal vein, there was a substantial increase in the pressor response to hepato-portal vein infusions of angiotensin II although the intravenous and superior mesenteric artery responses were unaltered.

Similar effects were observed in all dogs and the pooled responses for 7 dogs appear in Table 5.5. After hepato-portal vein infusion of Sar^1-Ile^8-angiotensin II, the pressor response to hepato-portal vein infusions of angiotensin II was significantly potentiated (before 28.7 ± 3.9 mm Hg x min, after 77.1 ± 19.6 mm Hg x min, $p<0.005$). This response is now significantly greater than the pressor response to superior mesenteric artery infusions (29.7 ± 4.3 mm Hg x min, $p<0.05$), although it remained significantly less than the intravenous response (159.0 ± 21.7 mm Hg x min, $p<0.01$).

Superior mesenteric blood flow

The pooled superior mesenteric blood flow responses for the 7 dogs studied with this protocol are recorded in Table 5.5. Following superior mesenteric artery administration of the antagonist, the fall in superior mesenteric blood flow to direct infusions of angiotensin II was dramatically attenuated (before -909.8 ± 144.5 ml, after -74.8 ± 98.1 ml, $p<0.005$).

However, after a hepato-portal vein infusion of Sar^1-Ile^8-angiotensin II, the superior mesenteric blood flow response to direct angiotensin II

infusions was significantly greater than the response following superior mesenteric artery administration of the antagonist (-582.8 ± 95.9 ml, $p<0.005$), although it remained significantly attenuated when compared to the control response ($p<0.01$).

This may indicate a decline in angiotensin II receptor-antagonism in the superior mesenteric circulation with time.

CALCULATED ANGIOTENSIN II INACTIVATION

The degree of angiotensin II inactivation in the splanchnic and hepato-portal circulations was calculated by the attenuated systemic pressor response technique.

Initial administration of Sar^1-Ile^8-angiotensin II via the superior mesenteric artery failed to alter the degree of angiotensin II inactivation in either of these vascular beds (splanchnic before 80.6 ± 2.3%, after 81.7 ± 2.0%; hepato-portal before 85.3 ± 1.9%, after 82.6 ± 2.0%, Table 5.5). This treatment was followed by an additional hepato-portal vein infusion of the antagonist, which resulted in a significant decrease in the hepatic inactivation of angiotensin II (50.1 ± 8.2%, $p<0.01$), although the degree of inactivation on superior mesenteric artery infusion of angiotensin II was unaltered (78.7 ± 4.2%, Table 5.5).

DISCUSSION

The influence of receptor function on the ability of peripheral vascular beds to inactivate infused angiotensin II was studied with the competitive angiotensin II antagonist, Sar^1-Ile^8-angiotensin II.

Treatment of the renal and hepatic circulation with Sar^1-Ile^8-angiotensin II resulted in approximately a three-fold potentiation of the systemic pressor response to renal artery and hepato-portal vein infusions of angiotensin II. This reflected a substantial reduction in the renal and hepatic inactivation of angiotensin II.

In contrast, a similar treatment of the femoral circulation failed to potentiate the systemic pressor response to femoral artery infusions of angiotensin II and concurrently, hindquarter inactivation of infused angiotensin II was not decreased.

The varied effects of Sar^1-Ile^8-angiotensin II in the renal/hepatic and femoral circulations may be associated with the contrasting manner in which the femoral vascular bed handles infused angiotensin II. The nature of the haemodynamic responses of the femoral circulation to angiotensin II administration has been discussed in Section 3. Unlike the other vascular beds studied, intravenous infusion of angiotensin II fails to increase femoral vascular resistance and consequently there is a redistribution of the cardiac output to the skeletal musculature. Furthermore, control levels of angiotensin II inactivation in the hindquarter are considerably lower than in the other vascular beds examined and therefore, a further reduction may not have been demonstrable.

The effects of Sar^1-Ile^8-angiotensin II were also studied in the

splanchnic and hepatic circulations. Treatment of these vascular beds raised some problems due to their close anatomical relationship and the inability to measure hepato-portal blood flow. The superior mesenteric and hepato-portal circulations are in series, with blood flow from the superior mesenteric circulation passing through the liver before returning to the systemic circulation. Therefore, the responses to superior mesenteric artery infusions of angiotensin II represent the sum of activities in the superior mesenteric and hepato-portal circulations and for convenience in this study, has been called the splanchnic circulation.

Initially, Sar^1-Ile^8-angiotensin II was administered via the superior mesenteric artery. This treatment failed to alter the magnitude or nature of any of the recorded pressor responses, although adequate receptor antagonism was evident in the superior mesenteric circulation. There was the possibility however, that the effects of Sar^1-Ile^8-angiotensin II did not extend to the hepato-portal circulation and this was investigated by a supplementary infusion of the antagonist via the hepato-portal vein.

This additional treatment resulted in a marked potentiation of the pressor response to hepato-portal vein infusions of angiotensin II as the result of a decreased hepatic inactivation of angiotensin II. The magnitude of these effects was similar to those observed in the renal circulation.

The pressor response to superior mesenteric artery infusion was unaltered by this second antagonist infusion. While this may indicate that Sar^1-Ile^8-angiotensin II does not alter angiotensin II inactivation in the mesenteric circulation, any effect may have been masked by the remaining hepatic activity. Furthermore, on examining the superior mesenteric blood flow responses to direct angiotensin II infusions, it

was apparent that the antagonistic effects of the initial superior mesenteric artery infusion of Sar^1-Ile^8-angiotensin II, were declining with time. Therefore, although angiotensin II receptor antagonism reduces the ability of the liver to inactivate angiotensin II, the results are inconclusive in respect to this effect in the superior mesenteric circulation.

The mechanism by which Sar^1-Ile^8-angiotensin II partially inhibits angiotensin II inactivation in the renal and hepatic circulations is difficult to assess from these results. The major physiological route of angiotensin II inactivation is metabolism at tissue sites (Ryan, 1974) and it may well be that the antagonist interferes with this metabolic process. Sar^1-Ile^8-angiotensin II is known to be relatively resistant to amino-peptidase degradation *in vitro* (Regoli, Park and Rioux, 1974), but to my knowledge, competitive antagonists of angiotensin II have not been previously reported to inhibit the inactivation of angiotensin *in vivo*.

The effects of Sar^1-Ile^8-angiotensin II in the renal and hepato-portal circulations therefore, may represent the binding of the antagonist to aminopeptidases thus preventing the access of these enzymes to infused angiotensin II. The partial inhibition of angiotensin II inactivation may indicate the failure of Sar^1-Ile^8-angiotensin II to influence the function of alternative renal and hepatic angiotensinases. The lack of an effect of the antagonist on the femoral inactivation of angiotensin II may be due to the distribution of aminopeptidases within that circulation.

Alternatively, occupancy of angiotensin II receptor sites by the competitive antagonist may alter angiotensinase activity in some manner. Farruggia, Sachs and Palaic (1979) have observed an intimate relationship between angiotensinase activity and receptor function in rabbits. They

suggested that membrane-bound angiotensinase is involved in the regulation of agonist availability at receptor sites and to the activation of additional receptors. Conversely, it may be possible therefore, that receptor function modulates angiotensinase activity.

The nature of angiotensin II receptors in the renal and femoral vascular beds is thought to be different (Caldicott, Taub and Hollenberg, 1977; Caldicott, Taub, Korngold and Hollenberg, 1978; Caldicott and Hollenberg, 1979) and this variation in receptor populations may be involved in the contrasting effects of Sar^1-Ile^8-angiotensin II on the inactivation of infused angiotensin II.

Angiotensin I converting enzyme, angiotensinase and angiotensin II receptors are all membrane bound (Caldwell, Seegal and Hsu, 1976; Ploth and Navar, 1979) and Sar^1-Ile^8-angiotensin II may impair the complex interactions which occur between these elements, at the membrane level.

The effects of Sar^1-Ile^8-angiotensin II may also be mediated by redistributing internal organ blood flow from areas of high angiotensinase activity to areas of low activity, particularly in the kidney where the greatest angiotensinase activity is found in the outer cortex (Itskovitz and Miller, 1967). However, the present evidence for selective regional vasoconstriction by exogenous angiotensin II is controversial (Freeman and Davis, 1979).

If angiotensin II is producing a greater degree of vasoconstriction in the outer cortical regions with a subsequent shift in the intrarenal distribution of blood flow (Hollenberg, Soloman, Adams, Abrams and Merrill, 1972), Sar^1-Ile^8-angiotensin II administration would maintain cortical blood flow and as this area contains the greatest angiotensinase

concentration, an increase in angiotensin II inactivation would be expected and not the observed decrease. Therefore, changes in the intrarenal distribution of blood flow do not explain this effect of Sar^1-Ile^8-angiotensin II, nor do secondary changes in glomerular filtration rate.

Adequate angiotensin II receptor antagonism was defined in this study by the inhibition of the local blood flow response to direct infusions of angiotensin II. Therefore, local administration of Sar^1-Ile^8-angiotensin II should be associated with a maintenance of glomerular filtration at the control level during renal artery infusions of angiotensin II. If glomerular filtration was an important process in the renal inactivation of angiotensin II, an increase in inactivation would be expected with this treatment, in contrast to the observed decrease.

This is in agreement with previous findings which have indicated that the tubular destruction of angiotensin II is thought to be of minor importance in the *in vivo* fate of angiotensin II (Akinkugbe, Brown and Cranston, 1966; Leary and Ledingham, 1969; Bailie, Rector and Seldin, 1971; Oparil and Bailie, 1973; Bailie and Oparil, 1977).

The effects of Sar^1-Ile^8-angiotensin II in the renal and hepatic circulations were of similar magnitude and it is probable therefore, that a common mechanism mediates this effect, further questioning the importance of changes in internal blood flow distribution and urinary excretion.

The effects of Sar^1-Ile^8-angiotensin II on angiotensin II inactivation in the renal circulation are independent of renal transit time. In the presence of the antagonist, a concomitant reduction of renal blood flow failed to alter the ability of Sar^1-Ile^8-angiotensin II to impair the inactivation of angiotensin II in the renal circulation. These results

confirm the hypothesis that the predominant intrarenal site of angiotensin inactivation is the vascular compartment (Bailie and Oparil, 1977) and this is also the site at which Sar^1-Ile^8-angiotensin II is exerting its effects.

Finally, if the mechanisms by which Sar^1-Ile^8-angiotensin II partially inhibits angiotensin II inactivation in the renal and hepatic circulations are further examined, it may lead to a clearer understanding of the fate and handling of angiotensin II in peripheral vascular beds. This is particularly important in the renal vascular bed because of the potentially important physiological role of the intrarenal renin-angiotensin system.

SUMMARY

1. Local treatment of the renal and hepato-portal circulations with the competitive angiotensin II antagonist, Sar^1-Ile^8-angiotensin II, significantly potentiated the systemic pressor response to renal artery and hepato-portal vein infusions of angiotensin II.

2. This effect of Sar^1-Ile^8-angiotensin II in the renal and hepato-portal circulations represented a significant decrease in the degree of angiotensin II inactivation in these vascular beds.

3. The effects of Sar^1-Ile^8-angiotensin II on the kidney's ability to inactivate infused angiotensin II were independent of concurrent alterations in renal blood flow and renal transit time.

4. Treatment of the femoral and superior mesenteric circulations with Sar^1-Ile^8-angiotensin II, failed to alter significantly the systemic pressor responses to femoral and superior mesenteric artery infusions of angiotensin II. Similarly, angiotensin II inactivation was unaltered in these vascular beds.

SECTION 6

MECHANISMS OF NORADRENALINE INACTIVATION IN PERIPHERAL VASCULAR BEDS

INTRODUCTION

Peripheral vascular beds are generally believed to be approximately equiactive in their ability to inactivate infused noradrenaline (Ginn and Vane, 1968; Vane, 1969; Gryglewski and Vane, 1970). This was not found to be the case in Section 4 of this thesis, where a considerable variation in noradrenaline inactivation was observed in the vascular beds studied. Possible mechanisms for this variance have been the subject of investigation in this section where the rate of noradrenaline inactivation has been studied in the renal, femoral and splanchnic vascular beds.

Noradrenaline is removed from the circulation by neuronal and extraneuronal uptake (de la Lande, Frewin and Waterson, 1967) followed by intracellular metabolism and/or storage (Trendelenburg, 1977). These differences in noradrenaline inactivation between the vascular beds studied may therefore represent varying activities of these uptake processes. Furthermore, direct administration of noradrenaline in these vascular beds results in a range of haemodynamic responses, suggesting that these circulations may differ in their α and β-adrenoceptor populations. This may, in some way, influence noradrenaline inactivation.

These aspects have been investigated in this section by local antagonism of α and β-adrenoceptors within each vascular bed studied. Two α-adrenoceptor antagonists were used, phentolamine and phenoxybenzamine. At the doses used in this study, phentolamine should not affect noradrenaline uptake processes whereas phenoxybenzamine should inhibit both neuronal and extraneuronal uptake in addition to its α-adrenoceptor antagonistic action (Starke, Montel and Wagner, 1971). By studying the effects of both antagonists, the relative influences of uptake and α-adrenoceptors on noradrenaline inactivation may be dissociated. Local infusions of

propranolol have been employed to investigate the role of β-adrenoceptors and in some experiments the effects of α and β-adrenoceptor antagonism, with phentolamine and propranolol respectively, have been combined.

Previous results have indicated that the degree of noradrenaline inactivation in the hindquarters is intimately related to the degree of femoral blood flow reduction associated with the noradrenaline administration (Haas, Goldblatt, Lewis and Gipson, 1973). Furthermore, in the kidney, the influence of local blood flow on noradrenaline inactivation is particularly important where in addition to the uptake processes, noradrenaline may be removed from the circulation by the combined effects of glomerular filtration and tubular secretion (Silva, Landsberg and Besarab, 1979). These mechanisms may be substantially affected by changes in renal haemodynamics as a consequence of α-adrenoceptor blockade. An attempt has been made in the present section to dissociate the effects on noradrenaline inactivation of α-adrenoceptor blockade *per se* and the secondary changes in renal haemodynamics.

METHODS

As in previous sections, the experimental animal was the ex-racing greyhound (23-33 kg). The methods of anaesthesia induction and maintenance, drug administration and measurement of cardiovascular variables have been outlined in the General Methods.

Zero and controlled reductions in organ blood flow were achieved by mechanically occluding the supplying artery with a loose ligature applied proximal to the site of intra-arterial infusion and distal to the flow probe.

In all experiments noradrenaline was administered by a 5 min infusion at 10 μg/min (59.1 nmol/min). All adrenoceptor antagonist infusions were administered for 5 min into the blood supply of the vascular bed under study immediately prior to the noradrenaline infusion. The doses of antagonist chosen were such that their antagonistic effects were confined to the vascular bed under study. Two α-adrenoceptor antagonists were used, namely phenoxybenzamine and phentolamine, and the β-adrenoceptor antagonist chosen was propranolol.

The following drugs were used: alpha-chloralose ($C_8H_{11}O_6Cl_3$, B.D.H.), morphine sulphate (D.B.L.), l-noradrenaline bitartrate monohydrate (Levophed, Winthrop), adrenaline (USV, Knoll), phenoxybenzamine hydrochloride (Dibenyline, S.K.F.), phentolamine mesylate (Regitine, Ciba), propranolol (Inderal, I.C.I.). All drugs were dissolved in physiological saline (sodium chloride, 154 mmol/l) and infusions were administered by a constant speed, motor-driven, syringe infusion pump at 1 ml/min. Five milligrams of l-ascorbic acid (Sigma) was added to all noradrenaline solutions to prevent oxidation, the final concentration being 1×10^{-4} mol/l.

RESULTS

EFFECT OF LOCAL PHENOXYBENZAMINE TREATMENT OF THE FEMORAL AND RENAL VASCULAR BEDS ON THE CARDIOVASCULAR RESPONSES TO INTRAVENOUS, RENAL ARTERY AND FEMORAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

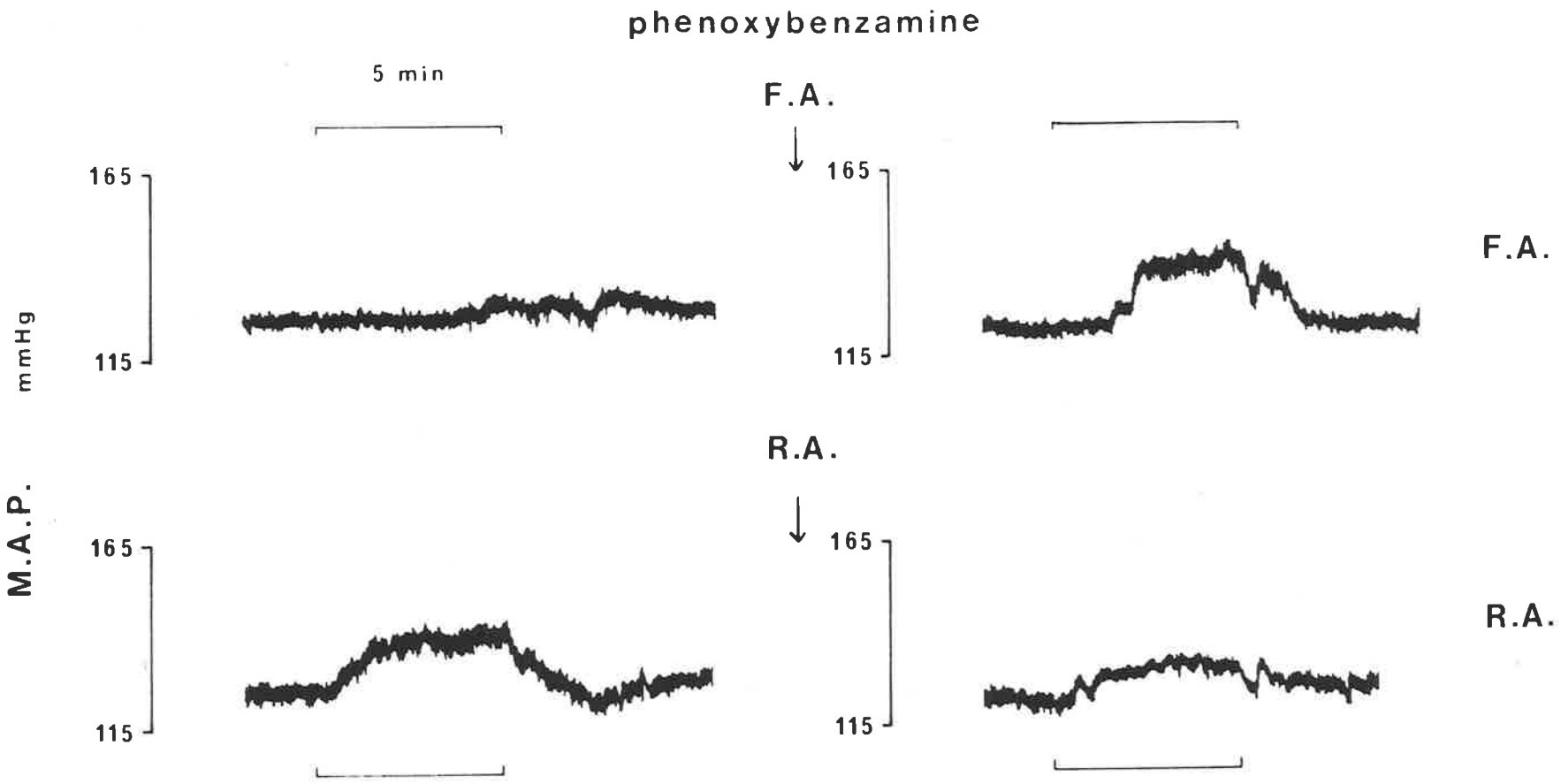
The responses of mean arterial pressure to femoral artery and renal artery infusions of noradrenaline in a single experiment before and after separate treatment of the femoral and renal vascular beds with phenoxybenzamine (100 μg/min [294 nmol/min] for 5 min) are found in Fig. 6.1. Similar results were obtained in each of the six dogs studied with this protocol and the pooled results appear in Table 6.1.

Local treatment of the femoral circulation with phenoxybenzamine (100 μg/min [294 nmol/min] for 5 min) resulted in a substantial potentiation of the mean arterial pressure response to femoral artery infusions of noradrenaline (before 11.0 ± 2.6 mm Hg x min, after 71.2 ± 8.1 mm Hg x min, $p<0.001$). While the pressor response to renal artery infusions of noradrenaline was reduced (before 103.5 ± 18.2 mm Hg x min, after 68.6 ± 8.9 mm Hg x min), the change was not statistically significant.

In contrast to the effect observed in the femoral vascular bed, infusion of the same dose of phenoxybenzamine into the renal circulation resulted in a significant attenuation of the renal artery pressor response (38.3 ± 8.1 mm Hg x min, $p<0.025$). The intravenous pressor response was also significantly reduced following combined femoral and renal phenoxybenzamine

FIG. 6.1. The changes in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min femoral artery (F.A.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after treatment of the femoral and renal circulations with phenoxybenzamine (100 μg/min [294 nmol/min] for 5 min).

NORADRENALINE



administration (control 127.8 ± 23.7 mm Hg x min, after 72.2 ± 8.7 mm Hg x min, $p<0.025$), suggesting that the effects of phenoxybenzamine were not confined to the renal and femoral vascular beds.

Blood flow

In both the femoral and renal vascular beds, effectiveness of α-adrenoceptor blockade was demonstrated by the significant attenuation of the vasoconstrictor responses to local administration of noradrenaline (femoral, before -995.6 ± 137.5 ml, after -325.3 ± 95.1 ml; renal, before -1354.4 ± 23.2 ml, after -122.0 ± 38.9 ml, $p<0.005$ in each vascular bed). Total blockade of local vascular responses with phenoxybenzamine could not be achieved without extension of effects to the systemic circulation.

CALCULATED NORADRENALINE INACTIVATION

Noradrenaline inactivation in the renal and femoral vascular beds has been calculated with the attenuated systemic pressor response technique.

Prior to phenoxybenzamine administration, there was a considerable disparity between these vascular beds in their values of noradrenaline inactivation (femoral 91.3 ± 2.5%, renal 17.3 ± 5.6%, $p<0.001$). Local treatment of the femoral vascular bed with phenoxybenzamine resulted in a dramatic reduction in noradrenaline inactivation in this vascular bed (10.8 ± 4.0%, $p<0.001$), whilst the degree of inactivation in the renal vascular bed was not significantly altered (14.4 ± 8.4%).

In contrast, subsequent treatment of the renal vascular bed with phenoxybenzamine significantly increased the renal inactivation of infused

noradrenaline (48.5 ± 9.1%, $p<0.025$) whilst noradrenaline inactivation in the previously treated femoral vascular bed remained reduced (16.2 ± 6.9%).

These effects of phenoxybenzamine administration on noradrenaline inactivation in the renal and femoral vascular beds are illustrated in Fig. 6.2 and recorded in Table 6.1.

THE EFFECT OF PHENTOLAMINE ON THE CARDIOVASCULAR RESPONSES TO RENAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

Unlike phenoxybenzamine, phentolamine at the dose chosen in this study (50 μg/min [132 nmol/min] for 5 min), does not inhibit noradrenaline uptake, although it remains a potent α-adrenoceptor antagonist.

In this group of 10 dogs noradrenaline was administered via both intravenous and renal artery routes of infusion and the mean arterial pressure responses before and after a renal artery infusion of phentolamine for 10 dogs appear in Table 6.3. Following phentolamine administration, the pressor response to renal artery infusions was significantly attenuated (before 92.0 ± 12.7 mm Hg x min, after 38.2 ± 6.0 mm Hg x min, $p<0.001$) whilst the intravenous response was not significantly altered (before, 130.9 ± 20.0 mm Hg x min). This effect is clearly demonstrated in Fig. 6.3, which contains results from a single experiment.

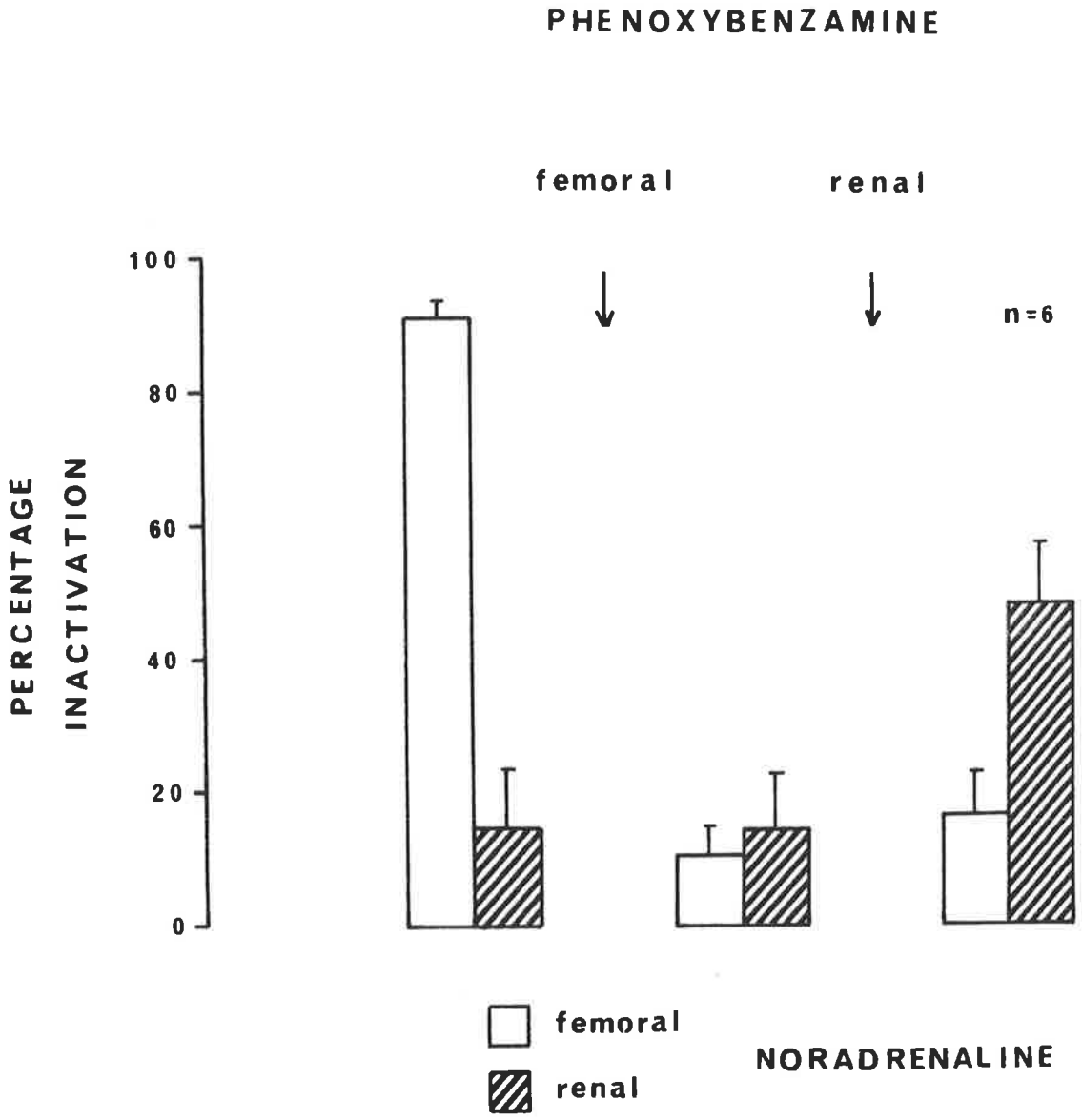


FIG. 6.2. The changes in calculated noradrenaline inactivation in femoral and renal circulations in response to 5 min femoral artery and renal artery administration of phenoxybenzamine (100 μg/min [294 nmol/min]).

Renal blood flow

Renal artery administration of phentolamine virtually abolished the decrease in renal blood flow (before -2397.6 ± 51.7 ml, after -372 ± 48.8 ml, Table 6.3) associated with renal artery infusion of noradrenaline indicating adequate renal α-adrenoceptor antagonism. This effect is demonstrated in Fig. 6.3.

CALCULATED NORADRENALINE INACTIVATION

Noradrenaline inactivation in the renal vascular bed was calculated by the attenuated systemic pressor response technique.

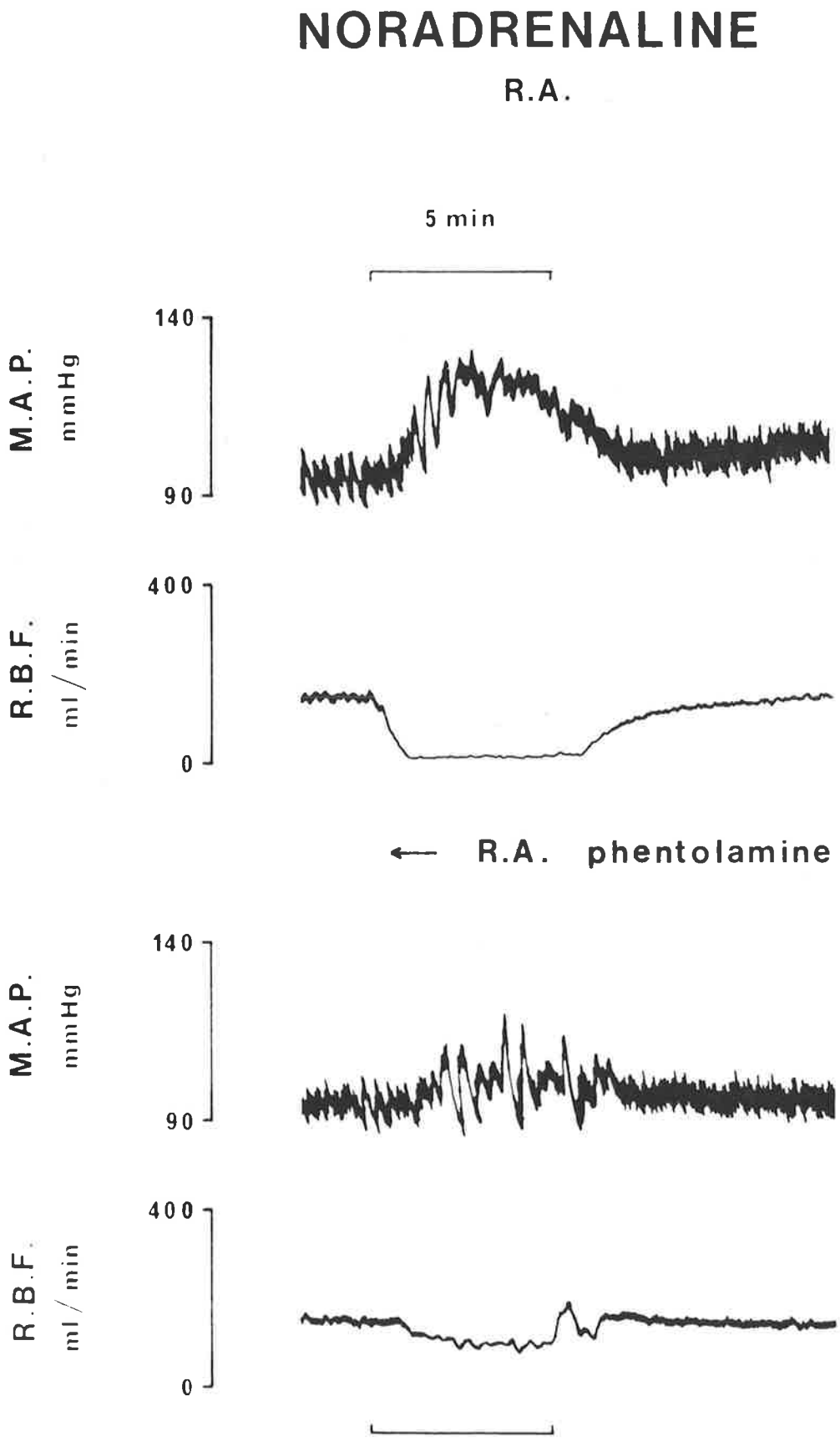
The control level of renal noradrenaline inactivation (26.8 ± 6.9%, n=7, Table 6.3) was similar to that observed in previous experiments (Table 6.1). Renal artery administration of phentolamine resulted in a significant increase in the degree of noradrenaline inactivation in the renal vascular bed (61.2 ± 3.2%, n=7, $p<0.005$).

THE EFFECT OF PHENTOLAMINE AND RENAL HAEMODYNAMICS ON THE CARDIOVASCULAR RESPONSES TO RENAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

The index of adequate renal α-adrenoceptor antagonism with phentolamine is an inhibition of the local vasoconstrictor effects of noradrenaline infusions. In this group of dogs, the relative effects of α-adrenoceptor antagonism and secondary changes in renal blood flow on noradrenaline

FIG. 6.3. The changes in mean arterial pressure (M.A.P., mm Hg) and renal blood flow (R.B.F., ml/min) in a single experiment in response to 5 min renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after renal artery administrations of phentolamine (50 μg/min [177.7 nmol/min] for 5 min).


NORADRENALINE
R.A.
5 min
M.A.P.
mmHg
140
90
R.B.F.
ml/min
400
0
← R.A. phentolamine
M.A.P.
mmHg
140
90
R.B.F.
ml/min
400
0

inactivation are dissociated by examining the effects of phentolamine alone and in conjunction with a mechanical reduction in renal blood flow.

The pressor responses in a single experiment to renal artery infusions of noradrenaline are found in Fig. 6.4 and the pooled results for 6 dogs are contained within Table 6.4. As in the previous group of dogs, renal artery administration of phentolamine resulted in a significant attenuation of the systemic pressor response (before 199.0 ± 54.3 mm Hg x min, after 81.3 ± 21.3 mm Hg x min, $p<0.025$) and the renal blood flow (before -1447.0 ± 157.4 ml, after -480.3 ± 67.8 ml, $p<0.005$) response to renal artery infusions of noradrenaline.

When the level of renal blood flow achieved during control renal artery infusions of noradrenaline was reproduced mechanically during infusions given after phentolamine treatment, the following effects were seen. The systemic pressor response to renal artery infusions of noradrenaline was enhanced (before mechanical flow reduction 81.3 ± 21.3 mm Hg x min, during mechanical flow reduction 104.0 ± 24.5 mm Hg x min) to become significantly greater than that seen following phentolamine alone ($p<0.01$), although it remained significantly less than the control response ($p<0.05$).

CALCULATED NORADRENALINE INACTIVATION

The degree of noradrenaline inactivation was calculated from the loss of systemic vasoactivity of renal artery infused noradrenaline when compared to the response on intravenous infusion. As in the preceding series, renal artery infusion of phentolamine significantly increased the renal inactivation of infused noradrenaline (before 34.0 ± 6.2%, after 63.0 ± 4.1%, $p<0.005$, Table 6.4).

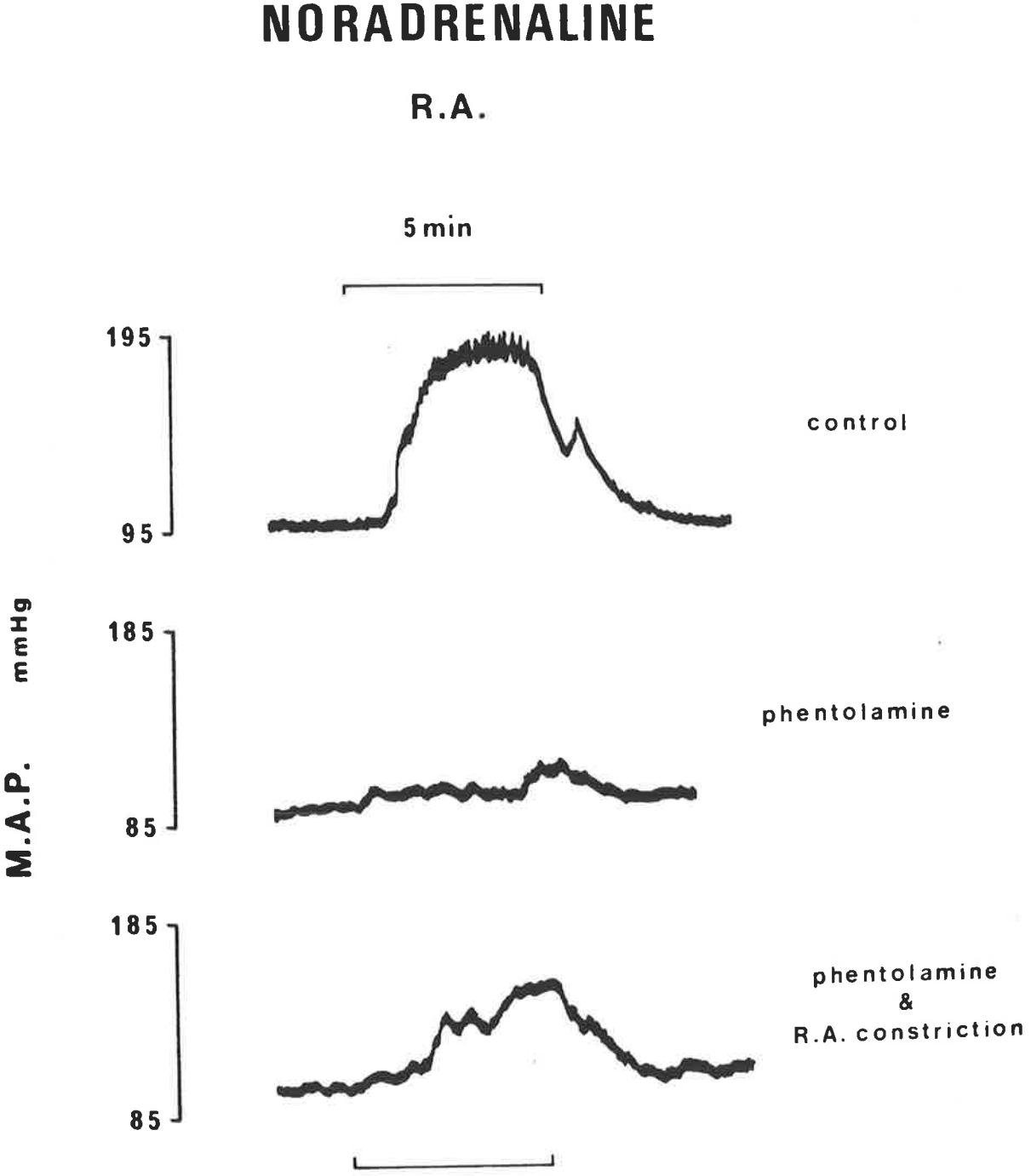


FIG. 6.4. The increases in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min renal artery infusion of noradrenaline (10 μg/min [59.1 nmol/min]) before and after renal artery administration of phentolamine (50 μg/min [132 nmol/min] for 5 min) alone and in conjunction with a renal artery constriction.

When the control degree of renal blood flow reduction was reproduced mechanically during the presence of α-adrenoceptor blockade with phentolamine, renal inactivation of noradrenaline decreased. This final level of noradrenaline inactivation was significantly less than that observed with phentolamine alone (43.6 ± 8.1%, $p<0.05$) and not significantly different from the control value.

THE EFFECT OF PHENTOLAMINE ON THE CARDIOVASCULAR RESPONSES TO SUPERIOR MESENTERIC ARTERY AND HEPATO-PORTAL VEIN INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

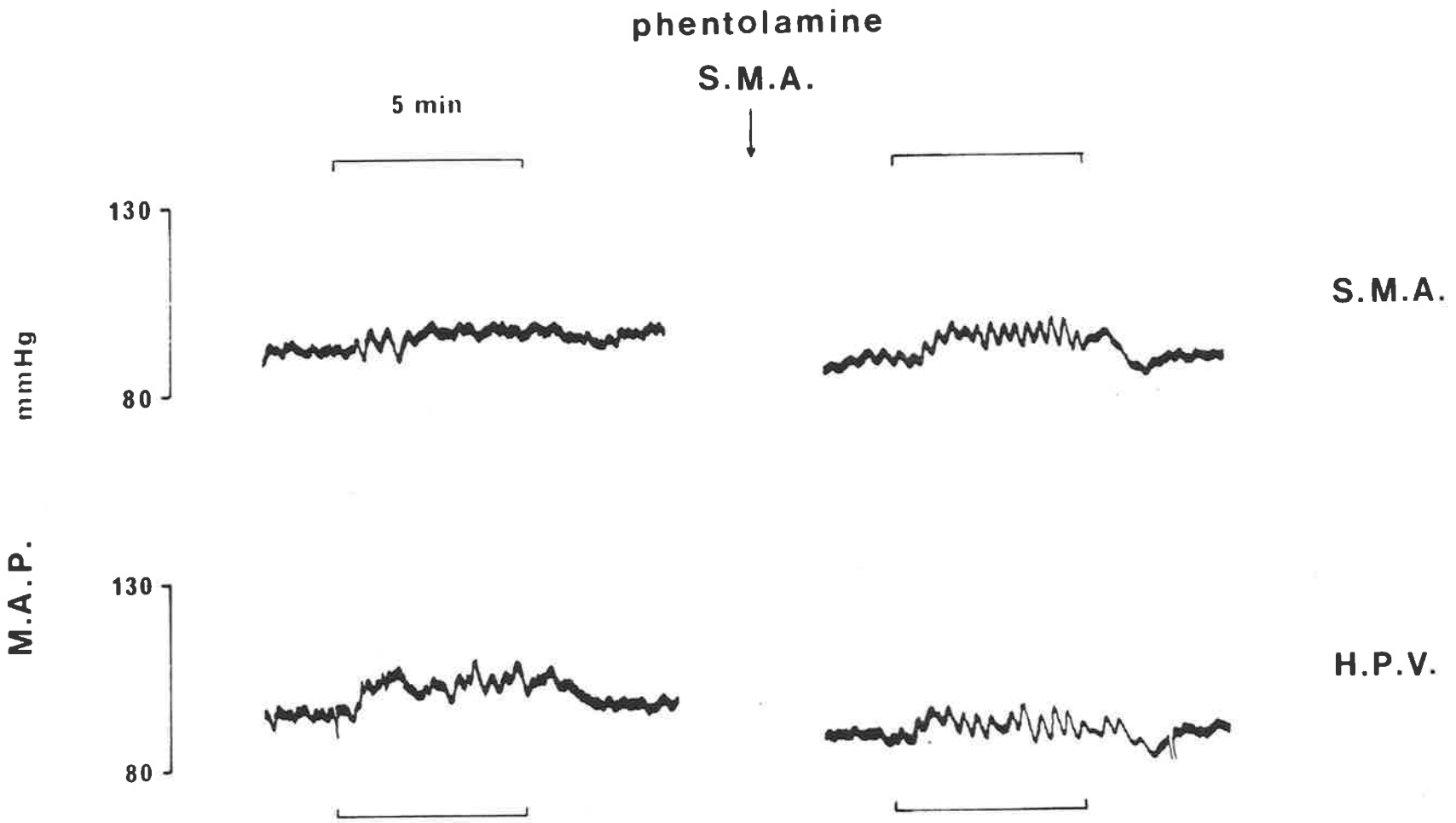
In this group of 6 dogs superior mesenteric artery and hepato-portal vein infusions of noradrenaline resulted in only minor elevations of mean arterial pressure (38.8 ± 7.2, 52.1 ± 9.2 mm Hg x min, respectively) when compared with the substantial pressor response on intravenous infusions of the same dose (157.3 ± 25.0 mm hg x min). The magnitude of these responses was unaltered by local treatment of the splanchnic circulation with phentolamine (200 μg/min [530 nmol/min] for 5 min; intravenous 156.8 ± 16.3 mm Hg x min, superior mesenteric artery 41.0 ± 7.3 mm Hg x min, hepato-portal vein 39.0 ± 6.6 mm Hg x min, Table 6.5, Fig. 6.5).

Superior mesenteric blood flow

Local administration of phentolamine in the splanchnic circulation significantly attenuated the fall in superior mesenteric blood flow

FIG. 6.5. The increases in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after superior mesenteric artery administration of phentolamine (200 μg/min [676 nmol/min] for 5 min).

NORADRENALINE



associated with superior mesenteric artery infusions of noradrenaline (before -809.7 ± 116.1, after -269.6 ± 74.7 ml, $p<0.005$, Table 6.5).

CALCULATED NORADRENALINE INACTIVATION

Noradrenaline inactivation in the splanchnic and hepato-portal circulations was calculated with the attenuated systemic pressor response. A high degree of noradrenaline inactivation was observed in the splanchnic and hepato-portal circulations (Table 6.5) and these values were unaltered by local α-adrenoceptor blockade with phentolamine (splanchnic before 73.3 ± 4.4%, after 73.0 ± 5.1%; hepato-portal before 62.5 ± 9.0%, after 74.0 ± 5.2%).

THE EFFECT OF PROPRANOLOL ON THE CARDIOVASCULAR RESPONSES TO RENAL ARTERY AND FEMORAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

Propranolol was infused into the renal and subsequently the femoral circulations, at a dose which was designed to confine its β-adrenoceptor antagonistic effects to the infused circulation (200 μg/min [676 nmol/min] for 5 min). Propranolol administration resulted in contrasting effects on the systemic pressor responses to renal artery and femoral artery infusions of noradrenaline, as demonstrated in Fig. 6.6 from a single experiment.

Local propranolol treatment resulted in a substantial attenuation of

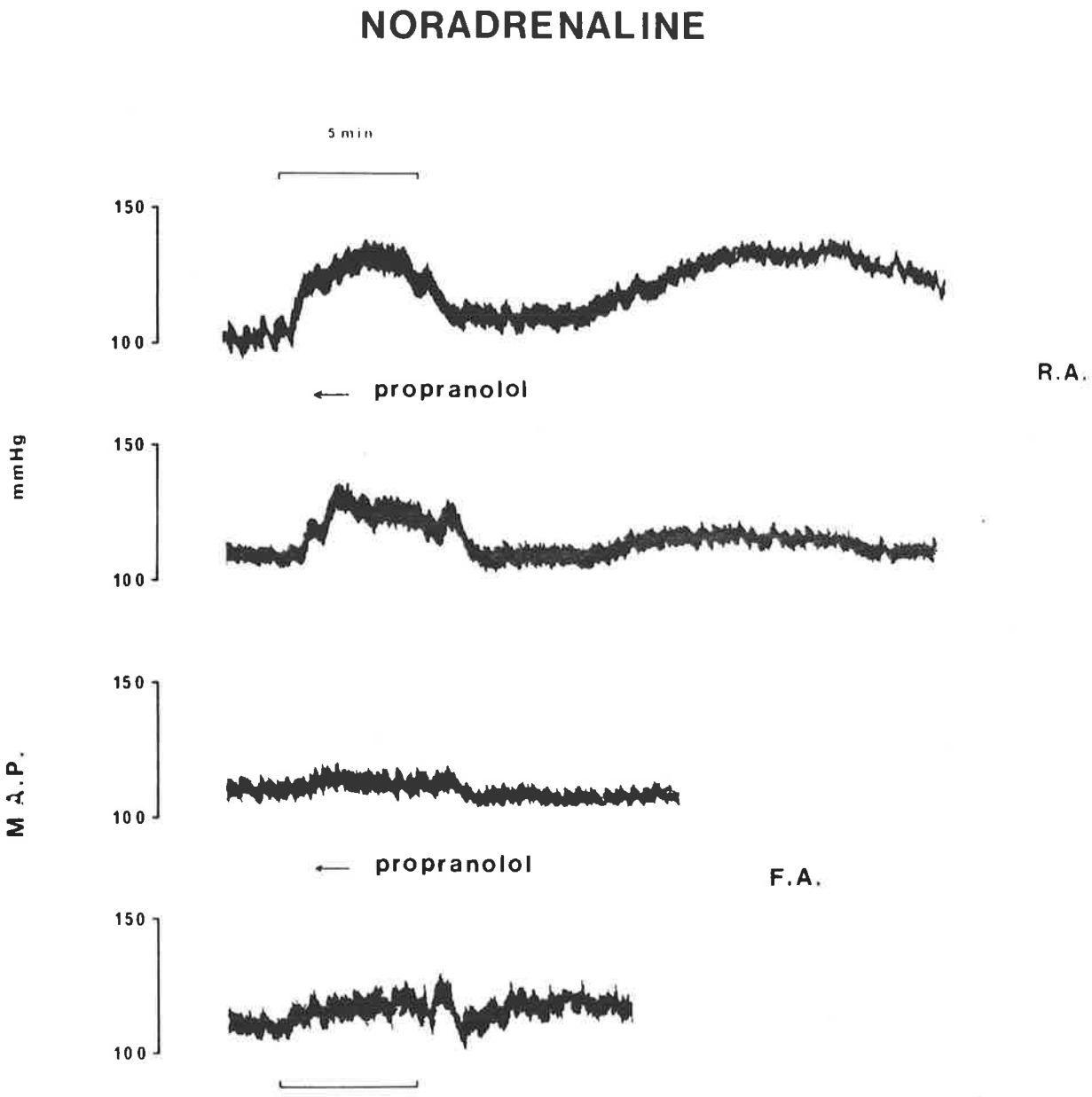


FIG. 6.6. The increases in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min renal artery (R.A.) and femoral artery (F.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after local administration of propranolol in the renal and femoral circulations (200 μg/min [676 nmol/min] for 5 min).

the pressor response to renal artery infusions of noradrenaline (before 175.4 ± 38.9 mm Hg x min, after 145.0 ± 33.6 mm Hg x min), whereas it was without effect on the femoral pressor response. Renal artery infusion of noradrenaline was characterized by a biphasic pressor response which was not observed on any other route of administration in this study, Fig. 6.6. The nature and mechanism of the secondary rise in mean arterial pressure is discussed in Section 7. Renal artery infusion of propranolol attenuated both the pressor response confined to the noradrenaline infusion period and the secondary pressor response.

Similar effects were observed in the 6 other dogs studied and the pooled results are contained in Table 6.6.

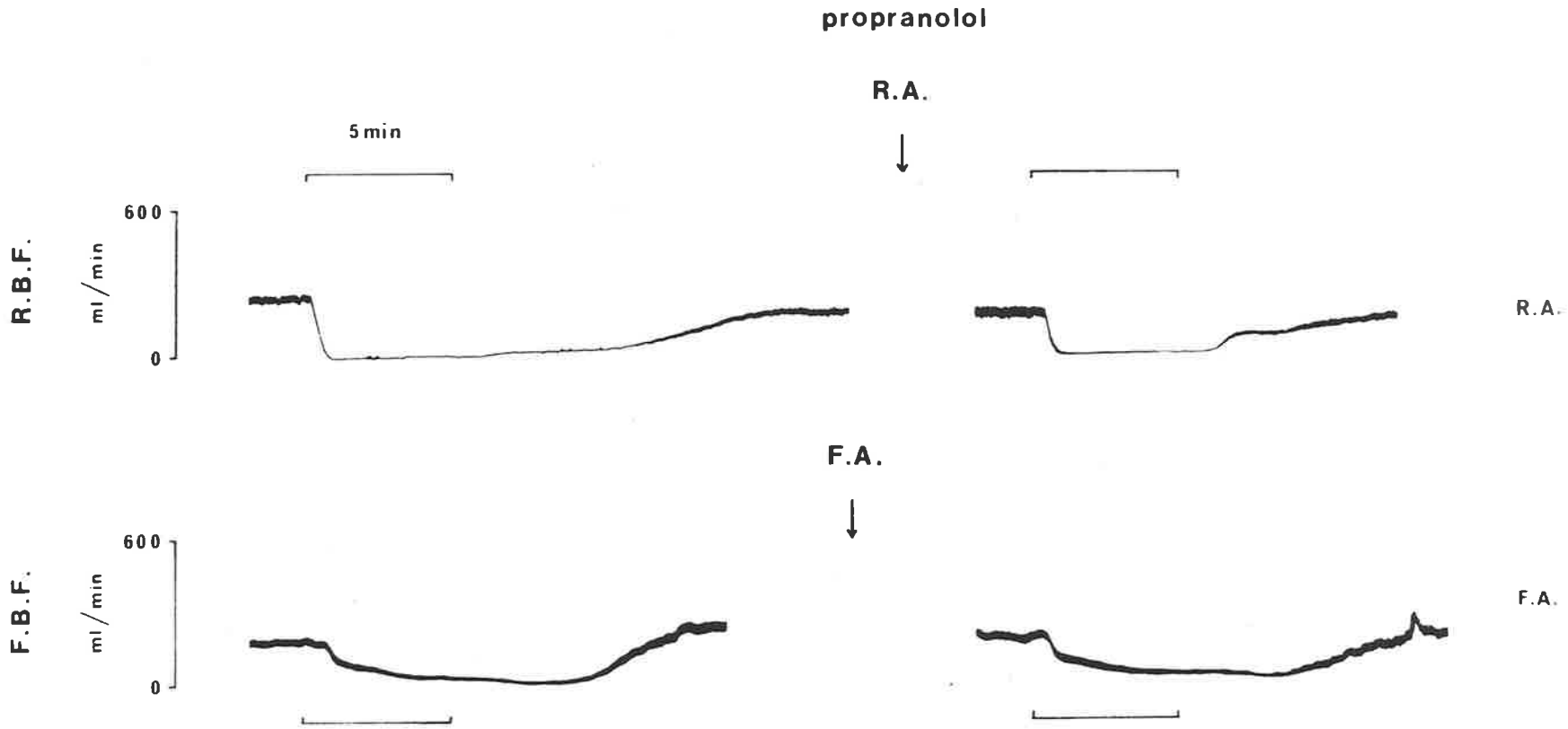
Blood flow

Treatment of the renal circulation with propranolol resulted in a significant attenuation of the renal blood flow response to renal artery infusions of noradrenaline (before -1693.7 ± 237.3 ml, after -1132.4 ± 174.0 ml, $p<0.01$, Table 6.6), whereas similar treatment of the femoral circulation failed to alter the femoral blood flow response to direct infusion of noradrenaline (before -1575.5 ± 343.9 ml, after -1676.3 ± 438.8 ml).

The nature of this attenuated renal blood flow response is demonstrated in the results from a single experiment, in Fig. 6.7. Propranolol does not alter the absolute fall in renal blood flow to direct noradrenaline infusions but decreases the duration of the response. This effect is in contrast to that observed with phentolamine, in which the duration and absolute fall in renal blood flow were attenuated.

FIG. 6.7. The reductions in renal blood flow (R.B.F., ml/min) and femoral blood flow (F.B.F., ml/min) in a single experiment in response to direct infusions of noradrenaline (10 ug 'min [59.1 nmol/min] for 5 min) before and after local administration of propranolol in the renal and femoral circulations (200 μg/min [676 nmol/min] for 5 min).

NORADRENALINE



CALCULATED NORADRENALINE INACTIVATION

Noradrenaline inactivation in the renal and femoral vascular beds was calculated with the attenuated systemic pressor response technique. Local β-adrenoceptor antagonism in the renal circulation with propranolol resulted in a significant increase in renal inactivation of noradrenaline (before 31.9 ± 5.2%, after 49.5 ± 6.2%, $p<0.001$). Similar treatment of the femoral circulation failed to alter the degree of noradrenaline inactivation in that vascular bed (before 85.1 ± 4.5%, after 86.1 ± 6.0%, Table 6.6).

THE EFFECT OF COMBINED PROPRANOLOL AND PHENTOLAMINE ADMINISTRATION ON THE CARDIOVASCULAR RESPONSES TO RENAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

Individually, propranolol and phentolamine administration in the renal circulation attenuated the systemic pressor response to renal artery infusion of noradrenaline. In this present group of five dogs, the effects of combined propranolol (200 μg/min [676 nmol/min] for 5 min) and phentolamine (100 μg/min [265 nmol/min] for 5 min) treatment have been studied, and the pooled results appear in Table 6.7.

Initially, the effects of propranolol alone were studied and similar results to the previous experiments were obtained. A subsequent renal artery infusion of phentolamine resulted in a further attenuation of the

renal artery pressor response ($p<0.001$). The pressor response to renal artery infusions of noradrenaline was now virtually abolished (control 151.2 ± 39.6 mm Hg x min, after renal artery propranolol and phentolamine 33.4 ± 10.1 mm Hg x min, Fig. 6.8).

Renal blood flow

Renal artery infusion of propranolol alone resulted in an attenuation of the renal blood flow response to renal artery infusions of noradrenaline (before -1108.0 ± 183.6 ml, after -898.2 ± 22.15 ml), the nature of which was discussed in the previous group of dogs. When phentolamine and propranolol treatment were combined, there was a further attenuation of the renal blood flow response (-344.8 ± 83.8 ml). Renal artery infusions of noradrenaline in this case marginally decreased renal blood flow (Fig. 6.8 and Table 6.7).

CALCULATED NORADRENALINE INACTIVATION

Noradrenaline inactivation in the renal vascular bed was calculated with the attenuated pressor response technique. In the experiments discussed above, similar increases in the renal inactivation of noradrenaline were observed on local administration of either propranolol or phentolamine.

In the present experiments, this effect of propranolol was confirmed (before 38.5 ± 9.7%, after 57.8 ± 9.2%, $p<0.02$) and further treatment of the renal vascular bed with phentolamine resulted in an additional increase in noradrenaline inactivation (85.8 ± 4.0%, $p<0.05$, Table 6.7, Fig. 6.9).

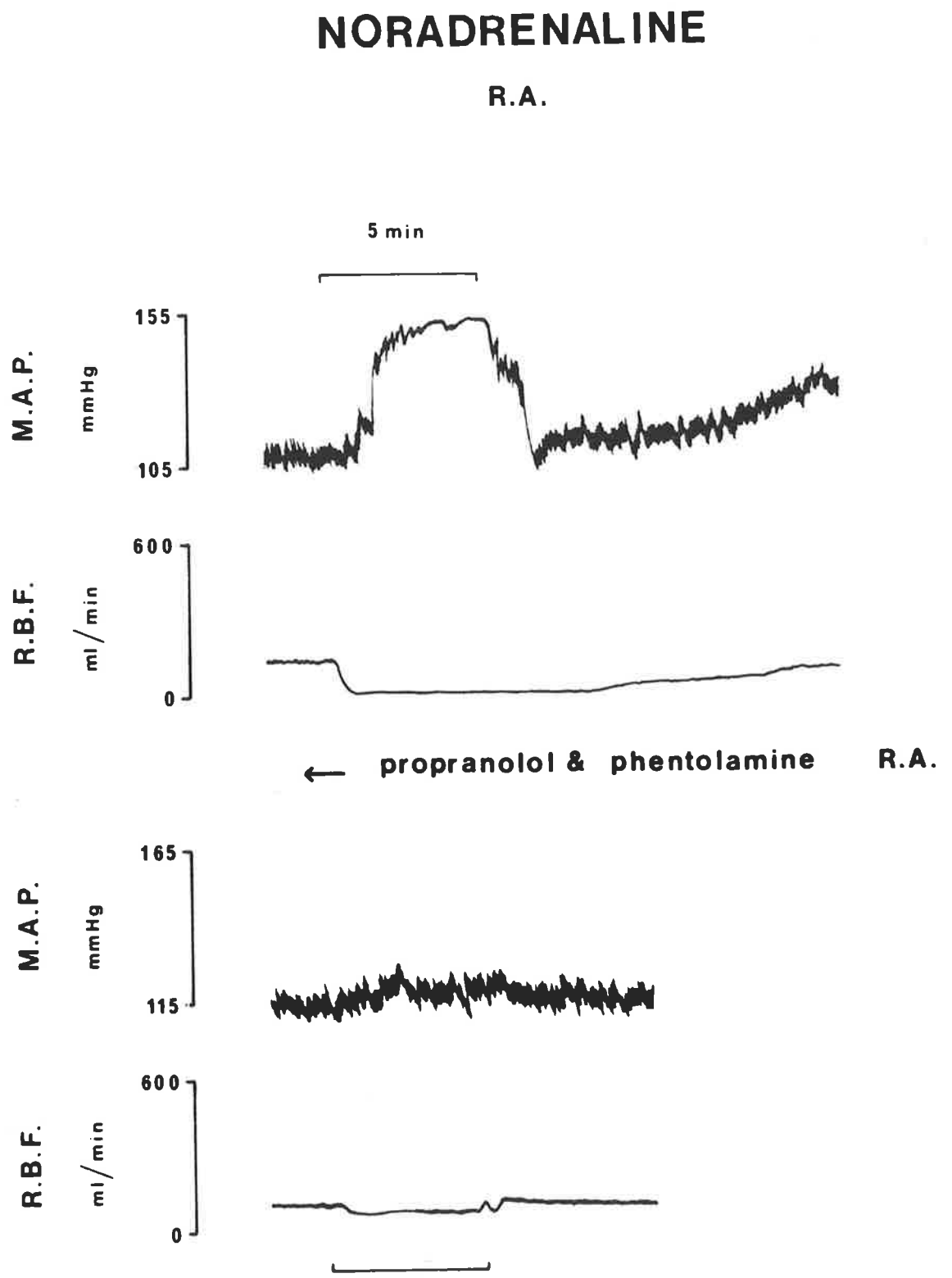
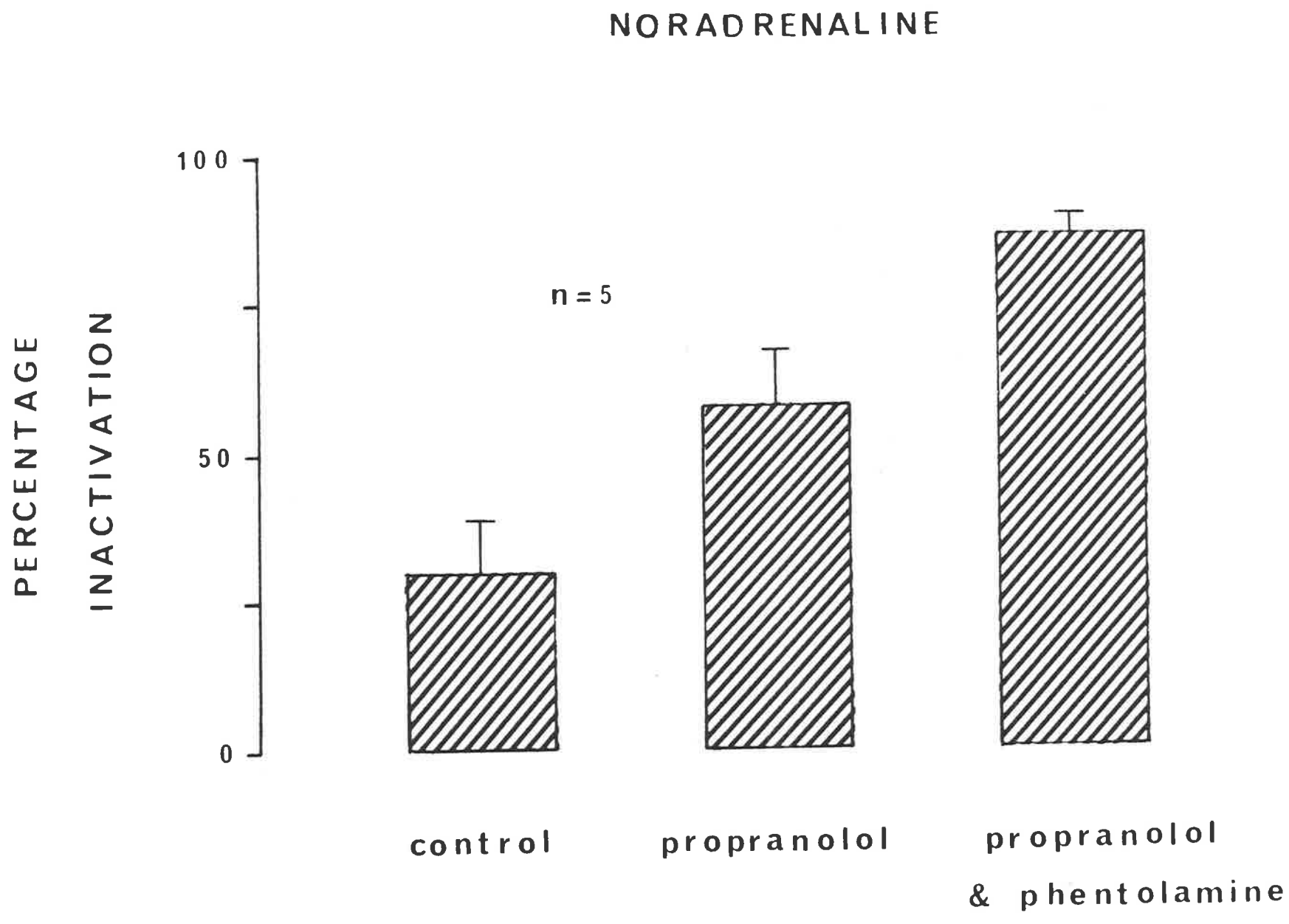


FIG. 6.8. The changes in mean arterial pressure (M.A.P., mm Hg) and renal blood flow (R.B.F., ml/min) in a single experiment in response to renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after combined treatment of renal circulation with phentolamine (100 μg/min [265 nmol/min] for 5 min) and propranolol (200 μg/min [676 nmol/min] for 5 min).

FIG. 6.9. The mean calculated percentage noradrenaline inactivation in the renal circulation for 5 dogs, before and after treatment of the renal circulation with propranolol alone (200 μg/min [676 nmol/min] for 5 min) or in conjunction with phentolamine (100 μg/min [265 nmol/min] for 5 min). The height of the columns represent the mean value for 5 dogs and the vertical line, one standard error of the mean.


NORADRENALINE
PERCENTAGE INACTIVATION
100
50
0
n = 5
control
propranolol
propranolol & phentolamine

DISCUSSION

It would appear that a number of mechanisms are responsible for the contrasting manner in which noradrenaline is inactivated in the renal, femoral, splanchnic and hepato-portal circulations. Of particular importance in the kidney are the influences of renal haemodynamics and the presumed subsequent alterations in glomerular filtration. The β-adrenoceptors also have a role, the contribution of which appears to be independent of renal haemodynamic alterations.

The marked differences in noradrenaline inactivation in the control state in these vascular beds studied reflect varying capacities to remove noradrenaline from the extracellular fluid, through neuronal and extraneuronal uptake. This possibility was examined by local administration of phenoxybenzamine in the renal and femoral vascular beds.

This treatment had remarkably contrasting effects in the respective vascular beds. In the femoral circulation, the systemic pressor response to femoral artery infusion of noradrenaline was considerably potentiated and noradrenaline inactivation dramatically reduced following local administration of phenoxybenzamine. However, following identical treatment of the renal vascular bed, the systemic pressor response to renal artery infusions of noradrenaline was significantly reduced and noradrenaline inactivation increased. There was a decline in the systemic vasoactivity of noradrenaline as indicated by the significantly smaller pressor response to intravenous infusions. This effect, however, does not explain the opposing action of phenoxybenzamine on noradrenaline inactivation in the femoral and renal vascular beds.

This contrasting effect of phenoxybenzamine in the renal circulation

suggests that alternative mechanisms are operating, as noradrenaline inactivation was increased following a dose of phenoxybenzamine which presumably inhibited neuronal and extraneuronal uptake in the femoral circulation.

It was decided, therefore, to investigate the influence of α-adrenoceptor antagonism alone, with phentolamine, in the renal and splanchnic vascular beds, at doses chosen to avoid the additional effects on noradrenaline uptake associated with phenoxybenzamine.

Local α-adrenoceptor blockade in the splanchnic circulation had no effect on the pressor responses to superior mesenteric artery or hepato-portal vein infusions of noradrenaline, nor in the degree of noradrenaline inactivation in the splanchnic or hepato-portal circulations. Alpha-adrenoceptor antagonism in the renal circulation, however, reduced the systemic pressor response to renal artery infusions of noradrenaline and increased the renal inactivation of noradrenaline. The degree and nature of these renal effects were similar to those previously observed with phenoxybenzamine.

It appears, therefore, that the effects of phenoxybenzamine on the renal circulation were mediated through its α-adrenoceptor antagonistic action and not through an effect on noradrenaline uptake. As the index of adequate α-adrenoceptor antagonism is an inhibition of the local blood flow response to direct infusion of noradrenaline, the altered state of renal haemodynamics of phentolamine and phenoxybenzamine administration, may be responsible for their effects rather than a direct effect on α-adrenoceptors *per se*.

This possibility was examined in a group of dogs in which the control

renal blood flow reduction was artifically reproduced by mechanical constriction of the renal artery, in the presence of phentolamine. This manoeuvre reversed the effects of phentolamine on the renal inactivation of noradrenaline, indicating that the effects of α-adrenoceptor antagonism were intimately related to the secondary changes in renal haemodynamics.

Noradrenaline is known to be excreted in the urine by the combined effects of glomerular filtration and tubular secretion (Silva, Landsberg and Besarab, 1979). Renal artery infusions of noradrenaline reduce renal blood flow and glomerular filtration and therefore impair this method of inactivation. Antagonism of blood flow effects by α-adrenoceptor blockade will therefore increase glomerular filtration and increase the degree of noradrenaline inactivation in the renal circulation. The influence of α-adrenoceptor antagonism on the tubular secretory mechanism is unknown. The considerably lower renal inactivation of noradrenaline in the control state, when compared with the other vascular beds studied, may therefore represent a greatly reduced capacity of the kidney to excrete noradrenaline through the urine.

This hypothesis was supported by the findings in Section 4, in which the degree of noradrenaline inactivation in the renal circulation was found to be inversely related to the fall in renal blood flow on direct infusion of noradrenaline. Greater reductions in renal blood flow resulted in an increased renal inactivation of noradrenaline. These results also indicate that an increase in renal transit time for infused noradrenaline does not result in an increase in noradrenaline inactivation, as was observed with angiotensin II by Bailie and Oparil (1977). A false positive result for angiotensin II and noradrenaline inactivation in the femoral vascular bed was reported by Haas, Goldblatt, Lewis and Gipson (1973) as a result of delayed transit of injected angiotensin II or noradrenaline

across the femoral vascular bed. This technical difficulty has been overcome in this thesis, as demonstrated by the results to α-adrenoceptor antagonism.

The effects of local β-adrenoceptor antagonism with propranolol were also studied in the renal and femoral vascular beds, again with contrasting results. Local treatment of the femoral vascular bed with propranolol did not alter the systemic pressor or femoral blood flow responses to femoral artery infusions of noradrenaline. Similar treatment of the renal circulation resulted in a significant increase in the renal inactivation of infused noradrenaline.

The renal blood flow response to renal artery infusions of noradrenaline was significantly attenuated following local propranolol administration, although the nature of this attenuation was different to that observed with phentolamine. With propranolol, the duration of renal blood flow reduction was considerably attenuated whilst the absolute fall in renal blood flow to renal artery infusions of noradrenaline was not altered. Therefore, unlike phentolamine, the mechanism for this increased renal inactivation of noradrenaline does not appear to involve urinary excretion. An additional mechanism must therefore be responsible. This is exemplified by the groups of dogs where the renal effects of propranolol were repeated, alone and in conjunction with renal administration of phentolamine. With this combined treatment their effects on noradrenaline inactivation were additive with the final degree of inactivation being of a similar magnitude to that observed in other vascular beds, suggesting that different mechanisms mediate their effects.

It is difficult to ascertain the mechanism of the effects of propranolol from these results. It does not appear to be a direct effect of

β-adrenoceptor antagonism, as similar treatment of the femoral vascular bed failed to alter any of the recorded variables. This observation is supported by the recent work of Jackson and Campbell (1980) who found that a noradrenaline-induced vasoconstriction of isolated mesenteric arteries was unaltered by a number of β-adrenoceptor antagonists.

It is well established that noradrenaline may stimulate renal renin release through a direct effect on the β-adrenoceptors of the juxtaglomerular cells (Winer, Chokshi, Yoon and Freedman, 1969; Davis and Freeman, 1976). Angiotensin II is known to have a presynaptic effect on vascular smooth muscle to increase its sensitivity to noradrenaline (Day and Moore, 1976) and intrarenally generated angiotensin II may indirectly, and directly through its vasoconstrictor action, be responsible for prolonged duration of the renal blood flow reduction to noradrenaline infusions. Propranolol may therefore reduce the duration of this renal blood flow response by inhibiting the noradrenaline induced increase in renal renin release. There has also been some controversy over the ability of angiotensin II to inhibit neuronal uptake (Starke, 1977; Westfall, 1977; Jackson and Campbell, 1979). The enhanced noradrenaline inactivation in the kidney following local propranolol may be explained in terms of an angiotensin II-induced inhibition of neuronal uptake. The possibility that local activation of the renin-angiotensin system is involved in these various effects has been examined in the following section.

SUMMARY

1. The importance of renal haemodynamics in the degree of noradrenaline inactivation within the kidney has been established.

2. Treatment of the renal and femoral circulations with phenoxybenzamine had contrasting effects on the ability of these two vascular beds to inactivate infused noradrenaline. Phenoxybenzamine substantially reduced the degree of noradrenaline inactivation in the hindquarters but significantly increased noradrenaline inactivation in the kidney.

3. Local α-adrenoceptor-antagonism with phentolamine in the splanchnic and hepato-portal circulations, failed to alter the degree of noradrenaline inactivation in these circulations.

4. Alpha-adrenoceptor antagonism with phentolamine in the renal circulation resulted in similar effects to those seen with phenoxybenzamine. This effect of phentolamine was reversed when the level of renal blood flow on control renal artery infusions of noradrenaline was reproduced mechanically.

5. The effects of local α-adrenoceptor antagonism on the kidney's ability to inactivate noradrenaline appear to be dependent on secondary changes in renal blood flow, with the mechanism for this increased noradrenaline inactivation possibly being an increased renal clearance of noradrenaline, with subsequent urinary excretion.

6. Treatment of the renal circulation with the β-adrenoceptor antagonist, propranolol, also increased the degree of noradrenaline inactivation within the kidney. The mechanism of this effect appears to be independent of an effect on glomerular filtration, as the absolute fall in renal blood flow to renal artery infusions of noradrenaline was unaltered by this treatment, although the duration of this blood flow reduction was attenuated.

7. Treatment of the femoral vascular bed with propranolol, failed to alter either the femoral blood flow or mean arterial pressure responses to femoral artery infusions of noradrenaline or the ability of the hindquarters to inactivate infused noradrenaline.

8. The effects of phentolamine and propranolol on the renal inactivation of noradrenaline summate on concomitant administration, suggesting that their effects are mediated through independent mechanisms.

SECTION 7

THE INFLUENCE OF THE RENIN-ANGIOTENSIN SYSTEM OF THE RENAL INACTIVATION OF NORADRENALINE

INTRODUCTION

In the previous section, the renal inactivation of infused noradrenaline was demonstrated to increase following local α and β-adrenoceptor antagonism. Although the degree of enhancement was similar with each treatment, their effects were considered to be mediated through independent mechanisms.

Renal artery infusions of noradrenaline at the dose employed resulted in a substantial fall in renal blood flow and glomerular filtration. Local α-adrenoceptor antagonism within the kidney inhibited these vasoconstrictor effects of infused noradrenaline and this is thought to enhance the inactivation of infused noradrenaline through an increased renal clearance of noradrenaline. However, the enhanced renal inactivation of noradrenaline following local β-adrenoceptor antagonism with propranolol appears to be independent of renal haemodynamics. Propranolol is not thought to influence catecholamine uptake directly (Jackson and Campbell, 1980) and as the observed increase in noradrenaline inactivation cannot be explained by an increase in the renal clearance of noradrenaline an alternative mechanism must be sought.

In view of the proposed involvement of the intrarenal renin-angiotensin system in the renal vascular responses to renal artery infusions of noradrenaline the possibility exists that the impaired intrarenal formation of angiotensin II following treatment with propranolol may, in some way, be responsible for the observed increase in renal inactivation of infused noradrenaline.

A number of interactions between angiotensin II and noradrenaline, both circulating and neuronally released, have been suggested in the literature (for review see Peach, 1977; Starke, 1977; Westfall, 1977).

Possibly the most significant with regard to the present study is the proposed inhibitory effect of angiotensin II on neuronal uptake, an effect which would be expected to decrease the rate of noradrenaline inactivation within a vascular bed. There is, however, considerable debate over the concentration of angiotensin II required for this effect (Starke, 1971; Khairallah, 1972), although recently Jackson and Campbell (1979) have demonstrated that subpressor doses of angiotensin II (3×10^{-11} M) inhibit neuronal uptake in isolated mesenteric arteries.

In the present section the effects of angiotensin II on the ability of the kidney to inactivate infused noradrenaline have been examined by the local inhibition of angiotensin I converting enzyme activity with SQ 20881. The results obtained have been compared with those observed in Section 6 with propranolol, which is also known to inhibit the local formation of angiotensin II through its well established inhibitory effects on renin release (Davis and Freeman, 1976).

METHODS

The experimental animal was the ex-racing greyhound (27-33 kg). The methods of anaesthesia induction and maintenance, drug administration, measurement of cardiovascular variables and radioimmunoassay techniques have been previously outlined in the General Methods.

The intrarenal renin-angiotensin II system was impaired by the renal artery administration of the angiotensin I converting enzyme inhibitor, SQ 20881 (200 μg/min [158.3 nmol/min] for 5 min) thereby inhibiting the formation of angiotensin II. Renal α-adrenoceptor antagonism was achieved with a renal artery infusion of phentolamine (100 μg/min [265 nmol/min] for 5 min) and studied in conjunction with angiotensin I converting enzyme inhibition.

The ability of renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min] for 5 min) to stimulate renal renin release was examined by estimating the plasma renin activity in blood samples taken from the inferior vena cava, centrally to the renal veins. Blood samples (5 ml) were withdrawn according to the following protocol: 2 pre-infusion control samples, 1 sample during the renal artery infusion of noradrenaline and 3 post-infusion samples. All samples were added to tubes containing 0.5 ml EDTA/dimercaprol (final concentration = 0.01 M) and stored in ice until centrifugation and separation of plasma.

The following drugs were used; alpha-chloralose ($C_8H_{11}O_6Cl_3$, B.D.H.), morphine sulphate (D.B.L.), l-noradrenaline bitartrate monohydrate (Levophed, Winthrop), phentolamine mesylate (Regitine, Ciba), angiotensin I (Sigma) and SQ 20881 (Squibb). All drugs were dissolved in physiological saline (sodium chloride 154 mmol/l) and all infusions were administered by a constant speed, motor-driven syringe infusion pump at 1 ml/min.

RESULTS

THE EFFECT OF RENAL ARTERY INFUSIONS OF SQ 20881 AND PHENTOLAMINE ON THE CARDIOVASCULAR RESPONSES TO INTRAVENOUS AND RENAL ARTERY INFUSIONS OF NORADRENALINE

GENERAL

Mean arterial pressure

The peptide SQ 20881 is a potent inhibitor of angiotensin I converting enzyme and the influence of local treatment of the renal vasculature with SQ 20881 alone and in conjunction with phentolamine on the systemic pressor response to renal artery infusions of noradrenaline in a single experiment is shown in Fig. 7.1

Following inhibition of converting enzyme activity within the kidney with SQ 20881 the systemic pressor response to renal artery infusions of noradrenaline was attenuated. Additional treatment of the renal vasculature with phentolamine further attenuated this pressor response.

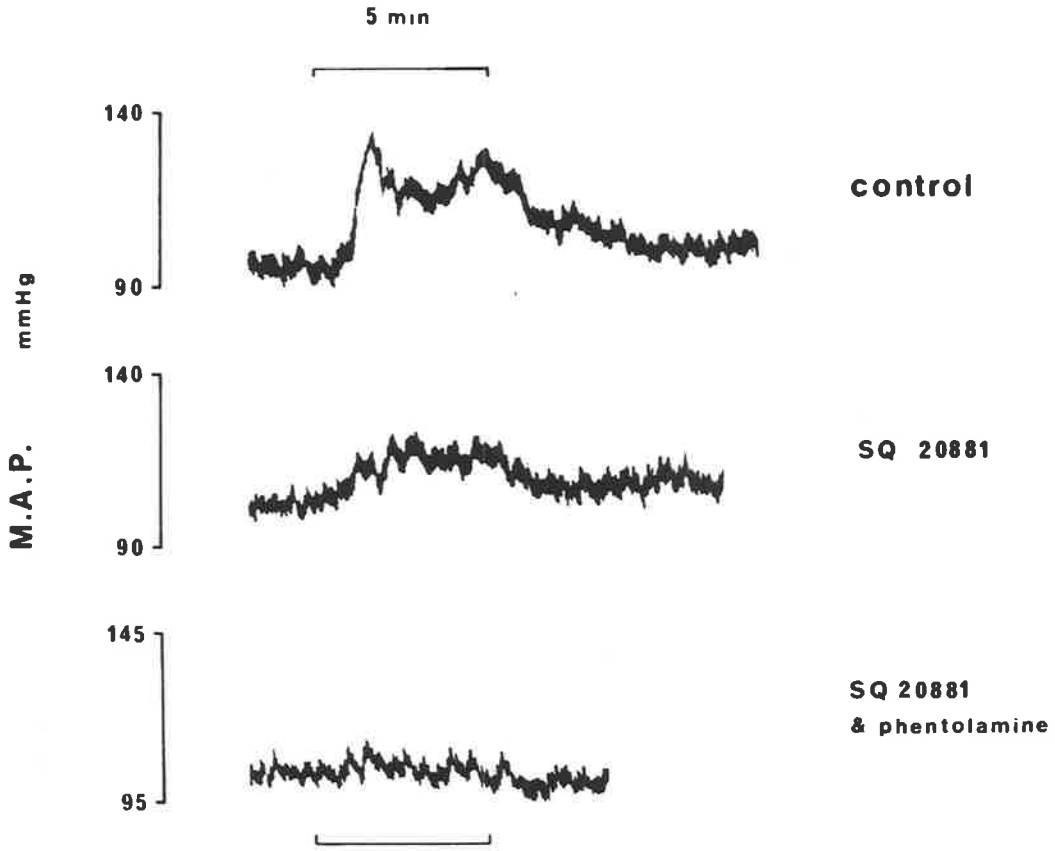
Similar effects were observed in each of the 6 dogs studied and the pooled results appear in Table 7.1. The systemic pressor response to renal artery infusions of noradrenaline was significantly attenuated following local treatment of the renal circulation with SQ 20881 (before 133.2 ± 35.0 mm Hg x min, after 78.2 ± 21.1 mm Hg x min, $p<0.05$). Concurrently, there was a slight decrease in the pressor response to intravenous infusions, although this effect was not significant.

Additional treatment of the renal vasculature with phentolamine

FIG. 7.1. The increases in mean arterial pressure (M.A.P., mm Hg) in a single experiment in response to 5 min renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after treatment of the renal circulation with SQ 20881 alone (200 μg/min [158 nmol/min] for 5 min) and in conjunction with phentolamine (100 μg/min [265 nmol/min] for 5 min).

NORADRENALINE

R.A.



virtually abolished the systemic pressor response to renal artery infusions of noradrenaline (23.0 ± 11.5 mm Hg x min). This response was significantly less than both the control response ($p<0.01$) and that seen after SQ 20881 treatment alone ($p<0.02$).

Following combined renal artery administration of SQ 20881 and phentolamine there was a slight decrease in the systemic pressor response to intravenous infusions of noradrenaline (122.3 ± 44.8 mm Hg x min). This response was significantly less than the control response to intravenous infusions (182.5 ± 55.4 mm Hg x min, $p<0.02$) although it was significantly different from the intravenous pressor response following treatment of the renal circulation with SQ 20881 alone (139.2 ± 53.5 mm Hg x min).

Renal blood flow

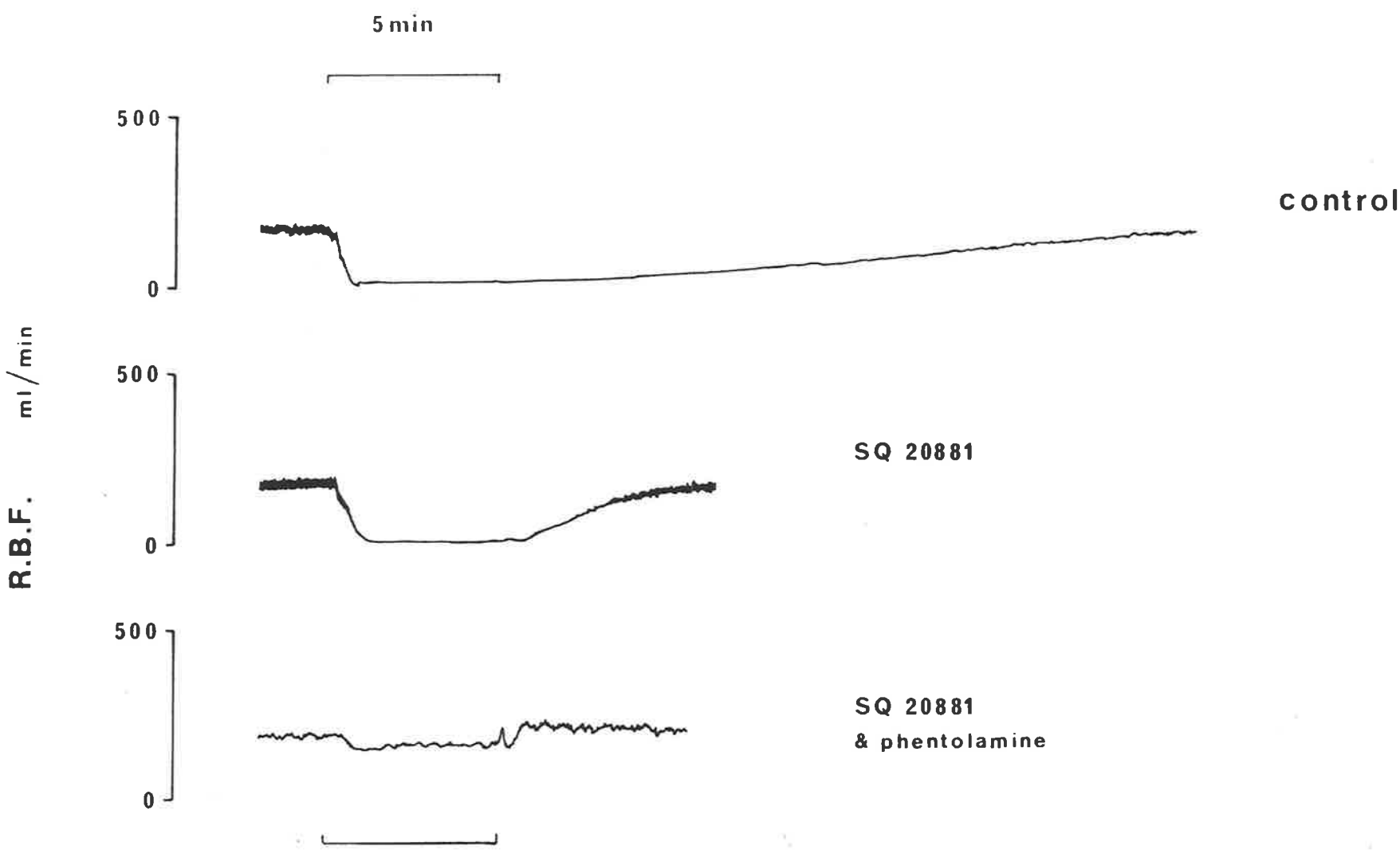
As discussed in Sections 4 and 6, renal artery infusions of noradrenaline, at the dose employed, result in a substantial reduction in renal blood flow, the duration of which greatly exceeds the noradrenaline infusion period. A similar effect has been observed in the present group of dogs. The blood flow responses in a single experiment both before and after renal artery SQ 20881 administration alone and in conjunction with phentolamine appear in Fig. 7.2.

The dose of SQ 20881 chosen was sufficient to substantially attenuate the fall in renal blood flow on renal artery injections of angiotensin I (500 ng; before -82.4 ± 17.0 ml, after -15.6 ± 3.6 ml), indicating an inhibition of converting enzyme activity within the renal circulation. The dose of phentolamine chosen was that shown previously to antagonise α-adrenoceptor activity with the kidney.

Treatment of the renal circulation with SQ 20881 failed to alter the absolute fall in renal blood flow to renal artery infusions of noradrenaline

FIG. 7.2. The reductions in renal blood flow (R.B.F., ml/min) in a single experiment in response to 5 min renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min] before and after treatment of the renal circulation with SQ 20881 alone (200 μg/min [158.3 nmol/min] for 5 min) and in conjunction with phentolamine (100 μg/min [265 nmol/min] for 5 min).

NORADRENALINE



but substantially shortened the duration of the renal blood flow reduction. The overall effect was a significant decrease in the integrated renal blood flow response (before -2018.8 ± 267.0 ml, after -1201.7 ± 153.8 ml, $p<0.01$, Table 7.1). Following the additional treatment of the renal vasculature with phentolamine the absolute fall in renal blood flow was attenuated to the extent that renal artery infusions of noradrenaline resulted in only a minor fall in renal blood flow (-302.0 ± 44.2 ml, n=6, $p<0.001$).

CALCULATED NORADRENALINE INACTIVATION

The degree of noradrenaline inactivation in the renal vascular bed was calculated from the loss of systemic pressor activity of noradrenaline infused via the renal artery when compared to the response on intravenous infusions.

The pooled values of noradrenaline inactivation in the renal circulation for the 6 dogs studied with this protocol are recorded in Table 7.1 and mean results are represented in Fig. 7.3. Following intrarenal coverting enzyme blockade with SQ 20881 the renal inactivation of noradrenaline was significantly increased (before 23.0 ± 5.7%, after 44.4 ± 7.8%, $p<0.02$) despite a slight reduction in the systemic pressor response to intravenous infusions of noradrenaline.

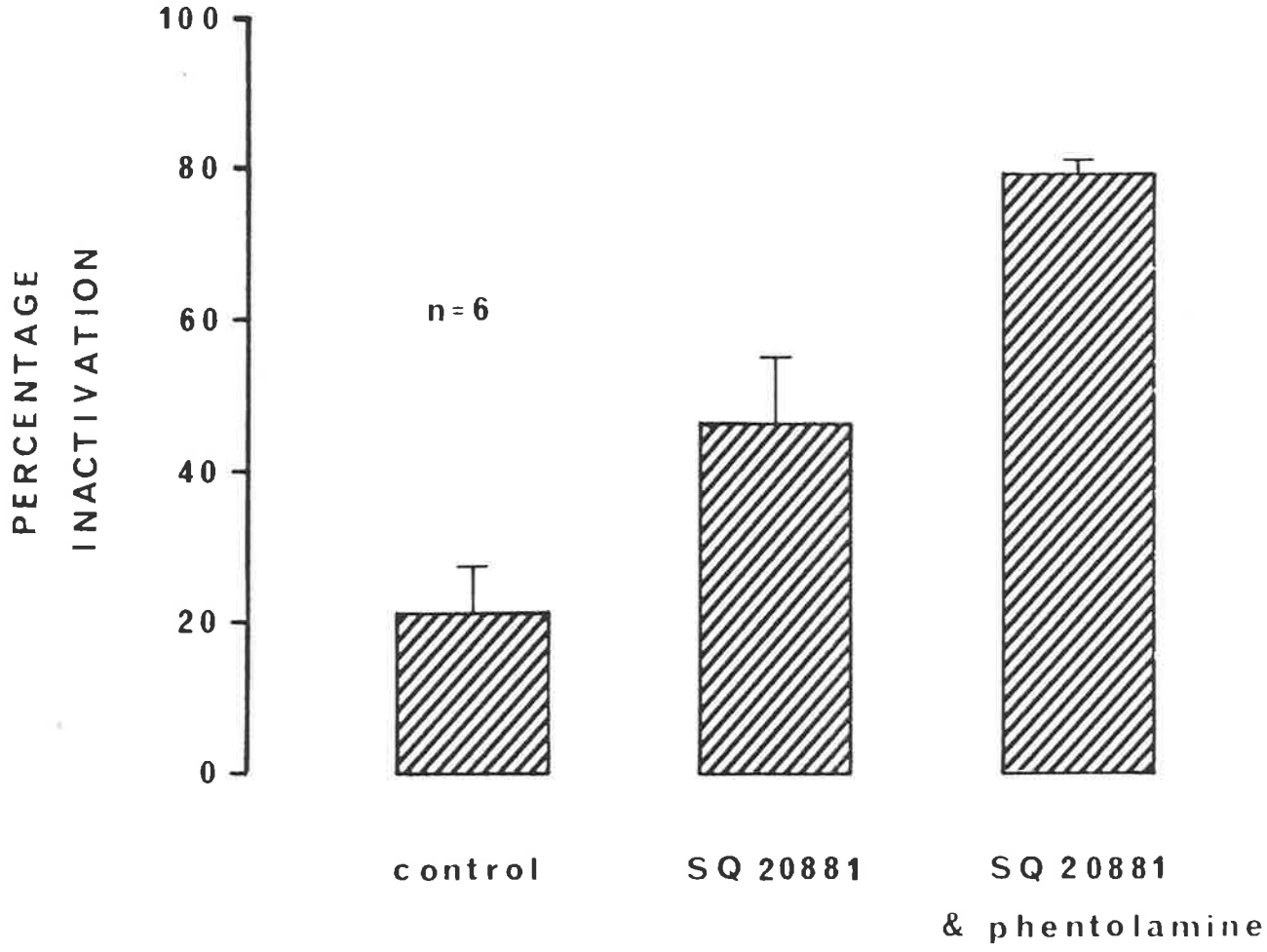
The degree of enhancement in the inactivation of noradrenaline following local inhibition of converting enzyme activity was not significantly different from that described for local propranolol treatment in Section 6.

A further increase in noradrenaline inactivation was observed on combined treatment of the renal vasculature with SQ 20881 and phentolamine (82.3 ± 3.6%, $p<0.02$).

FIG. 7.3. The mean calculated noradrenaline inactivation in the renal circulation for 6 dogs, before and after treatment of the renal circulation with SQ 20881 alone (200 μg/min [158.3 nmol/min] for 5 min) and in conjunction with phentolamine (100 μg/min [265 nmol/min] for 5 min). The height of the columns represent the mean value for 6 dogs and the vertical line, one standard error of the mean.

NORADRENALINE

R.A.



THE EFFECT OF RENAL ARTERY INFUSIONS OF NORADRENALINE ON SYSTEMIC PLASMA RENIN ACTIVITY

The effect of renal artery infusions of noradrenaline on systemic plasma renin activity was investigated by radioimmunoassay of high inferior vena cava blood samples taken before, during and after the infusion period. The results for 4 dogs appear in Table 7.2.

While there was a degree of variation in the resting levels of systemic plasma renin activity between dogs, in each dog studied plasma renin activity was unaltered until the renal artery infusion period had ended. The timing of this increase in plasma renin activity was similar to that of the secondary pressor response associated with renal artery infusions of noradrenaline (Fig. 6.5).

DISCUSSION

The experiments reported in this section demonstrate that treatment of the renal circulation with SQ 20881 results in a significant increase in the renal inactivation of infused noradrenaline. However, the mechanism for this enhanced noradrenaline inactivation is not clearly delineated from these experiments.

The drug, SQ 20881 is known to protect bradykinin from inactivation by kininase II, an enzyme present in high concentrations within the kidney (Ward and Mills, 1975), and elevated intrarenal levels of bradykinin may be responsible for this increased inactivation of noradrenaline. Bradykinin is a renal vasodilator (Stein, Congbalay, Karsh, Osgood and Ferris, 1972) and the effects of SQ 20881 may be renal blood flow dependent, in that bradykinin will inhibit the fall in renal blood flow associated with renal artery infusions of noradrenaline, thereby increasing the renal clearance of noradrenaline.

This possibility is unlikely as the absolute fall in renal blood flow to renal artery infusions of noradrenaline was unaltered by this treatment and as bradykinin is thought not increase the filtration fraction (Stein, Congbalay, Karsh, Osgood and Ferris, 1972), this effect of SQ 20881 is unlikely to be due to an increase in noradrenaline clearance. Furthermore, additional treatment of the renal circulation with phentolamine which does increase noradrenaline inactivation through a blood flow dependent mechanism, resulted in a further significant increase in noradrenaline inactivation. This suggests that the effects of SQ 20881 are likely to be independent of renal haemodynamics.

Interactions between bradykinin and the sympathetic nervous system

remain virtually unknown (Terragno and Terragno, 1979). Elevated intrarenal levels of bradykinin could conceivably enhance the renal inactivation of noradrenaline through a direct effect on the neuronal and extraneuronal metabolizing systems although there is no evidence in the literature to support such a mechanism.

The principal action of SQ 20881 of interest in the present study is its ability to inhibit the enzymatic conversion of angiotensin I to angiotensin II. Renal artery infusions of noradrenaline are known to stimulate the release of renin and the local generation of angiotensin II. Inhibition of angiotensin II formation during noradrenaline infusions may, therefore, in some way increase the rate of inactivation of noradrenaline within the kidney. Such an hypothesis for the action of SQ 20881 would also explain the similarity of its effect on noradrenaline inactivation with that of propranolol (Section 6) which is also known to inhibit angiotensin II formation by blocking renin release (Winer, Chokshi, Yoon and Freedman, 1969; Davis and Freeman, 1976).

The mechanism by which angiotensin II might protect noradrenaline from inactivation in the kidney, is not clear from the present studies. It does not appear to involve an increased renal clearance of noradrenaline as the absolute fall in renal blood flow to renal artery infusions of noradrenaline was unaltered by SQ 20881 treatment. Furthermore, the renal inactivation of infused noradrenaline was further significantly increased by additional treatment of the renal circulation with phentolamine, which is known to increase noradrenaline inactivation through a renal blood flow dependent mechanism. Phentolamine may also be acting by inhibiting the local formation of angiotensin II, by impairing the ability of macula densa to stimulate the release of renin on marked reductions in renal blood flow (Davis and Freeman, 1976).

Angiotensin II is known to stimulate the formation of prostaglandins within the kidney (McGiff, 1977). However, they are unlikely to have a role in the enhanced renal inactivation of noradrenaline, as they are not thought to affect the catecholamine uptake processes and of the prostaglandins studied in the literature, prostaglandin E, in contrast to, angiotensin II, inhibits noradrenergic transmission (Langer, 1976; Westfall, 1977).

Angiotensin II is known to have a number of interactions with the sympathetic nervous system, enhancing noradrenaline neuroeffector transmission (Peach, 1977; Starke, 1977). Angiotensin II increases the sensitivity of vascular smooth muscle to noradrenaline (Thoenen, Hurliman and Haefely, 1965; Panisset and Bourdois, 1968; Day and Moore, 1976). However, the pre-synaptic actions of angiotensin II are thought to be of greater physiological importance as the majority of investigations have demonstrated either that angiotensin II potentiates the effects of sympathetic nerve transmission to a greater extent than those of noradrenaline administration (Benelli, Della Bella and Gandini, 1964; Zimmerman and Gomez, 1965; Zimmerman and Gisslen, 1968; Kadowitz, Sweet and Brody, 1971), or that angiotensin II fails to potentiate the effects of exogenous noradrenaline at all (Hughes and Roth, 1971; Bell, 1972). This is with the exception of a recent report in which angiotensin II had a comparable potentiating action on the vasoconstriction elicited by either nerve stimulation (63%) or exogenous noradrenaline (62%, Jackson and Campbell, 1980).

The commonly accepted pre-synaptic action of angiotensin II is a facilitation of noradrenaline release per nerve impulse (Peach, 1977; Starke, 1977; Westfall, 1977). There is however, considerable difficulty in explaining the present changes in noradrenaline inactivation through this effect of angiotensin II. There may be a complex series of events,

whereby infused noradrenaline stimulates the intrarenal formation of angiotensin II, which in turn, facilitates the tonic release of noradrenaline from the sympathetic nerve terminals, which subsequently competes with the exogenously administered noradrenaline for neuronal uptake sites. However such an explanation seems unlikely.

Angiotensin II is also known to inhibit the neuronal uptake process, which is a major pathway of noradrenaline inactivation (Khairallah, 1972), although there is a considerable controversy over the dose required for this effect. Starke (1971) believes that an inhibition of neuronal uptake only occurs at high concentrations of angiotensin II (10^{-5} M) but recently, Jackson and Campbell (1979) found that subpressor doses of angiotensin II (3×10^{-9} M) inhibited neuronal uptake in isolated mesenteric arteries.

Previous investigators have studied the effects of exogenous angiotensin II administration and consequently in the present study, in which the effects of locally generated angiotensin II have been examined, it is difficult to ascertain the local concentration of angiotensin II and whether it is inhibiting neuronal uptake. While this mechanism would adequately describe the effects observed, further investigations are required to substantiate this hypothesis.

An interaction between SQ 20881 and noradrenaline in the renal circulation has been observed by two other groups. Seliq, Anderson and Korner (1979) found that treatment of the renal vascular bed with SQ 20881 attenuated the systemic pressor response to renal artery infusions of noradrenaline. However, these authors concluded that this effect was due to intrarenally generated angiotensin II contributing to the systemic pressor response to renal artery infusions of noradrenaline. This possibility seems remote

as in the present study an increase in systemic blood pressure associated with the renin-angiotensin system did not occur until several minutes after the conclusion of the noradrenaline pressor response. An impaired renal inactivation of noradrenaline due to elevated intrarenal levels of angiotensin II would seem a more likely explanation for their observations.

Similarly, Bomzon and Rosendorff (1975) noted that treatment of the kidney with SQ 20881 reduced the increase in renal vascular resistance in response to renal artery injections of noradrenaline. They concluded that intrarenally generated angiotensin II directly contributed to this renal vasoconstrictor action of noradrenaline although they failed to discuss the possibility that, in addition, angiotensin II may also be protecting noradrenaline from inactivation.

In conclusion, this study has demonstrated that the intrarenal levels of angiotensin II may substantially modulate the effects of circulating noradrenaline on the renal vasculature and the degree of noradrenaline inactivation which occurs during passage through the renal circulation.

The implications of this powerful interaction are extensive, particularly in those physiological and pathophysiological states where the intrarenal levels of angiotensin II are elevated. Furthermore, converting enzyme inhibitors are finding increasing clinical use in essential hypertension as well as classical renal hypertension. The demonstration that converting enzyme inhibition may modify catecholamine metabolism is an additional aspect which must be considered in understanding the mechanism of their hypotensive action.

SUMMARY

1. The influence of the intrarenal renin-angiotensin system on the kidney's ability to inactivate infused noradrenaline, was studied with the angiotensin I converting enzyme antagonist, SQ 20881.

2. Treatment of the renal circulation with SQ 20881 significantly attenuated the systemic pressor and renal blood flow responses to renal artery infusions of noradrenaline. The renal inactivation of noradrenaline was also significantly increased following local administration of SQ 20881.

3. Combined treatment of the renal circulation with SQ 20881 and phentolamine virtually abolished the systemic pressor response and local vasoconstrictor effects of renal artery infusions of noradrenaline and further significantly increased the degree of noradrenaline inactivation within the kidney from that seen following SQ 20881 treatment alone.

4. The nature and magnitude of these effects of SQ 20881 were virtually identical to those seen with propranolol in Section 6. The effects of SQ 20881 and propranolol are likely to be mediated through a common mechanism, namely, by inhibiting the intrarenal formation of angiotensin II.

5. It is concluded that the intrarenal levels of angiotensin II exert a marked influence on the local vascular effects of circulating noradrenaline as well as the extent to which noradrenaline is inactivated during passage through the kidney.

A P P E N D I X

TABLES

TABLE 3.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min), femoral blood flow (Δ F.B.F., ml) and renal blood flow (Δ R.B.F., ml) expressed as their integrals on 5 min intravenous, femoral artery and renal artery infusions of angiotensin II (500 ng/min, [50′ nmol/min]). The percentage inactivation of angiotensin II in the femoral and renal vascular beds is also shown.

TABLE 3.1

DATE	CIRCULATORY RESPONSE	ROUTE OF ANGIOTENSIN II INFUSION		
		INTRAVENOUS	FEMORAL ARTERY	RENAL ARTERY
9.08.78		216	187	75
17.08.78		210	167	21
9.09.78		190	106	7
23.09.78	Δ M.A.P.	217	160	39
30.09.77		113	124	14
21.10.77	mm Hg x min	161	113	18
26.10.77		257	132	23
4.11.77		134	74	38
9.11.77		98	68	19
17.11.77		102	144	32
18.11.77		362	283	24
25.11.77		148	82	51
	mean ± s.e.m.	184.0 ± 21.9	136.7 ± 17.1	30.1 ± 5.4
21.10.77		274	-115	-
26.10.77		44	-180	-
4.11.77	Δ F.B.F.	125	-540	28
9.11.77		112	-432	36
17.11.77	ml	274	-302	0
18.11.77		296	- 78	17
25.11.77		482	-181	77
	mean ± s.e.m.	243.7 ± 39.5	-261.1 ± 64.8	22.6 ± 10.6
9.08.78		-472	-280	-714
17.08.78		-288	-264	-804
9.09.78		-104	-304	-464
23.09.78	Δ R.B.F.	-244	-200	-500
30.09.77		-203	-218	-664
21.10.77	ml	-366	-318	-894
26.10.77		-284	-	-620
4.11.77		-224	-132	-560
9.11.77		- 44	- 80	-788
17.11.77		-212	-144	-420
18.11.77		-836	-484	-652
25.11.77		-160	-164	-680
	mean ± s.e.m.	-286.4 ± 59.6	-235.3 ± 33.8	-651.7 ± 39.8
9.08.78			13.4	65.3
17.08.78			20.5	90.0
9.09.78			44.2	96.3
23.09.78	Percentage		26.3	82.0
30.09.77			0	87.6
21.10.77	Inactivation		30.2	88.8
26.10.77			48.6	91.4
4.11.77	%		45.1	72.3
9.11.77			31.0	81.3
17.11.77			0	69.5
18.11.77			21.9	93.4
25.11.77			44.7	66.1
	mean ± s.e.m.		27.2 ± 4.9	82.0 ± 3.2

TABLE 3.2. The changes in femoral vascular resistance (Δ F.V.R., %) and renal vascular resistance (Δ R.V.R., %) expressed as a percentage of the pre-infusion value, on 5 min intravenous, femoral artery and renal artery infusions of angiotensin II (500 ng/min [0.5 nmol/min]).

DATE	CIRCULATORY RESPONSE	ROUTE OF ANGIOTENSIN II INFUSION		
		INTRAVENOUS	FEMORAL ARTERY	RENAL ARTERY
21.10.77		-12.0	41.6	3.0
26.10.77		20.6	94.4	6.4
4.11.77	Δ F.V.R.	- 5.1	164.7	-1.2
9.11.77		- 9.2	65.4	2.1
17.11.77	%	- 8.8	62.4	2.7
18.11.77		3.5	86.5	0.3
25.11.77		0.9	37.8	-2.4
	mean ± s.e.m.	-1.4 ± 4.2	79.0 ± 16.3	0.1 ± 2.3
21.10.77		47.9	34.3	72.3
26.10.77		82.4	12.1	175.4
4.11.77	Δ R.V.R.	32.0	11.5	51.3
9.11.77		6.9	8.1	79.9
17.11.77	%	39.5	15.0	64.2
18.11.77		102.3	82.6	123.8
25.11.77		26.6	11.0	103.7
	mean ± s.e.m.	48.2 ± 12.5	24.9 ± 10.2	95.8 ± 16.1

TABLE 3.3. The abdominal aortic, renal vein and femoral vein levels of angiotensin II as determined by radioimmunoassay, measured at 5 min intervals, before and after a 15 min intravenous infusion of angiotensin II at (500 ng/min [0.5 nmol/min]). The percentage inactivation of infused angiotensin II in the renal and femoral vascular beds as determined by the arterio-venous differences are also shown.

DATE	ANGIOTENSIN II pg/ml	SAMPLE						
		CONTROL		ANGIOTENSIN II 500 ng/min I.V.			CONTROL	
		1	2	3	4	5	6	7
DOG 1	Aortic	72.8	66.6	272.5	292.5	457.8	82.4	81.2
	Renal Vein	154.9	100.8	183.2	114.2	146.9	43.8	263.3
	Renal $\frac{A-V}{A}$ %	-	-	32.7	61.0	67.9	46.8	-
11/12/78	Femoral Vein	144.3	96.3	158.6	176.7	247.7	59.4	100.5
	Femoral $\frac{A-V}{A}$ %	-	-	41.7	39.6	45.9	27.9	-
DOG 2	Aortic	77.4	51.2	431.4	479.2	615.2	56.1	11.8
	Renal Vein	85.0	17.6	82.2	128.2	152.6	21.8	76.9
	Renal $\frac{A-V}{A}$ %	-	65.6	80.9	73.2	75.2	61.1	-
19/12/78	Femoral Vein	70.3	80.3	77.0	69.9	69.6	59.7	65.6
	Femoral $\frac{A-V}{A}$ %	78.5	54.6	53.3	66.1	57.6	30.3	-
DOG 3	Aortic	98.0	92.8	266.6	289.3	191.3	77.0	51.5
	Renal Vein	73.5	73.1	76.3	162.8	145.9	83.7	135.0
	Renal $\frac{A-V}{A}$ %	25.0	21.2	71.4	43.7	23.7	-	-
8/1/79	Femoral Vein	70.2	70.5	182.6	123.0	125.9	50.9	57.4
	Femoral $\frac{A-V}{A}$ %	28.4	24.0	31.5	57.5	34.2	33.9	-
DOG 4	Aortic	239.0	-	609.3	673.5	651.1	-	216.1
	Renal Vein	245.9	158.1	440.7	313.9	303.7	179.8	145.3
	Renal $\frac{A-V}{A}$ %	-	-	27.7	53.4	53.3	-	-
9/1/79	Femoral Vein	208.9	219.9	345.3	258.5	710.1	702.9	371.0
	Femoral $\frac{A-V}{A}$ %	12.6	-	43.3	61.6	-	-	-
DOG 5	Aortic	60.9	53.9	251.7	327.6	341.8	49.6	35.3
	Renal Vein	39.6	33.4	126.3	166.0	129.2	24.7	40.4
	Renal $\frac{A-V}{A}$ %	35.0	38.0	49.8	49.3	62.2	50.2	-
24/1/79	Femoral Vein	28.8	20.7	105.0	142.8	112.4	0	25.0
	Femoral $\frac{A-V}{A}$ %	52.7	61.6	58.3	56.4	67.1	100	29.2
mean ± s.e.m.	Aortic	109.4 ±36.8	66.1 ±11.0	366.4 ±77.1	412.4 ±82.7	451.4 ±95.7	66.3 ± 9.2	79.2 ±40.3
	Renal Vein	119.8 ±41.0	76.6 ±28.0	181.7 ±75.5	177.0 ±39.8	375.7 ±220.0	70.7 ±32.9	132.2 ±42.5
	Renal $\frac{A-V}{A}$ %			52.5 ±11.7	56.1 ± 5.7	56.5 ±10.0		
	Femoral Vein	104.3 ±35.7	97.5 ±37.0	173.7 ±52.3	154.2 ±35.0	253.1 ±131.9	174.6 ±148.2	123.9 ±70.3
	Femoral $\frac{A-V}{A}$ %			45.6 ± 5.2	56.2 ± 5.0	41.0 ±13.0		

TABLE 3.4. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) in response to 5 min intravenous (I.V.) and renal artery (R.A.) infusions of angiotensin II at the doses indicated. The percentage inactivation of angiotensin II in the renal vascular bed, for each dose of infused angiotensin II are also shown.

TABLE 3.4

DATE	CIRCULATORY RESPONSE	DOSE OF INFUSED ANGIOTENSIN II (nmol/min)					
		0.2		0.5		0.7	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
23.10.78		102	35	142	44	165	62
24.10.78		128	39	167	45	254	97
30.10.78	Δ M.A.P.	287	26	478	95	526	82
31.10.78		163	41	432	78	534	118
6.11.78	mm Hg x min	112	41	178	63	213	73
7.11.78		109	0	179	30	221	49
14.11.78		105	0	176	5	251	9
	mean ± s.e.m.	143.7 ± 25.2	26.0 ± 7.0	250.3 ± 53.3	51.4 ± 11.4	309.1 ± 58.1	70.0 ± 13.3
23.10.78		-250	- 496	- 451	- 730	-406	-784
24.10.78		- 80	- 291	- 35	- 320	-112	-413
30.10.78	Δ R.B.F.	-701	-1040	-1091	-1112	-976	-856
31.10.78		-224	- 448	- 260	- 644	-280	-532
6.11.78	ml	- 96	- 570	- 154	- 656	-259	-548
7.11.78		-131	- 547	- 272	- 634	-253	-560
14.11.78		- 74	- 339	- 134	- 416	-237	-474
	mean ± s.e.m.	-222.3 ± 84.0	-533.0 ± 93.1	-342.4 ± 134.3	-644.6 ± 95.6	-360.4 ± 107.6	-600.4 ± 61.1
23.10.78		65.7		69.0		63.6	
24.10.78	Percentage	69.5		73.0		61.8	
30.10.78		90.9		80.1		84.4	
31.10.78	Inactivation	74.8		81.9		77.9	
6.11.78	%	63.4		64.6		65.7	
7.11.78		100.0		83.2		77.8	
14.11.78		100.0		97.2		96.4	
	mean ± s.e.m.	80.6 ± 6.0		78.4 ± 4.1		75.4 ± 4.8	

TABLE 3.5. The mean arterial pressure (Δ M.A.P., mm Hg x min) and superior mesenteric blood flow (Δ S.M.B.F., ml) responses to 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]). The percentage inactivation of angiotensin II in the splanchnic and hepato-portal circulations are also shown.

DATE	CIRCULATORY RESPONSE	ROUTE OF ADMINISTRATION		
		I.V.	S.M.A.	H.P.V.
14.2.79		191	35	34
16.2.79		283	46	23
22.2.79	Δ M.A.P.	148	36	21
26.2.79		236	30	39
6.3.79	mm Hg x min	148	19	13
16.3.79		104	24	23
23.3.79		177	50	29
	mean ± s.e.m.	183.9 ± 22.7	34.3 ± 4.2	26.0 ± 3.3
14.2.79		-112	-1302	- 80
16.2.79		-708	-1380	-102
22.2.79	Δ S.M.B.F.	-218	- 544	- 54
26.2.79		-190	- 745	- 65
6.3.79	ml	-453	- 878	- 24
16.3.79		0	- 610	- 93
23.3.79		-	-	-
	mean ± s.e.m.	-280.2 ± 105.1	-909.8 ± 144.5	-69.7 ± 11.6
14.2.79			81.7	82.2
16.2.79			83.7	91.9
22.2.79	Percentage		75.7	85.8
26.2.79			87.3	84.3
6.3.79	Inactivation		87.2	91.2
16.3.79			76.9	77.9
23.3.79			71.7	83.6
	mean ± s.e.m.		80.6 ± 2.3	85.3 ± 1.9

TABLE 4.1.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min), femoral blood flow (Δ F.B.F., ml) and renal blood flow (Δ R.B.F., ml) expressed as their integrals, on 5 min intravenous, femoral artery and renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min]). The percentage inactivation of infused noradrenaline in the femoral and renal vascular beds is also shown.

DATE	CIRCULATORY RESPONSE	ROUTE OF NORADRENALINE INFUSION		
		INTRAVENOUS	FEMORAL ARTERY	RENAL ARTERY
21.10.77		102	0	68
26.10.77		86	0	78
4.11.77	Δ M.A.P.	78	0	39
9.11.77		49	0	68
17.11.77	mm Hg x min	73	0	53
18.11.77		652	43	393
25.11.77		86	0	41
	mean ± s.e.m.	160.8 ± 82.1	6.1 ± 6.1	105.7 ± 48.2
21.10.77		-245	- 864	- 850
26.10.77		- 90	- 108	- 46
4.11.77	Δ F.B.F.	- 89	- 814	- 36
9.11.77		-170	-2448	- 61
17.11.77	ml	-108	- 986	- 50
18.11.77		0	- 770	- 124
25.11.77		-128	-1411	- 102
	mean ± s.e.m.	- 67.7 ± 49.4	-1057.3 ± 273.6	- 181.3 ± 112.1
21.10.77		0	- 66	- 457
26.10.77		-148	0	-1180
4.11.77	Δ R.B.F.	-144	0	- 940
9.11.77		-120	0	-2472
17.11.77	ml	-136	0	-1484
18.11.77		-180	0	- 528
25.11.77		-264	0	-2568
	mean ± s.e.m.	-160.7 ± 21.7	- 9.4 ± 9.4	-1375.6 ± 324.6
21.10.77			100.0	33.8
26.10.77	Percentage		100.0	9.7
4.11.77			100.0	50.1
9.11.77	Inactivation		100.0	0
17.11.77			100.0	28.1
18.11.77	%		93.2	39.5
25.11.77			100.0	41.1
	mean ± s.e.m.		99.0 + 1.0	28.9 ± 6.8

TABLE 4.1.2. The changes in femoral vascular resistance (Δ F.V.R., %) and renal vascular resistance (Δ R.V.R., %) expressed as a percentage of the pre-infusion value, on 5 min intravenous, femoral artery, and renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min]).

DATE	CIRCULATORY RESPONSE	ROUTE OF NORADRENALINE INFUSION		
		INTRAVENOUS	FEMORAL ARTERY	RENAL ARTERY
21.10.77		114.4	156.2	69.4
26.10.77		54.6	40.9	36.8
4.11.77	Δ F.V.R.	69.3	510.8	25.7
9.11.77		37.9	200.1	19.7
17.11.77	%	37.6	200.7	24.2
18.11.77		120.0	704.4	102.0
25.11.77		33.3	416.5	17.9
	mean ± s.e.m.	66.7 ± 13.8	318.5 ± 80.2	42.2 ± 12.0
21.10.77		14.1	3.1	86.2
26.10.77		23.2	-9.2	161.5
4.11.77	Δ R.V.R.	24.4	0.6	214.1
9.11.77		15.5	2.8	591.4
17.11.77	%	23.7	-0.5	197.4
18.11.77		131.0	-3.0	177.6
25.11.77		39.1	-0.5	560.3
	mean ± s.e.m.	38.7 ± 15.7	- 0.8 ± 1.6	284.1 ± 76.9

TABLE 4.1.3. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) in response to 5 min intravenous (I.V.) and renal artery (R.A.) infusions of noradrenaline at the doses indicated. The percentage inactivation of noradrenaline in the renal vascular bed is shown for each dose.

TABLE 4.1.3.

DATE	CIRCULATORY RESPONSE	DOSE OF INFUSED NORADRENALINE (nmol/min)					
		14.7		29.5		59.1	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
23.10.78		33	5	37	38	81	70
24.10.78		63	6	57	21	83	70
30.10.78	Δ M.A.P.	44	25	104	61	167	137
31.10.78		81	3	120	77	376	137
6.11.78	mm Hg x min	29	7	47	14	109	97
7.11.78		52	17	85	24	278	230
14.11.78		33	15	81	64	197	133
	mean ± s.e.m.	46.9 ± 7.2	11.1 ± 3.0	75.9 ± 11.5	42.7 ± 9.3	184.4 ± 41.6	124.1 ± 20.8
23.10.78		-107	- 496	-197	-1488	-216	-1640
24.10.78		- 61	- 384	- 90	- 794	-105	-1016
30.10.78	Δ R.B.F.	-150	-1920	-314	-2448	-403	-2152
31.10.78		0	- 189	- 84	-1584	-148	-1792
6.11.78	ml	-102	- 296	-163	- 784	-253	-1576
7.11.78		- 96	- 608	-178	- 760	-307	-1005
14.11.78		- 55	- 426	- 54	- 541	- 61	- 496
	mean ± s.e.m.	-81.6 ± 18.1	-617.0 ± 223.0	-154.3 ± 33.5	-1200.0 ± 256.0	-213.3 ± 45.1	-1382.1 ± 214.1
23.10.78		84.8		0		13.6	
24.10.78	Percentage	90.5		63.1		15.7	
30.10.78		43.2		41.3		21.0	
31.10.78	Inactivation	96.3		35.8		63.6	
6.11.78		75.8		70.2		11.0	
7.11.78	%	67.3		71.8		17.3	
14.11.78		54.5		21.0		32.5	
	mean ± s.e.m.	73.2 ± 7.3		43.2 ± 10.2		21.1 ± 3.7	

TABLE 4.1.4. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and superior mesenteric blood flow (Δ S.M.B.F., ml) expressed as the integrals, in response to 5 min intravenous, superior mesenteric artery and hepato-portal vein infusions of noradrenaline (10.0 μg/min [59.1 nmol/min]). The percentage inactivation of noradrenaline in the splanchnic and hepato-portal circulations is also shown.

TABLE 4.1.4.

DATE	CIRCULATORY RESPONSE	ROUTE OF NORADRENALINE INFUSION		
		INTRAVENOUS	SUPERIOR MESENTERIC ARTERY	HEPATO-PORATL VEIN
8.6.79	Δ M.A.P. mm Hg x min	182	69	78
19.6.79		76	28	58
20.6.79		250	44	70
27.6.79		152	42	56
6.7.79		177	18	18
9.7.79		107	32	23
	mean ± s.e.m.	157.3 ± 25.0	38.8 ± 7.2	52.1 ± 9.2
8.6.79	Δ S.M.B.F. ml	552	- 720	150
19.6.79		-917	-1368	-345
20.6.79		-351	- 804	390
27.6.79		0	- 564	-472
6.7.79		-244	- 685	424
9.7.79		-340	- 717	-200
	mean ± s.e.m.	-216.7 ± 196.8	-809.7 ± 116.1	-97.5 ± 95.1
8.6.79	Percentage Inactivation %		62.1	57.1
19.6.79			63.2	23.7
20.6.79			82.4	72.0
27.6.79			72.4	63.2
6.7.79			89.8	89.8
9.7.79			70.1	69.1
	mean ± s.e.m.		73.3 ± 4.4	62.5 ± 9.0

TABLE 4.2.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min), femoral blood flow (Δ F.B.F., ml) and renal blood flow (Δ R.B.F., ml) expressed as their integrals, on 5 min intravenous, femoral artery and renal artery infusions of adrenaline (10 μg/min [54.6 nmol/min]). The percentage inactivation of adrenaline in the femoral and renal vascular beds is also shown.

DATE	CIRCULATORY RESPONSE	ROUTE OF ADRENALINE INFUSION		
		INTRAVENOUS	FEMORAL ARTERY	RENAL ARTERY
11.9.79	Δ M.A.P. mm Hg x min	168	16	162
14.9.79		188	8	92
18.9.79		178	6	116
19.9.79		41	4	36
21.9.79		173	8	96
	mean ± s.e.m.	149.6 ± 27.3	8.4 ± 2.0	100.4 ± 20.3
11.9.79	Δ F.B.F. ml	-348	- 996	- 188
14.9.79		0	-1163	-
18.9.79		-828	-1080	-1256
19.9.79		-119	-1200	0
21.9.79		-	-	-
	mean ± s.e.m.	-323.7 ± 182.9	-1109.7 ± 45.4	
11.9.79	Δ R.B.F. ml	-172	0	-1496
14.9.79		-200	0	- 552
18.9.79		-237	0	-2196
19.9.79		0	0	-1848
21.9.79		-269	0	-2832
	mean ± s.e.m.	-175.6 ± 46.9	0	-1784.8 ± 379.0
11.9.79	Percentage Inactivation %		90.0	3.6
14.9.79			95.7	51.1
18.9.79			96.6	34.8
19.9.79			90.2	12.2
21.9.79			95.4	44.5
	mean ± s.e.m.		93.7 ± 1.4	29.2 ± 9.2

TABLE 4.3.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and superior mesenteric blood flow (Δ S.M.B.F., ml) expressed as their integral, on 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of isoprenaline (1.0 μg/min [4.7 nmol/min]). The percentage inactivation of isoprenaline in the splanchnic and hepato-portal circulations is also shown.

DATE	CIRCULATORY RESPONSE	ROUTE OF ISOPRENALINE INFUSION		
		I.V.	S.M.A.	H.P.V.
8.6.79	Δ M.A.P.	- 53	-19	-15
19.6.79		-103	-19	-15
20.6.79	mm Hg x min	-161	-42	-76
27.6.79		- 63	- 9	-20
6.7.79		- 52	-20	-28
	mean ± s.e.m.	-86.4 ± 20.8	-21.8 ± 5.4	-30.8 ± 11.5
8.6.79		116	962	62
19.6.79	Δ S.M.B.F.	306	2028	0
20.6.79		-204	1120	-96
27.6.79	ml	96	1568	0
6.7.79		0	712	0
	mean ± s.e.m.	52.8 ± 76.1	1278.0 ± 233.6	- 6.8 ± 25.3
8.6.79	Percentage		64.1	71.7
19.6.79			81.5	85.4
20.6.79	Inactivation		73.9	52.8
27.6.79			85.7	68.2
6.7.79	%		61.5	46.1
	mean ± s.e.m.		73.3 ± 4.7	64.8 ± 7.0

TABLE 5.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min), renal blood flow (Δ R.B.F., ml) and calculated percentage increase in renal vascular resistance (Δ R.V.R., %) during renal artery infusions of angiotensin II (0.5 nmol/min), and the changes in mean arterial pressure (Δ M.A.P., mm Hg x min), femoral blood flow (Δ F.B.F., ml) and calculated percentage increase in femoral vascular resistance (Δ F.V.R., %) in 8 dogs, during femoral artery infusions of angiotensin II. The responses tabulated are those obtained before and after renal artery administration of Sar^{1}-Ile^{8}-AII (3.3 μg/min [3.5 nmol/min] for 5 min) with the changes in mean arterial pressure and blood flow being expressed as the integrals of the areas contained by the responses.

TABLE 5.1.

DATE	CIRCULATORY RESPONSE	RENAL ARTERY AII		CIRCULATORY RESPONSE	FEMORAL ARTERY AII	
		Control	After R.A. Sar^1-Ile^8-AII		Control	After R.A. Sar^1-Ile^8-AII
26.1.78		53	103		168	172
1.2.78		3	111		65	158
2.2.78		37	92		149	81
9.2.78	Δ M.A.P.	10	66	Δ M.A.P.	90	167
10.2.78		28	55		77	52
10.3.78	mm Hg x min	20	31	mm Hg x min	154	124
13.3.78		11	112		152	105
6.4.78		25	57		133	150
	mean ± s.e.m.	23.4 ± 5.7	78.4 ± 10.7		123.5 ± 14.1	126.1 ± 15.4
26.1.78		-592	-116		- 44	- 38
1.2.78		-252	-192		- 61	- 66
2.2.78		-732	-244		-163	- 97
9.2.78	Δ R.B.F.	-912	-196	Δ F.B.F.	-251	- 91
10.2.78		-436	- 88		-320	-285
10.3.78	ml	-684	-160	ml	-333	-334
13.3.78		-480	-252		- 94	-135
6.4.78		-304	- 64		-134	-255
	mean ± s.e.m.	-549.0 ± 79.2	-164.0 ± 24.7		-175.0 ± 40.1	-162.6 ± 24.7
26.1.78		128.7	27.1		43.2	23.2
1.2.78		229.7	18.7		17.0	0.6
2.2.78		172.2	59.1		158.5	29.6
9.2.78	Δ R.V.R.	210.0	32.4	Δ F.V.R.	64.6	42.5
10.2.78		53.2	17.3		32.5	238.3
10.3.78	%	127.1	16.7	%	113.9	126.7
13.3.78		27.9	30.5		28.9	40.0
6.4.78		30.2	7.0		56.9	58.8
	mean ± s.e.m.	74.2 ± 20.8	28.6 ± 7.6		73.0 ± 17.5	53.0 ± 19.4

TABLE 5.2. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) to 5 min intravenous (I.V.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]). The responses tablated are those before and after renal artery administration of Sar^1-Ile^8-angiotensin II (3.3 μg/min [3.5 nmol/min] for 5 min) alone and in conjunction with a renal artery constriction.

TABLE 5.2.

DATE	CIRCULATORY RESPONSE	ANGIOTENSIN II					
		Control		After R.A. Sar^1-Ile^8-AII		R.A. Sar^1-Ile^8-AII and R.A. Constriction	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
14.6.78		197	29	178	87	167	56
17.6.78		183	4	104	64	151	2
19.6.78	Δ M.A.P.	123	55	135	74	119	88
5.7.78		116	23	175	38	157	39
9.7.78	mm Hg x min	212	30	250	97	247	126
10.7.78		132	28	99	60	84	79
15.8.78		99	30	97	60	120	66
	mean ± s.e.m.	151.7 ± 16.8	28.4 ± 5.6	156.9 ± 20.1	68.6 ± 7.4	149.3 ± 19.5	65.1 ± 14.8
14.6.78		-912	-1648	-560	-396	-532	-1464
17.6.78		-188	- 604	- 96	- 80	-156	- 452
19.6.78	Δ R.B.F.	-404	- 944	-468	-280	-304	- 856
5.7.78		-108	- 356	-224	-192	-148	- 324
9.7.78	ml	-533	-1048	-508	-271	-468	- 792
10.7.78		-308	- 880	-260	-296	-224	-1000
15.8.78		-106	- 378	0	- 60	0	- 472
	mean ± s.e.m.	-356.6 ± 108.7	-836.9 ± 169.7	-302.3 ± 81.4	-225.0 ± 46.0	-261.7 ± 70.9	-756.7 ± 148.9
14.6.78		85.3		51.1		66.5	
17.6.78	Percentage	97.8		60.9		98.7	
19.6.78		55.3		45.2		26.0	
5.7.78	Inactivation	80.2		78.3		75.1	
9.7.78		85.1		61.2		49.0	
10.7.78	%	78.8		39.4		5.9	
15.8.78		69.7		37.4		45.0	
	mean ± s.e.m.	79.2 ± 5.2		53.5 ± 5.5		52.3 ± 11.7	

TABLE 5.3. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) in response to 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]) and the calculated percentage inactivation of angiotensin II in the femoarl and renal vascular beds. All responses tablated, were recorded before and after femoral artery infusion of Sar^1-Ile^8-angiotensin II (2.5 μg/min [2.7 nmol/min]).

DATE	CIRCULATORY RESPONSE	ANGIOTENSIN II					
		CONTROL			AFTER FEMORAL SAR^1-ILE^8-AII		
		I.V.	F.A.	R.A.	I.V.	F.A.	R.A.
29.5.78	Δ M.A.P. mm Hg x min	247	140	36	212	151	58
1.6.78		181	122	56	218	100	63
9.6.78		363	303	45	340	192	110
10.6.78		178	142	71	183	117	58
12.6.78		154	115	60	117	95	61
	mean ± s.e.m.	224.6 ±37.9	164.4 ±35.0	53.5 ±6.0	214.0 ±36.2	131.0 ±18.1	65.5 ±4.5
29.5.78	Percentage Inactivation %		43.3	85.4		28.8	72.6
1.6.78			32.5	80.1		54.1	71.1
9.6.78			16.5	87.6		43.5	67.6
10.6.78			20.2	60.1		36.1	68.3
12.6.78			25.3	61.0		18.8	47.9
	mean ± s.e.m.		27.6 ±4.8	74.8 ±6.0		36.6 ±6.0	65.5 ±4.5

TABLE 5.4. The changes in femoral blood flow (Δ F.B.F., ml) and femoral vascular resistance (Δ F.V.R., %) to femoral artery infusions of angiotensin II (AII 500 ng/min [0.5 nmol/min]) and the changes in renal blood flow (Δ R.B.F., ml) and renal vascular resistance (Δ R.V.R., %) to renal artery infusions of angiotensin II at the same dose. All tablated responses were recorded before and after femoral artery administration of Sar^1-Ile^8-angiotensin II (2.5 μg/min [2.7 nmol/min] for 5 min).

DATE	CIRCULATORY RESPONSE	FEMORAL ARTERY AII		CIRCULATORY RESPONSE	RENAL ARTERY AII	
		Control	After femoral Sar^1-Ile^8-AII		Control	After femoral Sar^1-Ile^8-AII
29.5.78	Δ F.B.F. ml	-496	75	Δ R.B.F. ml	-	-564
1.6.78		-187	66		-640	-488
9.6.78		-168	25		-908	-548
10.6.78		-312	0		-692	-600
12.6.78		-346	106		-904	-820
	mean ± s.e.m.	-381.8 ± 59.5	36.3 ± 6.0	mean ± s.e.m.	-786.0 ± 70.1	-604.0 ± 50.9
29.5.78	Δ F.V.R. %	120.4	- 7.4	Δ R.V.R. %	114.9	85.3
1.6.78		92.6	28.5		80.9	80.2
9.6.78		195.8	19.0		210.3	88.4
10.6.78		64.7	15.9		86.4	58.5
12.6.78		146.6	-11.5		50.1	76.2
	mean ± s.e.m.	124.0 ± 22.6	8.9 ± 7.8	mean ± s.e.m.	108.5 ± 27.4	79.5 ± 4.6

TABLE 5.5. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and superior mesenteric blood flow (Δ S.M.B.F., ml) on 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of angiotensin II (500 ng/min [0.5 nmol/min]). The responses tablated were recorded before and after 5 min superior mesenteric artery and hepato-portal vein infusions of Sar^1-Ile^8-angiotensin II (5.0 μg/min [5.3 nmol/min]).

TABLE 5.5.

DATE	CIRCULATORY RESPONSE	ANGIOTENSIN II								
		Control			After S.M.A. Sar^1-Ile^8-AII			After H.P.V. Sar^1-Ile^8-AII		
		I.V.	S.M.A.	H.P.V.	I.V.	S.M.A.	H.P.V.	I.V.	S.M.A.	H.P.V.
14.2.79		191	35	34	177	31	38	137	30	67
16.2.79		283	46	23	238	30	19	217	30	13
22.2.79	Δ M.A.P.	148	36	21	118	29	24	74	34	47
26.2.79		236	30	39	231	51	47	241	38	172
6.3.79	mm Hg x min	148	19	13	139	21	30	134	24	73
16.3.79		104	24	23	112	27	19	129	22	53
23.3.79		177	50	29	187	23	24	181	30	115
	mean ± s.e.m.	183.9 ±22.7	34.4 ±4.2	26.0 ±3.3	171.7 ±19.3	30.3 ±3.7	28.7 ±3.9	159.0 ±21.7	29.7 ±4.3	77.1 ±19.6
14.2.79		-1112	-1302	- 80	-384	-376	-168	-220	-618	-126
16.2.79		- 708	-1380	-102	924	348	0	0	-780	0
22.2.79	Δ S.M.B.F.	- 218	- 544	- 54	182	0	0	48	-208	0
26.2.79		- 190	- 745	- 65	235	-156	-125	154	-456	108
6.3.79	ml	- 453	- 878	- 24	432	-117	0	78	-571	0
16.3.79		0	- 610	- 93	171	-148	0	-	-	0
23.3.79		-	-	-	-	-	-	-451	-804	-178
	mean ± s.e.m.	-280.2 ±105.1	-909.8 ±144.5	-69.9 ±11.9	260.0 ±173.2	-74.8 ±98.1	-48.8 ±31.4	-98.5 ±88.4	-582.8 ± 95.9	-28.0 ±35.8
14.2.79			81.7	82.2		82.5	78.5		78.1	51.1
16.2.79	Percentage		83.7	91.9		87.4	92.0		86.2	94.0
22.2.79			75.7	85.8		75.4	79.7		54.0	36.5
26.2.79	Inactivation		87.3	84.3		77.9	79.6		84.2	28.6
6.3.79			87.2	91.2		84.9	78.4		82.1	45.5
16.3.79	%		76.9	77.9		75.9	83.0		82.1	58.9
23.3.79			71.7	83.6		87.7	87.2		83.4	36.5
	mean ± s.e.m.		80.6 ±2.3	85.3 ±1.9		81.7 ±2.0	82.6 ±2.0		78.7 ±4.2	50.1 ±8.2

TABLE 6.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) on 5 min intravenous, femoral artery and renal artery infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after femoral artery and renal artery infusions of phenoxybenzamine (P.B.Z. 100 μg/min, [293.8 nmol/min]). The calculated percentage inactivation of noradrenaline in the femoral and renal vascular beds is also shown.

DATE	CIRCULATORY RESPONSE	NORADRENALINE								
		CONTROL			FEMORAL P.B.Z.			RENAL P.B.Z.		
		I.V.	F.A.	R.A.	I.V.	F.A.	R.A.	I.V.	F.A.	R.A.
31.7.78	Δ M.A.P.	122	17	81	85	66	51	69	63	60
3.8.78		228	12	183	85	80		113		59
7.8.78		60	0	48	46	44	44	51	30	10
10.8.78	mm Hg x min	106	18	104	81	80	80	72	88	43
14.8.78		96	0	104	75	57	89	67	57	34
15.8.78		155	9	101	109	100	78	61	51	24
	mean ± s.e.m.	127.8 ±23.7	11.0 ±2.6	103.5 ±18.2	80.2 ±8.3	71.2 ±8.1	68.6 ±8.9	72.2 ±8.7	57.8 ±9.4	38.3 ±8.1
31.7.78	Percentage		86	34		22	40		8.7	13
3.8.78			95	20		6				48
7.8.78			100	20		4	4		41.2	80
10.8.78	Inactivation		83	2		1	0		0	40
14.8.78			90	0		24	0		14.9	49
15.8.78	%		94	28		8	28		16.4	61
	mean ± s.e.m.		91.3 ±2.5	17.3 ±5.6		10.8 ±4.0	14.4 ±8.3		16.2 ±6.9	48.5 ±9.1

TABLE 6.2. The changes in renal blood flow (Δ R.B.F., ml) and femoral blood flow (Δ F.B.F., ml) on 5 min direct infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after femoral artery and renal artery infusions of phenoxybenzamine (P.B.Z. 100 μg/min [293.8 nmol/min]).

DATE	CIRCULATORY RESPONSE	RENAL ARTERY NORADRENALINE			CIRCULATORY RESPONSE	FEMORAL ARTERY NORADRENALINE		
		CONTROL	FEMORAL P.B.Z.	RENAL P.B.Z.		CONTROL	FEMORAL P.B.Z.	RENAL P.B.Z.
31.7.78	Δ R.B.F.	-1620	-1524	-224	Δ F.B.F.	-1150	-252	- 40
3.7.78		-2308				- 724		
7.8.78		- 812	- 776	0		- 608	-241	-375
8.8.78	ml	-1200	-1488	-130	ml	- 840	-124	-256
14.8.78		-1400	-1288	-170		-1130	-616	-544
15.8.78		- 787	- 710	- 86		-1522	-544	-497
	mean ± s.e.m.	-1354.4 ± 23.2	-1157.2 ±174.1	-122.0 ±38.0	mean ± s.e.m.	-995.6 ±137.5	-355.4 ±95.1	-342.4 ±90.7

TABLE 6.3. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) on 5 min intravenous (I.V.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after a 5 min renal artery infusion of phentolamine (50 μg/min [132.5 nmol/min]). The calculated percentage inactivation of noradrenaline in the renal vascular bed, before and after phentolamine administration, is also shown.

TABLE 6.3.

DATE	CIRCULATORY RESPONSE	CONTROL		AFTER RENAL PHENTOLAMINE	
		I.V.	R.A.	I.V.	R.A.
3.08.78		40	48	-	18
1.09.78		241	123	170	59
4.09.78		117	73	73	21
5.09.78	Δ M.A.P.	127	79	-	26
11.09.78		177	69	128	36
25.09.78	mm Hg x min	114	72	89	29
26.09.78		108	119	-	44
2.10.78		72	97	68	30
9.10.78		219	183	177	80
10.10.78		94	57	77	39
	mean ± s.e.m.	130.9 ±20.0	92.0 ±12.7	113.0 ±18.4	38.2 ±6.0
3.08.78		- 82	-1976	-	-262
1.09.78		-126	-1040	-114	-216
4.09.78		-195	-2120	-282	-440
5.09.78	Δ R.B.F.	-176	-1260	-	-256
11.09.78		-326	-2272	-	-402
25.09.78		-120	-2112	- 94	-382
26.09.78	ml	-256	-1176	-	-222
2.10.78		-739	-6748	-442	-296
9.10.78		-237	-2840	-370	-582
10.10.78		-392	-2432	-349	-666
	mean ± s.e.m.	-264.9 ±60.7	-2397.6 ± 51.7	-275.2 ±58.1	-372.4 ±48.8
3.08.78		0		-	
1.09.78		49.2		67.0	
4.09.78	Percentage	37.8		71.2	
5.09.78		38.6		-	
11.09.78	Inactivation	61.0		64.1	
25.09.78		36.8		67.4	
26.09.78	%	0		-	
2.10.78		0		55.9	
9.10.78		16.4		54.8	
10.10.78		28.7		48.0	
	mean ± s.e.m.	26.8 ± 6.9		61.2 ± 3.2	

TABLE 6.4. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) on 5 min intravenous (I.V.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after a 5 min renal artery infusion of phentolamine (50 μg/min [132.5 nmol/min]) alone and in conjunction with a renal artery constriction. The calculated percentage inactivation of noradrenaline in the renal vascular bed during these three states are also shown.

TABLE 6.4.

DATE	CIRCULATORY RESPONSE	NORADRENALINE					
		Control		R.A. phentolamine		R.A. phentolamine and R.A. constriction	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
7.5.79	Δ M.A.P. mm Hg x min	164	146	144	66	164	74
14.5.79		690	420	404	155	302	186
17.5.79		462	266	302	138	274	172
19.5.79		154	82	127	49	98	52
22.5.79		79	64	77	27	57	50
29.5.79		398	216	286	53	322	90
	mean ± s.e.m.	324.5 ±95.4	199.0 ±54.3	223.3 ±51.6	81.3 ±21.3	202.8 ±45.8	104.0 ±24.5
7.5.79	Δ R.B.F. ml	-150	-1032	-240	-660	-236	-1692
14.5.79		- 60	-1218	- 86	-276	-140	- 912
17.5.79		-182	-1464	-148	-312	-128	-1344
19.5.79		-224	-1640	-158	-536	-190	-1936
22.5.79		-295	-1224	-269	-442	-274	-2040
29.5.79		-324	-2104	-184	-656	-156	-2248
	mean ± s.e.m.	-205.8 ± 39.7	-1447.0 ± 157.4	-180.8 ± 27.0	-486.3 ± 67.3	-187.3 ± 23.5	-1695.3 ± 201.7
7.5.79	Percentage Inactivation %	11.0		54.2		54.9	
14.5.79		39.1		61.6		38.4	
17.5.79		42.4		54.3		37.2	
19.5.79		46.7		61.4		46.9	
22.5.79		19.0		64.9		12.3	
29.5.79		48.7		81.5		72.0	
	mean ± s.e.m.	34.0 ± 6.2		63.0 ± 4.1		43.6 ± 8.1	

TABLE 6.5. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) and superior mesenteric blood flow (Δ S.M.B.F., ml) on 5 min intravenous (I.V.), superior mesenteric artery (S.M.A.) and hepato-portal vein (H.P.V.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after a 5 min superior mesenteric artery infusion of phentolamine (200 μg/min [530 nmol/min]). The calculated percentage inactivation of noradrenaline in the splanchnic and hepato-portal circulations before and after phentolamine administration are also shown.

TABLE 6.5.

DATE	CIRCULATORY RESPONSE	NORADRENALINE					
		Control			After S.M.A. Phentolamine		
		I.V.	S.M.A.	H.P.V.	I.V.	S.M.A.	H.P.V.
8.6.79	Δ M.A.P. mm Hg x min	182	69	78	224	38	46
19.6.79		76	28	58	136	39	27
20.6.79		250	44	70	108	23	33
27.6.79		152	42	56	173	60	36
6.7.79		177	18	18	163	22	24
9.7.79		107	32	33	137	64	68
	mean ± s.e.m.	157.3 ±25.0	38.8 ±7.2	52.1 ±9.2	156.8 ±16.3	41.0 ±7.3	39.0 ±6.6
8.6.79	Δ S.M.B.F. ml	552	- 720	150	733	0	115
19.6.79		-917	-1368	-345	-168	-401	- 93
20.6.79		-351	- 804	-390	-106	-295	-336
27.6.79		0	- 564	132	-472	- 88	-188
6.7.79		-244	- 685	0	424	-108	0
9.7.79		-340	- 717	-132	-200	-456	272
	mean ± s.e.m.	-216.7 ±196.8	-809.7 ±116.1	-97.5 ±95.1	36.8 ±184.4	-269.6 ± 74.7	-38.3 ±88.5
8.6.79	Percentage Inactivation %		62.1	57.1		83.0	79.5
19.6.79			63.2	23.7		71.3	80.1
20.6.79			82.4	72.0		78.7	69.4
27.6.79			72.4	63.2		65.3	79.2
6.7.79			89.8	89.8		86.5	85.3
9.7.79			70.1	69.1		53.2	50.4
	mean ± s.e.m.		73.3 ±4.4	62.5 ±9.0		73.0 ±5.1	74.0 ±5.2

TABLE 6.6. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min) on 5 min intravenous (I.V.), femoral artery (F.A.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after 5 min renal artery and femoral artery infusion of propranolol (200 μg/min [675.6 nmol/min]). The calculated percentage inactivation of noradrenaline in the femoral and renal vascular beds before and after propranolol administration are also shown.

DATE	CIRCULATORY RESPONSE	NORADRENALINE								
		CONTROL			R.A. PROPRANOLOL			F.A. PROPRANOLOL		
		I.V.	F.A.	R.A.	I.V.	F.A.	R.A.	I.V.	F.A.	R.A.
9.7.79		193	-	124	224	-	177	-	-	-
24.7.79		296	12	166	312	37	121	384	21	157
31.7.79	Δ M.A.P.	438	149	392	480	188	330	420	180	306
7.8.79		223	9	105	262	12	97	234	10	86
9.8.79	mm Hg x min	177	30	131	228	44	99	214	30	158
17.8.79		294	41	210	296	13	129	304	21	191
21.8.79		135	22	100	144	15	62	169	14	55
	mean ± s.e.m.	250.8 ±38.4	43.8 ±21.6	175.4 ±38.9	278.0 ±39.6	51.8 ±27.8	145.0 ±33.6	287.5 ±40.6	46.5 ±26.9	158.8 ±35.9
9.7.79			-	35.7		-	21.0		-	-
24.7.79	Percentage		95.9	43.9		88.1	61.2		94.5	59.1
31.7.79			66.0	10.5		60.8	31.2		57.1	27.1
7.8.79	Inactivation		95.9	52.9		95.4	63.0		95.7	63.3
9.8.79			83.0	26.0		80.7	56.6		84.6	26.2
17.8.79	%		86.0	28.6		95.6	56.4		93.1	37.2
21.8.79			83.7	25.9		89.6	56.9		91.7	67.4
	mean ± s.e.m.		85.1 ±4.5	31.9 ±5.2		84.1 ±6.4	49.5 ±6.2		86.1 ±6.0	46.7 ±7.6

TABLE 6.7. The changes in renal blood flow (Δ R.B.F., ml) and femoral blood flow (Δ F.B.F., ml) on direct infusion of noradrenaline (10 μg/min [59.1 nmol/min] for 5 min) before and after a 5 min renal artery (R.A.) and femoral artery (F.A.) infusion of propranolol (200 μg/min [675.6 nmol/min]).

DATE	CIRCULATORY RESPONSE	RENAL ARTERY NORADRENALINE			CIRCULATORY RESPONSE	FEMORAL ARTERY NORADRENALINE		
		CONTROL	R.A. PROPRANOLOL	F.A. PROPRANOLOL		CONTROL	R.A. PROPRANOLOL	F.A. PROPRANOLOL
9.7.79		-2156	-1872	-		-	-	-
24.7.79		-2664	-1284	-2072		- 984	- 667	- 475
31.7.79	Δ R.B.F.	- 915	- 465	- 480	Δ F.B.F.	- 535	- 319	- 447
7.8.79		-1095	- 870	-2025		-1046	-1740	-1604
9.8.79	ml	-1844	-1392	-2192	ml	-1908	-2256	-1836
17.8.79		-1860	-1240	-1480		-2328	-1800	-1908
21.8.79		-1272	- 804	-1208		-2651	-3276	-2076
	mean ± s.e.m.	-1693.7 ±237.3	-1132.0 ±174.0	-1576.2 ±269.1	mean ± s.e.m.	-1575.5 ±343.9	-1676.3 ±438.8	-1391.0 ±300.6

TABLE 6.8. The mean arterial pressure (Δ M.A.P., mm Hg x min) and renal blood flow (Δ R.B.F., ml) responses in 5 dogs on 5 min intravenous (I.V.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]) before and after a 5 min renal artery infusion of propranolol (200 μg/min [675.6 nmol/min]) alone and in conjunction with phentolamine (100 μg/min [355.4 nmol/min] for 5 min). The calculated percentage inactivation of noradrenaline in the renal vascular bed for each dog is also shown.

DATE	CIRCULATORY RESPONSE	NORADRENALINE					
		CONTROL		R.A. PROPRANOLOL		R.A. PROPRANOLOL + PHENTOLAMINE	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
11.9.79		290	214	272	159	248	63
14.9.79	Δ M.A.P.	298	268	404	256	294	19
18.9.79		154	55	125	15	112	6
19.9.79	ml	212	132	256	85	226	48
21.9.79		190	87	316	138	250	31
	mean ± s.e.m.	228.8 ±28.2	151.2 ±39.6	274.6 ±43.4	130.6 ±40.0	226.0 ±30.6	33.4 ±10.1
11.9.79		0	-1044	-128	- 612	0	-672
14.9.79	Δ R.B.F.	-127	- 656	-173	- 720	-158	-192
18.9.79		-118	-1560	-151	-1776	-235	-287
19.9.79	ml	- 89	- 780	- 79	- 615	- 42	-293
21.9.79		-210	-1500	0	- 768	0	-280
	mean ± s.e.m.	-108.8 ±33.8	-1108.0 ±183.6	-106.2 ±30.8	-898.2 ±221.5	-87.0 ±47.0	-344.8 ±83.8
11.9.79	Percentage	26.2		41.5		74.6	
14.9.79		10.1		36.6		93.5	
18.9.79	Inactivation	64.3		88.0		94.6	
19.9.79		37.7		66.8		78.8	
21.9.79	%	54.2		56.3		87.6	
	mean ± s.e.m.	38.5 ± 9.7		57.8 ± 9.2		85.8 ± 4.0	

TABLE 7.1. The changes in mean arterial pressure (Δ M.A.P., mm Hg x min), renal blood flow (Δ R.B.F., ml) in response to 5 min intravenous (I.V.) and renal artery (R.A.) infusions of noradrenaline (10 μg/min [59.1 nmol/min]), before and after renal artery infusion of SQ 20881 (200 μg/min [265.0 nmol/min] for 5 min) alone and in conjunction with phentolamine (100 μg/min [355.4 nmol/min] for 5 min). The changes in calculated noradrenaline inactivation in the renal vascular bed are also shown.

TABLE 7.1.

DATE	CIRCULATORY RESPONSE	NORADRENALINE					
		CONTROL		R.A. SQ 20881		R.A. SQ 20881 AND PHENTOLAMINE	
		I.V.	R.A.	I.V.	R.A.	I.V.	R.A.
20.11.79	Δ M.A.P. mm Hg x min	428	290	405	164	342	79
27.11.79		95	80	88	41	63	11
29.11.79		94	57	111	35	83	23
1.12.79		113	104	74	52	75	12
2.12.79		107	98	72	58	54	11
7.12.79		258	170	185	119	117	2
	mean ± s.e.m.	182.5 ± 55.4	133.2 ± 35.0	139.2 ± 53.5	78.2 ± 21.1	122.3 ± 44.8	23.0 ± 11.5
20.11.79	Δ R.B.F. ml	-136	-1710	-108	- 890	-170	-290
27.11.79		-478	-3160	-462	-1700	-424	-490
29.11.79		-168	-1440	- 92	- 710	0	-192
1.12.79		-162	-2365	-152	-1180	-175	-216
2.12.79		-130	-1950	-186	-1550	-210	-270
7.12.79		-416	-1488	-266	-1180	-412	-354
	mean ± s.e.m.	-248.3 ± 63.6	-2018.8 ± 267.0	-211.0 ± 56.2	-1201.7 ± 153.8	-231.8 ± 66.0	-302.0 ± 44.2
20.11.79	Percentage Inactivation %	32.2		59.5		76.9	
27.11.79		15.8		53.4		82.5	
29.11.79		39.4		68.5		72.3	
1.12.79		8.0		29.7		84.0	
2.12.79		8.4		19.4		79.6	
7.12.79		34.1		35.7		98.3	
	mean ± s.e.m.	23.0 ± 5.7		44.4 ± 7.8		82.3 ± 3.6	

TABLE 7.2. Systemic plasma renin activity (ng AI/ml/hr) in 4 dogs before and after 5 min renal artery infusion of noradrenaline (10 µg/min [59.1 nmol/min]). Blood samples (5 ml) were taken from the high inferior vena cava at 5 min intervals.

DATE		INFERIOR VENA CAVA SAMPLE					
				N.A. INFUSION			
		1	2	3	4	5	6
20.11.79	SYSTEMIC	1.59	1.73	1.28	1.36	2.68	-
27.11.79	PLASMA RENIN	0.76	0.32	0.08	0.68	1.00	0.28
29.11.79	ACTIVITY	0.08	0	0.08	0.24	0.60	0.16
1.12.79	ng AI/ml/hr	0	1.12	0.40	0.68	2.96	6.40
	mean ± s.e.m.	0.61 ± 0.43	0.79 ± 0.45	0.45 ± 0.32	0.74 ± 0.27	1.81 ± 0.68	2.28 ± 2.52

B I B L I O G R A P H Y

B I B L I O G R A P H Y

ACKERLY, J., BLUMBERG, A. and PEACH, M. (1976). Angiotensin interactions with myocardial sympathetic neurons: enhanced release of dopamine-β-hydroxylase during nerve stimulation. Proc. Soc. Exp. Biol. Med. 151, 650-653.

AIKEN, J. W. and VANE, J. R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor response of angiotensin. J. Pharmacol. Exp. Ther. 184, 678-687.

AKINKUGBE, O. O., BROWN, W. C. B. and CRANSTON, W. I. (1966). Pressor effects of angiotensin infusions into different vascular beds in the rabbit. Clin. Sci. 30, 409-416.

AUKLAND, K. (1976). Renal blood flow. Int. Rev. Physiol., Kidney and Urinary Tract Physiology II, ed. Thurau, K. Vol. 11, 23-77.

AXELROD, J., WEIL-MALHERBE, H. and T OMCHICK, R. (1959). The physiological disposition of H^3-epinephrine and its metabolite metanephrine. J. Pharmacol. Exp. Ther. 127, 251-256.

BAILIE, M. D. and OPARIL, S. (1977). Relation of renal haemodynamics to metabolism of angiotensin II by the canine kidney. Circ. Res. 41, 283-287.

BAILIE, M. D., RECTOR, F. C. and SELDIN, D. W. (1971). Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. J. Clin. Invest. 50, 119-125.

BAKHLE, Y. S., REYNARD, A. M. and VANE, J. R. (1969). Metabolism of angiotensins in isolated perfused tissues. Nature (London) 222, 956-999.

BARER, G. R. (1961). A comparison of the circulatory effects of angiotensin, vasopressin and adrenaline in the anaesthetized cat. J. Physiol. 156, 49-66.

BAUDOIN, M., MEYER, P. and WORCEL, M. (1971). Specific binding of ^{3}H-angiotensin II in rabbit aorta. Biochem. Biophys. Res. Comm. 42, 434-440.

BAUM, T. (1963). Vascular reactivity of reserpine-pretreated dogs. J. Pharmacol. Exp. Ther. 46. 30-35.

BELL, C. (1972). Mechanism of enhancement by angiotensin II of sympathetic adrenergic transmission in the guinea pig. Circ. Res. 31, 348-353.

BENELLI, G., DELLA BELLA, D. and GANDINI, A. (1964). Angiotensin and peripheral sympathetic nerve activity. Brit. J. Pharmacol. 22, 211-219.

BIRON, P. And CAMPEAU, L. (1971). Pulmonary and extra pulmonary fate of angiotensin I. Rev. Can. Biol. 30, 27-34.

BIRON, P., CAMPEAU, L. and DAVID, P. (1969). Fate of angiotensin I and II in the human pulmonary circulation. Amer. J. Cardiol. 24, 544-547.

BIRON, P., MEYER, P. and PANISSET, J-C. (1968). Removal of angiotensins from the systemic circulation. Can. J. Physiol. Pharmacol. 46, 175-178.

BLUMBERG, A. L., ACKERLY, J. A. and PEACH, M. J. (1975). Differentiation of neurogenic and myocardial angiotensin II receptors in isolated rabbit atria. Circ. Res. 36, 719-726.

BOADLE, M. C., HUGHES, J. and ROTH, R. H. (1969). Angiotensin accelerates catecholamine biosynthesis in sympathetically innervated tissues. Nature (London) 222, 987-988.

BOCK, K. D., KRECKE, H. J. and KUHN, H. M. (1958). Untersuchungen über die Wirkung von synthetischem Hypertensin II auf Blutdruck, Atmung und Extremitätendurchblutung des Meschen. klin. Wschr. 36, 254.

BOMZON, L. and ROSENDORFF, C. (1975). Renovascular resistance and noradrenaline. Amer. J. Physiol. 229, 1649-1653.

BRAUN-MENÉNDEZ, E., FASCIOLO, J. C., LELOIR, L. F. and MUÑOZ, J. M. (1939). La substancia hipertensora de la sangre del rinon isquemiada. Rev. Soc. Arg. Biol. 15, 420-425.

BROD, J., HEJL, Z., HORNYCH, A., JIRKA, J., SLECHTA, V. and BURIANOVA, B. (1969). Comparison of the haemodynamic effects of equipressor doses of intravenous angiotensin and noradrenaline in man. Clin. Sci. 36, 161-172.

BROUGHTON PIPKIN, F. (1972). Hepatic inactivation of val^5 angiotensin II amide (hypertensin), val^5 angiotensin II free acid and adrenaline in immature and adult rabbits. J. Physiol. (London) 225, 35-36P.

BROUGHTON PIPKIN, F., MOTT, J. C. and ROBERTON, N. R. C. (1971). Angiotensin II-like activity in circulating arterial blood in immature and adult rabbits. J. Physiol. (London) 218, 385-403.

BROWN, M. J. (1976). Cardiovascular actions of perhexiline. Ph.D. Thesis, University of Melbourne, Australia.

BUMPUS, F. M., SMEBY, P. R., PAGE, I. H. and KHAIRALLAH, P. A. (1964). Distribution and metabolic fate of angiotensin II and various derivatives. Can. Med. Ass. J. 90, 190-193.

BUÑAG, R. D. (1974). Circulatory effects of angiotensin. IN: Handbook of Experimental Pharmacology, 37, "Angiotensin", Page, I. H. and Bumpus, F. M. (Eds) Springer-Verlag.

CAIN, M. D., CATT, K. J. and COGHLAN, J. P. (1969). Effect of circulating fragments of angiotensin II on radioimmunoassay in arterial and venous blood. J. Clin. Endocrinol. Metab. 29, 1639-1643.

CAIN, M. D., CATT, K. J., COGHLAN, J. P. and BLAIR-WEST, J. R. (1970). Evaluation of angiotensin II metabolism in sheep by radioimmunoassay. Endocrinology 86, 955-964.

CALDICOTT, W. J. H. and HOLLENBERG, N. K. (1979). Offset of actions of angiotensin II, angiotensin III, and their analogue antagonists in renal and femoral vascular beds: further evidence for different vascular angiotensin receptors. Life Sci. 24, 503-512.

CALDICOTT, W. J. H., TAUB, K. J. and HOLLENBERG, N. K. (1977). Identical mesenteric femoral and renal vascular responses to angiotensin II and III in the dog. Life Sci. 20, 517-522.

CALDICOTT, W. J. H., TAUB, K. J., KORNGOLD, E. and HOLLENBERG, N. K. (1978). Heptapeptide analogues induce greater blockade of renal than femoral vascular responses to angiotensin. Life Sci. 23, 105-110.

CALDWELL, P. R. B., SEEGAL, B. C. and HSU, K. C. (1976). Angiotensin-converting enzyme: vascular endothelial localization. Science 191, 1050-1051.

CALLINGHAM, B. A. and BURGEN, A. S. V. (1966). The uptake of isoprenaline and noradrenaline by the perfused rat heart. Mol. Pharmacol. 2, 37-42.

CELANDER, O. and MELLANDER, S. (1955). Elimination of adrenaline and noradrenaline from the circulating blood. Nature (London) 176, 973-974.

CHAMBERLAIN, M. J., BROWSE, N. L., GIBSON, D. G. and GLEESON, J. A. (1964). Angiotensin and reno-portal anastomosis. Brit. Med. J. II, 1507-1508.

CHEVILLARD, C. and ALEXANDRE, J. (1970). Action, *in vitro* et *in vivo*, de l'angiotensine II sur le captage cardiaque de la noradrénaline. Experentia (Basel) 26, 1334-1336.

CHEVILLARD, C., DUCHÊNE, N. and ALEXANDRE, J. M. (1975). How does angiotensin II increase cardiac dopamine-β-hydroxylation? J. Pharm. Pharmac. 27, 193-196.

COLLIER, J. G. (1972). New dialysis technique for the continuous measurement of the concentration of vaso-active hormones. Brit. J. Pharmacol. 44, 383P.

COLLIS, M. G. and ALPS, B. J. (1975). Vascular reactivity to noradrenaline, potassium chloride, and angiotensin II in the rat perfused mesenteric vasculature preparation, during the development of renal hypertension. Cardiovasc. Res. 9, 118-126.

COLLIS, M. G. and ALPS, B. J. (1976). The renin-angiotensin system, dietary salt, and increased sensitivity to noradrenaline in mesenteric vasculature preparations from renal/salt hypertensive rats. Cardiovasc. Res. 10, 232-235.

COLLIS, M. G. and VANHOUTTE, P. M. (1978). Increased renal vascular reactivity to angiotensin II but not to nerve stimulation or exogenous norepinephrine in renal hypertensive rats. Circ. Res. 43, 544-552.

COX, R. H. (1972). Influence of chloralose anaesthesia on cardiovascular function in trained dogs. Amer. J. Physiol. 223, 660-667.

DANON, A. and SAPIRA, J. D. (1972). Binding of catecholamines to human serum albumin. J. Pharmacol. Exp. Ther. 182, 295-302.

DAVILA, D. and KHAIRALLAH, P. A. (1970). Effect of ions on inhibition of norepinephrine uptake by angiotensin. Arch. Int. Pharmacodyn. 185, 357-364.

DAVIS, J. O. and FREEMAN, R. H. (1976). Mechanisms regulating renin release. Physiol. Rev. 56, 1-56.

DAY, M. D. and MOORE, A. F. (1976). Interaction of angiotensin II with noradrenaline and other spasmogens on rabbit isolated aortic strips. Arch. Int. Pharmacodyn. 219, 29-44.

DAY, M. D. and OWEN, D. A. A. (1969). Potentiation by angiotensin of responses to endogenously released noradrenaline in the pithed rat. Arch. Int. Pharmacodyn. 179, 469-479.

DE LA LANDE, I. S. and JELLETT, L. B. (1972). Relationship between the roles of monoamine oxidase and sympathetic nerves in the vasoconstrictor response of the rabbit ear artery to norepinephrine. J. Pharmacol. Exp. Ther. 180, 47-55.

DE LA LANDE, I. S., FREWIN, D. and WATERSON, J. G. (1967). The influence of sympathetic innervation of vascular sensitivity to noradrenaline. Brit. J. Pharmacol. Chemother. 31, 82-93.

DE LEEUW, P. W., FALKE, H. E., PUNT, R. and BIRKENHÄGER, W. H. (1978). Noradrenaline secretion from the human kidney. Clin. Sci. Mol. Med. 55, 85s-87s.

DENGLER, H. and REICHEL, G. (1960). Untersuchungen zur intrazellulären Lokalisation der renin-und hypertensinase-activitat. Experientia 16, 36-38.

DI SALVO, J. and FELL, C. (1970). Effects of angiotensin on canine renal blood flow. Proc. Soc. Exp. Biol. Med. 133, 1432-1434.

DISTLER, A., LIEBAU, H. and WOLFF, H. P. (1965). Action of angiotensin on sympathetic nerve endings in isolated blood vessels. Nature (London) 207, 764-765.

DRIVER, R. L. and VOGT, M. (1950). Carotid sinus reflex and contraction of the spleen. Brit. J. Pharmacol. Chemother. 5, 505-509.

ECKERT, G. E. and ROSE, J. C. (1959). A study of angiotensin in the pulmonary circulation. Georgetown Med. Bull. 13, 72.

EISEMAN, B., BRYANT, L. and WALTUCH, T. (1964). Metabolism of vasomotor agents by the isolated perfused lung. J. Thoracic Cardiovasc. Surg. 48, 798-806.

ELLIOT, T. R. (1905). The action of adrenalin. J. Physiol. (London) 32, 401-467.

FARRUGGIA, P., SACHS, S. and PALAIC, D. (1979). Effect of angiotensinase inhibitors on angiotensin receptors in rabbit aorta. Mol. Pharmacol. 15, 525-530.

FERRARIO, C. M., GILDENBERG, P. L. and McCUBBIN, J. W. (1972). Cardiovascular effects of angiotensin mediated by the central nervous system. Circ. Res. 30, 257-262.

FORSYTH, R. P., HOFFBRAND, B. I. and MELMON, K. L. (1971). Haemodynamic effects of angiotensin in normal and environmentally stressed monkeys. Circulation 44, 119-129.

FREEMAN, R. H. and DAVIS, J. L. (1979). Physiological actions of angiotensin II on the kidney. Fed. Proc. 38, 2276-2279.

GASCON, A. L. and WALASZEK, E. J. (1968). Mechanism of the musculotropic activity of angiotensin on the isolated guinea pig seminal vesicle. Arch. Int. Pharmacodyn. 175, 265-272.

GEDDES, L. A. (1969). The measurement of blood pressure. Year Book Medical Publishers Inc., Chicago.

GINN, R. W. and VANE, J. R. (1968). Disappearance of catecholamines from the circulation. Nature (London) 219, 740-742.

GOMER, S. K. and ZIMMERMAN, B. G. (1973). Effect of angiotensin II on uptake and release of dl-^{3}H-metaraminol. Proc. Soc. Exp. Biol. (N.Y.) 142, 787-792.

GOMORI, P., TAKACS, L. and KALLAY, K. (1962). The effect of synthetic angiotensin II on the redistribution and shifting of blood. Arch. Int. Pharmacodyn. 138, 254-262.

GREENWAY, C. V., LAWSON, A. D. and MELLANDER, S. (1967). The effects of stimulation of the hepatic nerves, infusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anaesthetized cat. J. Physiol. (London) 192, 21-47.

GREENWAY, C. V. and OSHIRA, G. (1972). Comparison of the effects of hepatic nerve stimulation on arterial flow, distribution of arterial and portal flows and blood content in the livers of anaesthetized cats and dogs. J. Physiol. (London) 192, 21-41.

GREENWAY, C. V. and STARK, R. D. (1971). Hepatic vascular bed. Physiol. Rev. 51, 23-65.

GRYGLEWSKI, R. and VANE, J. R. (1970). The inactivation of noradrenaline and isoprenaline in dogs. Brit. J. Pharmacol. 39, 573-584.

HAAS, E., GOLDBLATT, H., LEWIS, L. and GIPSON, E. C. (1968). Inhibition of angiotensin and its protection by a cofactor in blood. Amer. J. Physiol. 215, 1420-1428.

HAAS, E., GOLDBLATT, H., LEWIS, L. and GIPSON, E. C. (1969). Angiotensin-protective cofactor. Amer. J. Physiol. 217, 981-987.

HAAS, E., GOLDBLATT, H., LEWIS, L. and GIPSON, E. C. (1973). Interplay of vasodilator and vasoconstrictor substances in the femoral arterial bed: Identification of the angiotensin cofactor. Lab. Invest. 28, 1-7.

HALL, J. E., GUYTON, A. C., JACKSON, T. E., COLEMAN, T. G., LOHMEIER, T. E. and TRIPPODO, N. C. (1977). Control of glomerular filtration rate by renin - angiotensin system. Amer. J. Physiol. 233, F366-F373.

HERTTING, G. and SUKO, J. (1966). Influence of angiotensin, vasopressin or changes in flow rate on vasoconstriction, changes in volume and [^{3}H]-noradrenaline release following postganglionic nerves stimulation in the isolated cat spleen. Brit. J. Pharmacol. 26, 686-696.

HODGE, R. L., NG, K. K. F. and VANE, J. R. (1967). Disappearance of angiotensin from the circulation of the dog. Nature (London) 215, 138-141.

HOFFBRAND, B. I. and FORSYTH, R. P. (1973). Regional blood flow changes during norepinephrine, tyramine and methoxamine infusions in the unanaesthetized rhesus monkeys. J. Pharmacol. Exp. Ther. 184, 656-661.

HOLLENBERG, N. K., SOLOMAN, H. S., ADAMS, D. F., ABRAMS, H. L. and MERRILL, J. P. (1972). Renal vascular responses to angiotensin and norepinephrine in normal man. Effect of sodium intake. Circ. Res. 31, 750-757.

HUGHES, J. and ROTH, R. H. (1969). Enhanced release of transmitter during sympathetic nerve stimulation in the presence of angiotensin. Brit. J. Pharmacol. 37, 516-517P.

HUGHES, J. and ROTH, R. H. (1971). Evidence that angiotensin enhances transmitter release during sympathetic stimulation. Brit. J. Pharmacol. 41, 239-255.

ICHIKAWA, S., JOHNSON, J. A., FOWLER, W. L., PAYNE, C. G., KUEZ, K. and KEITZER, W. F. (1978). Pressor responses to norepinephrine in rabbits with 3-day and 30-day renal artery stenosis: The role of angiotensin II. Circ. Res. 43, 437-446.

ITSKOVITZ, H. D. and MILLER, L. (1967). Differential studies of angiotensinase activities from several tissue sources. Amer. J. Med. Sci. 254, 659-666.

IVERSEN, L. L. (1967). The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press.

JACKSON, E. K. and CAMPBELL, W. B. (1979). Inhibition of neuronal noradrenaline uptake by angiotensin II in the rat mesentery. Can. J. Physiol. Pharmacol. 57, 1443-1447.

JACKSON, E. K. and CAMPBELL, W. B. (1980). Inhibition of angiotensin II potentiation of sympathetic nerve activity by beta-adrenergic antagonists. Hypertension 2, 90-96.

JANOWSKY, D. S., DAVIS, J. M., FANN, W. E., FREEMAN, J., NIXON, R. and MICHELAKIS, A. A. (1972). Angiotensin effect on uptake of noradrenaline by synaptosomes. Life Sci. 11, 1-11.

JOHNSON, D. C. and RYAN, J. W. (1968). Degradation of angiotensin II by a carboxypeptidase of rabbit liver. Biochim. Biophys. Acta (Amsterdam) 160, 196-203.

JOHNSTON, C. I., HUTHINSON, J. S. and MENDELSOHN, F. A. (1970). Biological significance of renin-angiotensin immunization. Circ. Res. 27 (Suppl.), 215.

JONES, R. T. (1968). Renal excretion of l-epinephrine in the dog. Amer. J. Physiol. 215, 371-374.

KADOWITZ, P. J., SWEET, C. S. and BRODY, M. J. (1971). Potentiation of adrenergic venomotor responses by angiotensin, prostaglandin $F_{2\alpha}$ and cocaine. J. Pharmacol. Exp. Ther. 176, 167-173.

KADOWITZ, P. J., SWEET, C. S. and BRODY, M. J. (1972). Influence of angiotensin I, angiotensin II and cocaine on adrenergic vasoconstrictor responses in the dog hindpaw. J. Pharmacol. Exp. Ther. 183, 275-283.

KANEKO, Y., TAKEDA, T., NAKAJIMA, K. and UEDA, H. (1966). Effect of angiotensin on the pressor response to tyramine in normotensive subjects and hypertensive patients. Circ. Res. 19, 673-680.

KAPITOLA, J., KUCHEL, O., SCHREIBEROVA, O. and JAHODA, I. (1968). Blood flow through organs of the rat after an intravenous injection of angiotensin. Physiol. Bohemoslov. 17, 437-443.

KHAIRALLAH, P. A. (1972). Action of angiotensin on adrenergic nerve endings: inhibition of norepinephrine uptake. Fed. Proc. 31, 1351-1357.

KHAIRALLAH, P. A., BUMPUS, F. M., PAGE, I. H. and SMEBY, R. R. (1963). Angiotensinase with a high degree of specificity in plasma and red cells. Science 140, 672-674.

KHAIRALLAH, P. A., PAGE, I. H., BUMPUS, F. M. and TURKER, R. K. (1966). Angiotensin tachyphylaxis and its reversal. Circ. Res. 19, 247-254.

KIRAN, B. K. and KHAIRALLAH, P. A. (1969). Angiotensin and norepinephrine efflux. Europ. J. Pharmacol. 6, 102-108.

KRASNEY, J. A. (1968). Effects of angiotensin on stroke volume and regional blood flow and resistance. Amer. J. Physiol. 215, 1454-1461.

KRASNEY, J. A., PAUDLER, F. T., HOGAN, P. M., LOWE, R. F. and YOUMANS, W. B. (1966). Peripheral adrenergic basis for cardiovascular action of angiotensin. Amer. J. Physiol. 211, 1447-1450.

KUNOS, G. (1976). Adrenoceptors. Ann. Rev. Pharmacol. Toxicol. 18, 291-311.

LAFONTAINE, J. J., NIVEZ, M. P. and ARDAILLOU, R. (1979). Hepatic binding sites for angiotensin II in the rat. Clin. Sci. 56, 33-40.

LANGER, S. Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. Brit. J. Pharmacol. 60, 481-497.

LEARY, W. P. and LEDINGHAM, J. G. (1969). Removal of angiotensin by isolated perfused organs of the rat. Nature (London) 222, 959-960.

LEARY, W. P. and LEDINGHAM, J. G. (1970). Renal and hepatic inactivation of angiotensin in rats: Influence of sodium balance and renal artery compression. Clin. Sci. 38, 573- 582.

LEARY, W. P. and LEDINGHAM, J. G. (1970b). Inactivation of angiotensin II analogues by isolated perfused rat liver and kidney. Nature (London) 227, 178-179.

LEDINGHAM, J. G. and LEARY, W. P. (1974). Catabolism of angiotensin II. IN: Handbook of Experimental Pharmacology, 37, "Angiotensin", Page, I. H. and Bumpus, F. M. (Eds), Springer-Verlag.

MALIK, K. U. and McGiff, J. C. (1976). Cardiovascular actions of prostaglandins. IN: Prostaglandins: Physiological, Pharmacological and Pathological Aspects, Karim, S. M. M. (Ed), Lancaster, MTP Press, 103-200.

MALIK, K. U. and NASJLETTI, A. (1976). Facilitation of adrenergic transmission by locally generated angiotensin II in rat mesenteric arteries. Circ. Res. 38, 26-30.

MANDEL, M. J. and SAPIRSTEIN, L. A. (1962). Effect of angiotensin infusion of regional blood flow and regional vascular resistance in the rat. Circ. Res. 10, 807-816.

MATSUNAGA, M. (1971). Nature of lysosomal angiotensinase activity. Jap. Circ. J. 35, 333-338.

MATSUNAGA, M., SAITO, KIRA, J., OGINO, K. and TAKAYASU, M. (1969). Acid angiotensinase as a lysosomal enzyme. Jap. Circ. J. 33, 545-551.

MAY, P., SANDERS, F. H. and DONABEDIAN, R. K. (1974). Binding of catechol derivatives to human serum proteins. Experentia (Basel) 30, 304-305.

McCUBBIN, J. W. and PAGE, I. H. (1963). Renal pressor system and neurogenic control of arterial pressure. Circ. Res. 12, 553-559.

McGIFF, J. C. (1977). Bartter's syndrome results from an inbalance of vasoactive hormones. Ann. Int. Med. 87, 369-372.

McGIFF, J. C., CROWSHAW, K., TERRAGNO, N. A. and LONIGRO, A. I. (1970). Release of prostaglandin-like substance into renal venous blood in response to angiotnsin II. Circ. Res. 27, Suppl 1, I-121-I-130.

MERRILL, J. E., PEACH, M. J. and GILMORE, J. P. (1973). Angiotensin I conversion in the kidney and its modulation by sodium balance. Ann. J. Physiol. 224, 1104-1108.

MELLANDER, S. and JOHANSSON, B. (1968). Control of resistance, exchange and capacitance functions in the peripheral circulation. Pharmacol. Rev. 20, 117-196.

METHOT, A. L., MEYER, P., BIRON, P., LORAIN, M. F., LAGRUE, G. and MILLIEZ, P. (1964). Hepatic inactivation of angiotensin. Nature (London) 203, 531-532.

MEYER, P., PAPADIMITRIOU, A. and WORCEL, M. (1970). Possible existence of different types of angiotensin II receptors. Brit. J. Pharmacol. 40, 541P-542P.

MOORE, A. F. and POVINELLI, M. A. (1979). Specific membrane-binding of angiotensin II - the role of degrading enzymes. J. Pharm. Pharmac. 31 375-377.

NICHOLAS, T. E. (1970). Potentiation of the effects of noradrenaline and of sympathetic stimulation of the perfused rat caudal artery by angiotensin. J. Pharm. Pharmac. 22, 37-41.

OPARIL, S. and BAILIE, M. D. (1973). Mechanism of renal handling of angiotensin II in the dog. Circ. Res. 33, 500-507.

OSBORN, E. C., HUGHES, S. G., PURIE, A. T., WILLICOMBE, P. R. and MAHLER, R. F. (1969). Clearance studies of angiotensin II and (^{35}S) phenylthiocarbanyl-angiotensin II in the sheep and dog. Cardiovasc. Res. 3, 134-146.

OSBORN, E. C., TILDESLEY, G., LEACH, K. G. and RIGBY, G. V. (1974). Effects of angiotensin I and angiotensin II on renal blood flow in sheep. Amer. J. Physiol. 226, 518.

OSBORNE, M. J., ANGLES d'AURIAC, G., MEYER, P. and WORCEL, M. (1970). Mechanism of extraction of angiotensin II in coronary and renal circulations. Life Sci. 9, 859-867.

OSSWALD, W. and BRANCO, D. (1973). The effects of drugs and denervation on removal and accummulation of noradrenaline in the perfused hindlimb of the dog. Naunyn-Schmiedeberg's Arch. Pharmacol. 227, 175-190.

OVERY, H. R., PFISTER, R. and CHIDSEY, C. A. (1967). Studies on the renal excretion of noradrenaline. J. Clin. Invest. 46, 482-489.

PAGE, I. H. and HELMER, O. M. (1939). A crystalline pressor substance, angiotonin. Proc. Cent. Soc. Clin. Res. 12, 17.

PALAIC, D. and KHAIRALLAH, P. A. (1967). Effect of angiotensin on uptake and release of norepinephrine by brain. Biochem. Pharmacol. 16, 2291-2298.

PANISSET, J-C. and BOURDOIS, P. (1968). Effect on angiotensin on the response to noradrenaline and sympathetic nerve stimulation and on ^{3}H-noradrenaline uptake in cat mesenteric blood vessels. Can. J. Physiol. Pharmacol. 46, 125-131.

PEACH, M. J. (1977). Renin-angiotensin system: biochemistry and mechanisms of action. Physiol. Rev. 57, 313-370.

PEACH, M. J. and CHIU, A. T. (1974). Stimulation and inhibition of aldosterone biosynthesis *in vitro* by angiotensin II and analogs. Circ. Res. 34-35, Suppl: I-7-I-13

PEACH, M. J., CLINE, W. H., DAVILA, D. and KHAIRALLAH, P. A. (1970). Angiotensin-catecholamine interactions in the rabbit. Europ. J. Pharmacol. 11, 286-292.

PEACH, M. J., BUMPUS, F. M. and KHAIRALLAH, P. A. (1971). Release of adrenal catecholamines by angiotensin I. J. Pharmacol. Exp. Ther. 176, 366-376.

PLOTH, D. W. and NAVAR, L. G. (1979). Intrarenal effects of the renin-angiotensin system. Fed. Proc. 38, 2280-2285.

POWIS, G. (1975). The binding of catecholamines to human serum proteins. Biochem. Pharamcol. 24, 707-712.

PULMAN, T. N., OPARIL, S. and CARONE, F. A. (1975). Fate of labelled angiotensin II microinfused into individual nephrons in the rat. Amer. J. Physiol. 228, 747-751.

QUEBBEMANN, A. and RENNICK, B. (1970). Inhibition of renal tubular transport of catecholamines by cocaine: an organic base mechanism. J. Pharmacol. Exp. Ther. 175, 248-258.

REGOLI, D. and GAUTHER, R. (1971). Site of action of angiotensin and other vasoconstrictors on the kidney. Can. J. Physiol. Pharmacol. 49, 608-612.

REGOLI, D., PARK, W. K. and RIOUX, F. (1974). Pharmacology of angiotensin. Pharmacol. Rev. 26, 69-123.

REGOLI, D. and VANE, J. R. (1964). Sensitive method for the assay of angiotensin. Brit. J. Pharmacol. Chemother. 23, 351-359.

REID, I. A., MORRIS, B. J. and GANONG, W. F. (1978). The renin-angiotensin system. Ann. Rev. Physiol. 40, 377-410.

REIT, E. (1972). Action of angiotensin on the adrenal medulla and autonomic ganglia. Fed. Proc. 31, 1338-1343.

RENNICK, B. (1968). Dopamine: renal tubular transport in the dog and plasma binding studies. Amer. J. Physiol. 215, 532-534.

RENNICK, B. and QUEBBEMANN, A. (1970). Site of excretion of catechol and catecholamines: renal metabolism of catechol. Amer. J. Physiol. 218, 1307-1312.

RENNICK, B. R. and YOSS, N. (1962). Renal tubular excretion of DL-epinephrine-2-C^{14} in the chicken. Amer. J. Physiol. 215, 347-350.

RYAN, J. W. (1974). The fate of angiotensin II. IN: Handbook of Experimental Pharmacology, 37, "Angiotensin", Page,I. H. and Bumpus, F. M. (Eds), Springer-Verlag.

SALT, P. J. (1972). Inhibition of noradrenaline uptake in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. Eur. J. Pharmacol. 20, 329-340.

SCHUMANN, H. J. (1970). Discussion p 210. IN: New Aspects of Storage and Release Mechanisms of Catecholamines, Schumann, H. J. and Kroneberg, G. (Eds), Berlin-Heidelberg-New York: Springer.

SCROOP, G. C. (1967). An investigation of the cardiovascular actions of angiotensin in man. M.D. Thesis, University of Adelaide, Australia.

SCROOP, G. C. and LOWE, R. D. (1969). Efferent pathways of the cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci. 37, 605-619.

SCROOP, G. C., WALSH, J. A. and WHELAN, R. F. (1965). A comparison of the effects of intra-arterial and intravenous infusions of angiotensin and noradrenaline on the circulation in man. Clin. Sci. 29, 315-326.

SELIQ, S. E., ANDERSON, W. P. and KORNER, P. I. (1979). Renal vascular responses to noradrenaline are partially mediated by angiotensin II in conscious dogs. Proc. Aust. Physiol. Pharmacol. Soc. 10, 191P.

SEVERS, W. B. and DANIELS-SEVERS, A. (1973). Effects of angiotensin on the central nervous system. Pharmacol. Rev. 25, 415-449.

SHABETAI, R., FOWLER, N. O. and HURLBURT, O. (1963). Hemodynamic studies of dogs under pentobartitol and morphine chloralose anaesthesia. J. Surg. Res. 3, 263-267.

SILVA, P., LANDSBERG, L. and BESARAB, A. (1979). Excretion and metabolism of catecholamines by the isolated perfused rat kidney. J. Clin. Invest. 64, 850-857.

STARKE, K. (1970). Interactions of angiotensin and cocaine on the output of noradrenaline from isolated rabbit hearts. Naunyn-Schmiedeberg's Arch. Pharmacol. 265, 383-386.

STARKE, K. (1971). Action of angiotensin on uptake, release and metabolism of ^{14}C-noradrenaline by isolated rabbit hearts. Europ. J. Pharmacol. 14, 112-123.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77, 1-124.

STARKE, K., ENDO, T., TAUBE, H. D. and BOROWSKI, E. (1975). Presynaptic receptor systems on noradrenergic nerves. IN: Chemical Tools in Catecholamine Research, Almergren, O., Carlssen, A. and Engle, J. (Eds), 2, 193-200. Amsterdam-Oxford North Holland.

STARKE, K., MONTEL, H. and WAGNER, J. (1971). Effect of phentolamine on noradrenaline uptake and release. Naunyn-Schmiedeberg's Arch. Pharmacol. 271, 181-192.

STEIN, J. H., CONGBALAY, R. C., KARSH, K. L., OSGOOD, R. W. and FERRIS, T. F. (1972). The effect of bradykinin on proximal tubular sodium reabsorption in the dog. Evidence for functional nephron heterogeneity. J. Clin. Invest. 51, 1709-1721.

STREWLER, G. J., HINRICHS, K. J., GUIOD, L. R. and HOLLENBERG, N. K., (1972). Sodium intake and vascular smooth muscle responsiveness to norepinephrine and angiotensin II in the rabbit. Circ. Res. 31, 758-766.

TERRAGNO, N. A. and TERRAGNO, A. (1979). Release of vasoactive substances by kinins. IN: Handbook of Experimental Pharmacology, Erdös, E. G. (Ed), 25, Suppl., 402-426, Springer-Verlag.

THOENEN, H., HURLIMANN, A. and HAEFELY, W. (1965). The effect of angiotensin on the response to postganglionic sympathetic stimulation of the cat spleen; lack of facilitation of norepinephrine liberation. Med. Pharmacol. Exp. 13, 379-387.

TRENDELENBURG, U. (1977). Catecholamine metabolism and vascular reactivity: an analysis of neuronal and extraneuronal storage and metabolizing systems. IN: Factors Influencing Vascular Reactivity by O. Carrier and S. Shibatai, Tokyo, Igaku-Shoin.

TURKER, R. K. (1973). Effect of angiotensin on the response to norepinephrine and peri-arterial stimulation of the isolated perfused cat terminal ileum. Europ. J. Pharmacol. 21, 171-177.

TURKER, R. K., YAMAMOTO, M., BUMPUS, F. M. and KHAIRALLAH, P. A. (1971). Lung perfusion with angiotensin I and II. Evidence of release of myotropic and inhibition substances. Circ. Res. 28, 559-567.

VANE, J. R. (1966). The estimation of catecholamines by biological assay. Pharmacol. Rev. 18, 317-324.

VANE, J. R. (1969). The release and fate of vaso-active hormones in the circulation. Brit. J. Pharmacol. 35, 209-242.

WARD, P. E. and MILLS, I. H. (1975). Distribution of renal kininases in relation to the kallikrein-bradykinin system and water excretion. J. Endocrinol. 67, 60P.

WAUGH, W. H. (1972). Angiotensin II: Local renal effects of physiological increments in concentration. Can. J. Physiol. Pharmacol. 50, 711-716.

WESTFALL, T. C. (1977). Local regulation of adrenergic transmission. Physiol. Rev. 57, 659-728.

WHITBY, L. G., AXELROD, J. and WEIL-MALHERBE, H. (1961). The fate of H^3-norepinephrine in animals. J. Pharmacol. Exp. Ther. 132, 193-201.

WINER, N., CHOKSHI, D. S., YOON, M. S. and FREEDMAN, A. D. (1969). Adrenergic receptor mediation of renin secretion. J. Clin. Endocrinol. Metab. 29, 1168-1175.

ZIMMERMAN, B. G. (1962). Effect of acute sympathectomy on responses to angiotensin and noradrenaline. Circ. Res. 11, 780-787.

ZIMMERMAN, B. G. (1967). Evaluation of peripheral and central components of action of angiotensin II on the sympathetic nervous systm. J. Pharmacol. Exp. Ther. 158, 1-10.

ZIMMERMAN, B. G. and GISSLEN, J. (1968). Pattern of renal vasoconstriction and transmitter release during sympathetic stimulation in presence of angiotensin and cocaine. J. Pharmacol. Exp. Ther. 163, 320-329.

ZIMMERMAN, B. G., GOMER, S. K. and LIAO, J. C. (1972). Action of angiotensin on vascular adrenergic nerve endings: Facilitation of norepinephrine release. Fed. Proc. 31, 1344-1350.

ZIMMERMAN, B. G. and GOMEZ, J. (1965). Increased response to sympathetic stimulation in the cutaneous vasculature in presence of angiotensin. Int. J. Neuropharmacol. 4, 185-193.

ZIMMERMAN, B. G. and WHITMORE, L. (1967). Effect of angiotensin and phenoxybenzamine on release of norepinephrine in vessels during sympathetic nerve stimulation. Int. J. Neuropharmacol. 6, 27-38.