STUDIES OF THE INTERACTION BETWEEN THE RENIN-ANGIOTENSIN-SYSTEM AND SYMPATHETIC NERVOUS SYSTEM IN MAN

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DECLARATION

I declare that the thesis has been composed by myself. The work described in the thesis was performed by myself alone or with coworkers as follows. The study described in chapter 3 was performed by myself, Dr S. Pai and Dr A.D. Struthers. The studies described in chapters 4-6 were performed in full and equal collaboration by myself and Dr J. McMurray (Research Fellow, Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee). The studies described in chapters 7-9 were performed by myself alone. The studies described in chapters 10-12 were performed by myself in the laboratory of and with the collaboration of Dr D.J. Webb (Lecturer, Department of Clinical Pharmacology, St. Georges Hospital Medical School, London).

I confirm that I have not presented the studies in the thesis in candidature for any other degree, diploma or professional qualification.

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SECTION THREE: DISCUSSION

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PREFACE

Experimental animal evidence suggests that there is a major interaction between the renin-angiotensin system and the sympathetic nervous system. The present studies investigate whether this interaction occurs in healthy man. The activity of the renin-angiotensin system was altered by infusion of low doses of angiotensin II, angiotensin converting enzyme inhibitors and saralasin infusion. The sympathetic nervous system was stimulated either by physiological stimuli or by infusion of noradrenaline, tyramine or isoprenaline. The studies were performed on the general circulation by intravenous infusion and on the localised circulation of the forearm by very low dose infusion into the brachial artery.

ABSTRACT

There is considerable evidence for an interaction between the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) from animal studies but the evidence in man is conflicting. The present investigations sought evidence for such an interaction in healthy man. The studies were in two main groups, those that sought a postsynaptic interaction whereby angiotensin II (AII) augments the effect of released noradrenaline (NA), and those that sought a presynaptic interaction whereby AII augments the release of NA.

The first study sought a postsynaptic interaction by infusion of AII and NA alone and in combination. There was a significant synergistic effect of AII/NA with respect to systolic blood pressure (BP). There was no interaction with regard to diastolic BP. The fact that this interaction was limited to systolic BP suggests that this interaction occurs in the myocardium rather than the peripheral vasculature or may be an effect on intravascular volume. A renal AII/NA interaction causing retention of sodium/fluid might account for this.

A series of experiments were performed to investigate a possible renal tubular antinatriuretic interaction. These showed that both AII and NA had direct antinatriuretic effects. There was however no synergistic antinatriuretic effect. It seems unlikely that the postsynaptic AII/NA interaction is due to sodium/fluid retention.

The next study therefore investigated the possibility that AII augments the inotropic response to beta receptor stimulation. The combination of AII/isoprenaline caused a marked increase in stroke volume above that seen with isoprenaline alone. There are several possible mechanisms but a regional redistribution of venous blood from the splanchnic bed increasing cardiac filling seems most likely.

In contrast when looking for a presynaptic interaction AII did not alter the release of NA nor haemodynamic responses to physiological stimulation by bicycle exercise, cold pressor test, forearm isometric handgrip, standing from lying nor pharmacological stimulation of NA release by tyramine infusion.

These studies relied on measurement of plasma NA as an index of sympathetic tone which has recognised limitations. The next studies sought evidence for a presynaptic interaction by an alternative methodology. By very low dose infusion of hormone into the brachial artery the isolated forearm vasculature of man in vivo was studied. It was shown that AII did not augment the vasoconstriction caused by the incremental infusion of NA. By contrast AII was shown to augment the vasoconstrictor response to sympathetic stimulation by lower body negative pressure. These studies suggest that in the peripheral vasculature AII acts by a presynaptic mechanism, probably through increased NA release.

Conclusions: These studies suggest that AII increases the presynaptic local release of NA but this effect is not of sufficient magnitude to spill over into plasma altering venous plasma NA. With regard to the effect of NA after it has been released (postsynaptic effect), AII does not influence the effects of NA on the peripheral resistance vessels, but synergistically augmentes the rise in systolic BP seen with NA and increases the effect of beta-agonism on stroke volume. In intact man during systemic infusion a regional redistribution of venous blood from the splanchnic bed increasing cardiac filling seems the most likely mechanism.

LIST OF ABBREVIATIONS

AII	-	angiotensin II
ACE	-	angiotensin converting enzyme
BP	-	blood pressure
FBF	-	forearm blood flow
HR	-	heart rate
ISO	-	isoprenaline
LBNP	-	lower body negative pressure
NA	-	noradrenaline
RAS	-	renin-angiotensin system
SNS	-	sympathetic nervous system
SV	-	stroke volume
TYR	-	tyramine

A list of abbreviations relevant to the renal sodium excretion studies described in chapters four to six may be found on page 70

OVERVIEW OF INVESTIGATIONS

Each study has been coded for the following features:

1. Systemic or local: This describes whether the study involves systemic intravenous infusions and measures haemodynamic and venous plasma hormone responses to infusion and/or stimuli or whether the study involves the local intra-arterial infusion of drug and measures the local forearm blood flow response to infusion and/or stimulus

2. Manipulation of the renin-angiotensin system: This describes whether the study has involved "activating" the RAS by AII infusion or salt depletion thereby comparing normal AII with Raised AII or whether the "activity" of the RAS has been reduced by pharmacological interruption thereby comparing normal AII with Low AII.

3. Manipulation of the sympathetic nervous system: The method of stimulation of the SNS has been given. Stimulation was achieved by infusion of pharmacological agents or NA, or by "physiological" stimuli.

4. Site of interaction investigated: The site of interaction investigated by the study is indicated. Broadly this may be either presynaptic, whereby AII enhances the release of NA, or postsynaptic whereby AII augments the effects of previously released NA. There is considerable overlap for site of interaction in the studies and the reader is referred to the Discussion for further information.

The OVERVIEW OF INVESTIGATIONS is intended as a quick reference guide for the reader to give a general plan of the studies in this thesis. The overview is given below and at the beginning of the Discussion (Section 3; chapter 13).

OVERVIEW OF INVESTIGATIONS

Chapter number:

3. The effect of intravenous infusion of angiotensin II and noradrenaline alone and in combination on blood pressure, heart rate and plasma noradrenaline.
[Systemic: Raised AII: NA infusion: Postsynaptic]

4. The effect of angiotensin II on renal sodium excretion.
[Systemic: Raised AII: None: Direct AII action]

5. The effect of noradrenaline on renal sodium excretion.
[Systemic: None: NA infusion: Direct NA action]

6. The effect of angiotensin II and noradrenaline alone and in combination on renal sodium excretion.
[Systemic: Raised AII: NA infusion: Postsynaptic]

7. The effect of angiotensin II on the stroke volume response to beta agonism.
[Systemic: Raised AII: Beta-agonist infusion: Postsynaptic]

8. The effect of angiotensin II on endogenous noradrenaline release.
[Systemic: Raised AII: Physiological stimuli of SNS: Presynaptic]

9. The effect of angiotensin II on the haemodynamic and plasma noradrenaline responses to tyramine infusion.
[Systemic: Raised AII: NA release by tyramine infusion: Presynaptic]

10. The effect of local angiotensin II on the forearm blood flow response to noradrenaline.
[Local: Raised AII: NA infusion: Postsynaptic]

11. The effect of local angiotensin II on the forearm blood flow response to lower body negative pressure.
[Local: Raised AII: Physiological stimulus (LBNP): Presynaptic]

12. Studies employing pharmacological interruption of the renin-angiotensin system.

(a) The effect of local angiotensin converting enzyme inhibition on the forearm blood flow response to lower body negative pressure.
[Local: Low AII: Physiological stimulus (LBNP): Role of local ACE activity]

(b) The effect of systemic angiotensin converting enzyme inhibition and reinfusion of angiotensin II on the forearm blood flow response to lower body negative pressure
[Local: Low AII: Physiological stimulus (LBNP): Role of circulating ACE]

(c) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium replete subjects.
[Local: Low AII: Physiological stimulus (LBNP): Presynaptic]

(d) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium deplete subjects.
[Local: Raised AII: Physiological stimulus (LBNP): Presynaptic]

SECTION ONE: INTRODUCTION

CHAPTER ONE

General Introduction

The stimulus for the studies presented in this thesis derived from my developing clinical interest in the treatment of hypertension and heart failure. With the wider recognition of the neuro-endocrine changes that occur in these pathological conditions a number of approaches have been used to treat hypertension and heart failure more or less specifically. These include the sympathetic nervous system (SNS) blocking agents with varied sites of action including prazosin, bethanidine, guanethidine, beta-blocking agents, methyldopa and clonidine. The potent angiotensin converting enzyme (ACE) inhibitors interfere with the renin-angiotensin-system (RAS). The calcium channel blocking agents, hydrallazine, nitrate therapy and diuretic therapy also have important roles to play in the management of hypertension and/or heart failure.

Reviewing the literature it is clear that the complex physiological and pathophysiological interactions between different components of the neurohormonal systems involved remain uncertain. This thesis reports studies to systematically investigate the interaction between the RAS and the SNS in healthy human volunteers.

THE RENIN-ANGIOTENSIN-SYSTEM (RAS).

(Reviews: Laragh and Sealey, 1973; Oparil and Haber, 1974; Davis and Freeman, 1976; Fraser et al, 1979; Haber and Carlson, 1983; Hall et al, 1986)

The RAS is a major factor in the short and longterm regulation of arterial pressure. This is achieved by a hormonal cascade system which has been characterised in detail and is illustrated in figure 1.1. Renin is a circulating enzyme of molecular weight 40,000 daltons in its active form,

Figure 1.1

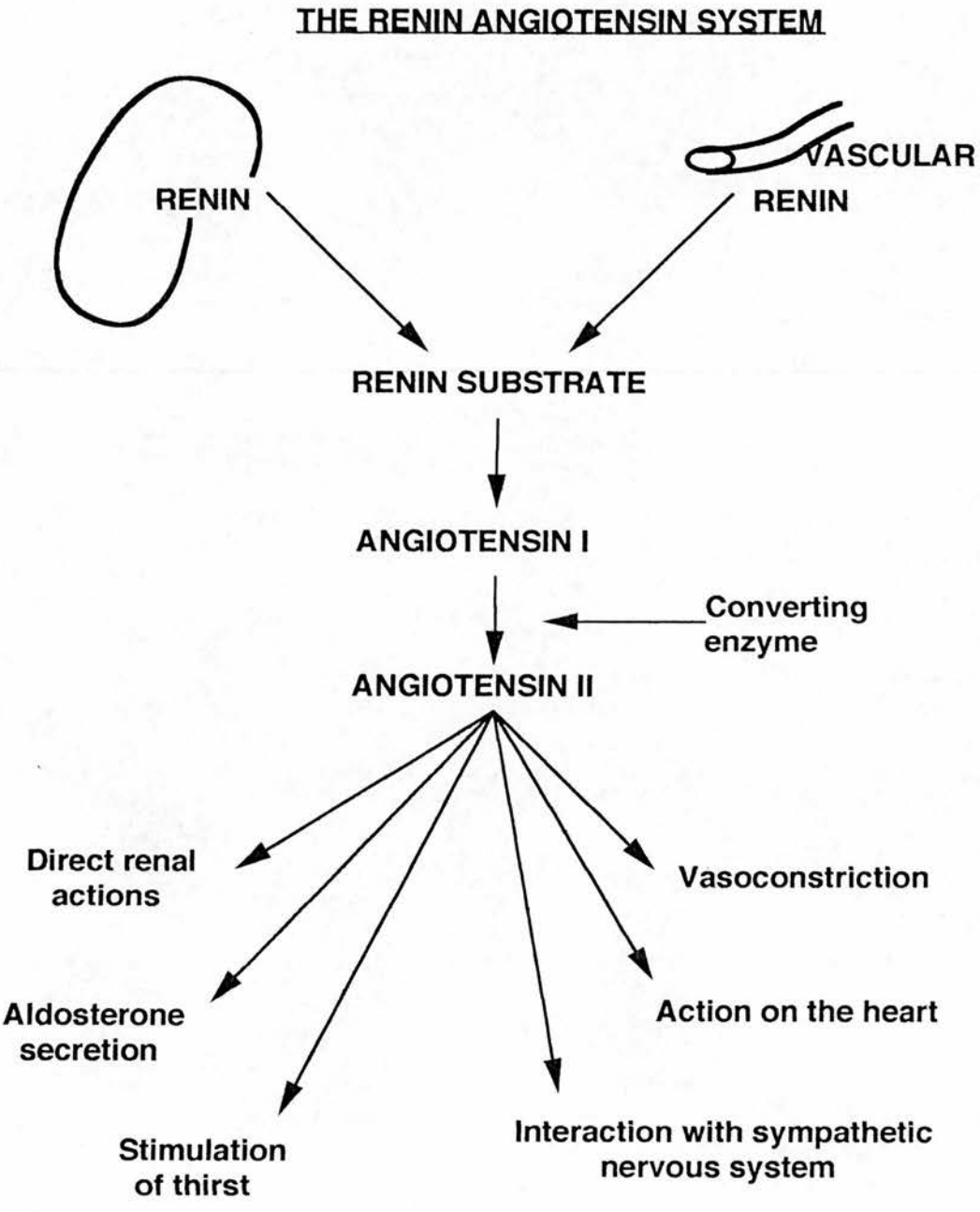

THE RENIN ANGIOTENSIN SYSTEM
RENIN
VASCULAR
RENIN
RENIN SUBSTRATE
ANGIOTENSIN I
Converting
enzyme
ANGIOTENSIN II
Direct renal
actions
Vasoconstriction
Aldosterone
secretion
Action on the heart
Stimulation
of thirst
Interaction with sympathetic
nervous system

Figure 1.1 legend

The Renin-angiotensin system. Renin from renal and vascular sources acts on circulating renin-substrate to produce the inactive decapeptide angiotensin I. In the presence of angiotensin converting enzyme angiotensin I is further split to give the active octapeptide angiotensin II.

Angiotensin II has many biological activities. It acts (1) on the adrenal cortex to release aldosterone, (2) directly on the kidney to cause efferent arteriolar constriction and affects renal tubular function, (3) to cause central stimulation of thirst and also causes water retention by stimulating the release of antidiuretic hormone from the posterior pituitary, (4) directly on vascular smooth muscle receptors to cause vasoconstriction of resistance vessels, (5) has an inotropic effect on the myocardium and (6) interacts with the sympathetic nervous system to augment the release and effect of noradrenaline.

produced mainly in the juxta glomerular cells of the afferent arteriole in the kidney. Renin is specific for and acts to cleave renin substrate (a circulating alpha$_2$-globulin, angiotensinogen) to produce the inactive decapeptide angiotensin I (A-I). A-I itself is of little biological activity though in high concentrations A-I may alter intrarenal blood flow, stimulate thirst and have a central pressor effect. The next step in the cascade is the cleavage of A-I to the octapeptide angiotensin II (AII). This is performed by angiotensin converting enzyme (ACE) which is located in endothelial plasma membranes. The main source of ACE is in the pulmonary vasculature though it has recently been shown that ACE and other components of the RAS are also located in a variety of tissues including brain and heart (Campbell, 1987; Jin et al, 1988). The AII generated is the primary active component of the RAS hormonal cascade (figure 1.1) and is discussed shortly. AII has a short half-life in the circulation estimated to be approximately 15-20 seconds or one circulation time. AII is rapidly taken up into tissues and enzymatically degraded or is broken down within the circulation by angiotensinases to angiotensin III (AIII) and smaller inactive fragments. A-III is a heptapeptide which retains some physiological activity. A-III binds tightly to AII receptors and can stimulate aldosterone release and has a weak pressor effect. The physiological role of AIII remains in doubt.

Control of the RAS cascade occurs at the beginning with renin release; no other stage is thought to be rate limiting though may be targets for pharmacological interference. Davis and Freeman (1976) have reviewed the mechanisms of control of renin secretion in detail. There are three main mechanisms influencing renin release from the kidney.

(1) Via intrarenal receptors which are of two types. Firstly renal vascular receptors which respond to changes in wall tension in the

afferent arteriole. The receptors are influenced by changes in transmural pressure, alterations in diameter of the afferent arteriole, renal sympathetic activity that alters renal arteriolar tone and to intrinsic myogenic factors. The second receptor is the macula densa which is influenced by changes in sodium and chloride in the tubular lumen and appears to respond to changes in electrolyte load rather than changes in concentration.

(2) The renal sympathetic nerves which end in the juxtaglomerular cells and in the smooth muscle of the renal arterioles. They alter renin release by exerting a direct action on the juxtaglomerular apparatus, by effecting renal arteriolar tone and the vascular receptor, and by altering glomerular capillary pressure and the filtered sodium load.

(3) Several humoral agents alter renin release including AII, vasopressin, adrenaline and noradrenaline (NA), sodium and potassium ions and prostaglandins.

Feedback control thus occurs. An increase in AII concentration results in decreased renin secretion by increased sodium retention and an increase in extracellular fluid volume, direct negative feedback, direct and indirect systemic vasoconstriction and direct sodium load effects on the macula densa (Oparil and Haber, 1974).

ANGIOTENSIN II.

(Reviews: Laragh and Sealey, 1973; Severs and Daniels-Severs, 1973; Oparil and Haber, 1974; Page and Bumpus, 1974; Simpson, 1981; Goodfriend, 1983; Haber and Carlson, 1983; Mendelsohn. 1985)

AII is the main active component of the RAS cascade described above. AII is a polar octapeptide of molecular weight 1031 daltons. The

principal physiological actions are illustrated in figure 1.1. AII acts on specific receptors in all its target tissues including adrenal, vascular smooth muscle, myocardium, kidney, brain and liver. The cellular consequences of AII binding to receptor remain for the most part obscure. Calcium flux and turnover of phosphatidyl inositol have been implicated as the intracellular "second messenger" in the response of vascular smooth muscle and adrenal zona glomerulosa cells to AII.

AII has many biological activities. The main physiological actions are as a direct vasoconstrictor of resistance vessels and as a major stimulus to secretion of aldosterone from the adrenal zona glomerulosa. AII is one of the most potent pressor substances known. This action has a rapid onset occurring within 10-20 minutes of a stimulus such as acute haemorrhage causing hypotension. AII is responsible for increasing the release of aldosterone which in turn causes sodium retention and potassium loss via an action on the distal tubule and collecting duct in the kidney.

In addition AII has a number of other actions which have been described but of which the physiological significance is unclear. Thus as well as an immediate direct vasoconstrictor effect a separate "slow-pressor" effect occurring after prolonged infusion of AII is well recognised. A continuous infusion of small doses of AII into animals and man produces a progressive rise in blood pressure. In contrast infusion and boluses of high doses of AII are associated with tachyphlaxis. AII has an inotropic action on the myocardium which may affect arterial pressure directly.

AII has a number of effects that alter volume homeostasis which influences the longer term regulation of arterial pressure. These include several direct intrarenal effects on sodium and water excretion which may

influence volume homeostasis. AII causes direct stimulation of tubular transport of sodium and water and also causes efferent arteriolar constriction. The latter increases tubular reabsorption by altering the physical forces of the peritubular capillary and possibly by lowering vasa recta blood flow.

AII also has stimulatory effects on thirst and release of antidiuretic hormone. These actions may be effects of AII on the central nervous system. Access of circulating AII appears to be limited to structures lacking a blood brain barrier. The three susceptible areas are (1) the area postrema which mediates a pressor action of AII in many species, (2) the subfornical organ which provokes thirst, secretion of antidiuretic hormone and a pressor action in response to AII and (3) the organum vasculosum which may also mediate these effects.

The pressor effect of AII action on the central nervous system is mediated by increased sympathetic outflow. AII affects the sympathetic nervous system at other sites. AII stimulates sympathetic ganglia and causes adrenaline release from the adrenal medulla. In addition facilitation of sympathetic neurotransmission has been recognised as an additional mechanism whereby AII can affect cardiovascular function.

Many of these effects of AII occur only at pharmacological doses or may be demonstrated only under abnormal experimental conditions and their physiological importance is uncertain. However the facilitation of sympathetic neurotransmission may occur at levels of AII within the physiological range and is discussed in detail below.

ANGIOTENSIN FACILITATION OF SYMPATHETIC NEUROTRANSMISSION

The evidence in experimental animals

It has been recognised for almost three decades that AII can facilitate peripheral sympathetic neurotransmission. In experiments on the dog hindpaw Zimmerman (1962) showed that the pressor response to AII was reduced by acute sympathectomy. Importantly the response to both NA and tyramine remained unchanged. In addition stimulation of the cut lumbar sympathetic nerves restored the pressor response to AII in most instances. That the effect on peripheral sympathetic neurotransmission can be considered separately from any putative central effect was also demonstrated at an early stage. Zimmerman (1967) showed that in conditions in which AII caused a marked increase in the pressor response to peripheral nerve stimulation in cutaneous and renal vascular beds of the dog there was no response to intracarotid and intravertebral injection of AII. The extensive work in isolated tissue preparations and experimental animal preparations has recently been reviewed (Westfall, 1977; Vanhoutte et al, 1981; Zimmerman et al, 1984: van Zwieten and de Jonge, 1986).

The effects of AII on peripheral sympathetic neurotransmission include potentiation of release of NA both from unstimulated nerves and in response to nerve stimulation, blockade of reuptake of NA, potentiation of the postsynaptic effects of NA and increased NA synthesis (figure 1.2).

Potentiation of release of noradrenaline from unstimulated nerves. Several laboratories have shown that AII can cause the release of NA from presynaptic sites. In early experiments Baum (1963) showed that the vasoconstrictor activity of AII was reduced in dogs pretreated with reserpine and Distler and coworkers (1965) showed that AII caused NA depletion of porcine arterial strips and that the contractile effect of

Figure 1.2

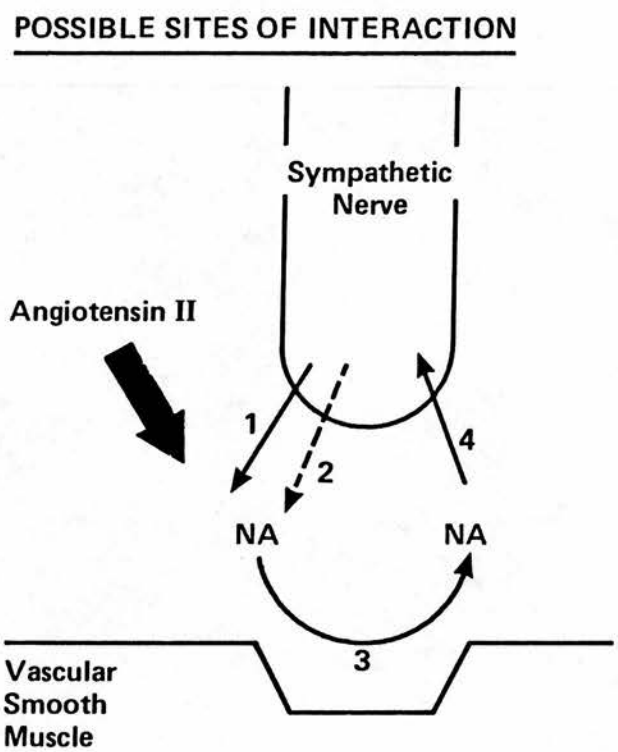

POSSIBLE SITES OF INTERACTION
Sympathetic Nerve
Angiotensin II
1
2
3
4
NA
NA
Vascular Smooth Muscle

Figure 1.2 legend

Possible sites of interaction between the renin-angiotensin system and the sympathetic nervous system. (1) Mobilisation and release of endogenous noradrenaline (NA) from presynaptic sites. (2) The sensitisation of vascular postsynaptic adrenoceptors. (3) The blockade of the neuronal reuptake of NA. (4) The presynaptic facilitation of the amount of NA released per nerve impulse.

AII was reduced by pretreatment with reserpine. It was concluded that the action of AII on vascular smooth muscle was mediated by liberation of NA from the sympathetic nerve endings. Later Drimal and Boska (1973) showed that the positive inotropic action of AII in the dog heart was reduced by prior NA depletion by reserpine. They suggest that AII acts in part by causing the release of NA in a tyramine-like fashion. Palaic and Panisset (1969) demonstrated that high doses of AII increase the release of ^{3}H-labelled NA and reduced reuptake and retention of NA in the unstimulated vas deferens of the guinea pig. Hughes and Roth (1971) demonstrated a small increase (5-10%) in the basal efflux of ^{3}H-labelled NA from isolated rabbit portal vein and coeliac artery however the same dose of AII increased the stimulated ^{3}H-labelled NA efflux by 30-150%. Knape and van Zwieten (1987) showed that reserpine pretreatment reduced the pressor response to high doses of AII in pithed rats in which the central nervous system effects can be excluded. In addition bilateral adrenalectomy did not alter the pressor response to AII. They conclude that part of the vasoconstriction at high doses of AII is caused by the activation of presynaptic AII receptors in sympathetic nerve terminals resulting in catecholamine release.

These studies and others indicate that AII may cause the release of preformed NA in a tyramine like manner. However it is also clear that the effect occurred only at very high doses of AII and in isolated tissue preparations and abnormal circumstances such as pithed rat preparations. It must be concluded that this effect of AII does not play a role under physiological conditions.

Potentiation of release of noradrenaline during nerve stimulation. The prejunctional facilitation of sympathetic neurotransmission in vascular smooth muscle by enhancement of the amount of NA released per nerve

impulse is well supported by a large volume of published work from many laboratories. Many authors have shown that the pressor response to peripheral nerve stimulation is potentiated by AII at doses which cause little or no change in the response to exogenous NA (Zimmerman, 1962; Review: Zimmerman, 1978; Panisset and Bourdois, 1968; Kadowitz et al, 1971; Antonaccio and Kerwin, 1981; Clough et al, 1982; de Jonge et al, 1982; Story and Ziogas, 1986). Other studies have confirmed that AII causes an increased release of radiolabelled NA during nerve stimulation and have confirmed this mechanism (Palaic and Panisset, 1969; Starke, 1971; Hughes and Roth, 1971; Zimmerman et al, 1972; Ziogas et al, 1985). This potentiating effect of AII on NA release during nerve stimulation can be blocked by saralasin, an AII analogue and antagonist (Zimmerman, 1973; Blumberg et al, 1975; Malik and Nasjletti, 1976; Review: Westfall, 1977; Kawasaki et al, 1982; Ziogas et al, 1985).

This finding suggests that the presynaptic facilitation is mediated by presynaptic AII receptors, located at sympathetic nerve terminals in vascular smooth muscle. Furthermore these effects have been demonstrated to occur at low circulating levels of AII that may well be of physiological and pathophysiological significance.

Blockade of reuptake of noradrenaline. AII was shown to block uptake of NA in tissue slices (Palaic and Khairallah, 1967a; Palaic and Khairallah, 1967b) and this was therefore postulated as an early explanation of the facilitating action of AII on sympathetic neurotransmission (Khairallah, 1972; Zimmerman and Gomez, 1965; Panisset and Bourdois, 1968). However this effect was seen at high doses of AII and was not seen at lower doses in preparations that showed potent inhibition of uptake by cocaine, a known inhibitor (Pals and Masucci, 1968; Schumann et al, 1970; Starke, 1970). Also the potentiating

effect of AII persisted in the presence of an uptake inhibitor, whereas the response to a second uptake inhibitor was abolished (Schumann et al, 1970; Starke, 1970). These findings are therefore incompatible with the inhibition of NA uptake as a major action of AII.

Potentiation of the postsynaptic effects of noradrenaline. A postsynaptic facilitation of sympathetic neurotransmission by AII has also been postulated. The evidence for this effect stems from three observations. Firstly that AII potentiates the response to exogenous NA in a number of tissue and vascular preparations. Secondly that reduction in AII by treatment with ACE inhibitors or its pharmacological blockade with saralasin reduces the response to exogenous NA in similar preparations including pithed rats. Thirdly that after treatment with ACE inhibitors or saralasin, infusion of AII restores the response to NA (Panisset and Bourdois, 1968; Zimmerman, 1973; Clough et al, 1983; de Jonge et al, 1983; Kaufman and Volmer, 1985; Weber et al, 1989). One repeated finding in such studies is that where investigated in the same preparation the facilitatory action of AII during nerve stimulation is more consistent and is of considerably greater magnitude (Zimmerman and Gomez, 1965; Malik and Nasjletti, 1976; Kawasaki et al, 1982; Zimmerman et al, 1987; Isaacson and Reid, 1990). However many studies demonstrating a facilitatory effect of AII during nerve stimulation have failed to show an effect of AII on exogenous NA (Benelli et al 1964; Bell, 1972; Kadowitz et al, 1972; Zimmerman et al, 1972; Turker, 1973; Johnson et al, 1974; Furukawa et al, 1983).

Taken together these findings suggest that there is a specific postsynaptic interaction of AII facilitating the effects of released NA, but which is of lesser magnitude than the presynaptic interaction demonstrable during nerve stimulation, and appears to occur at high rather than

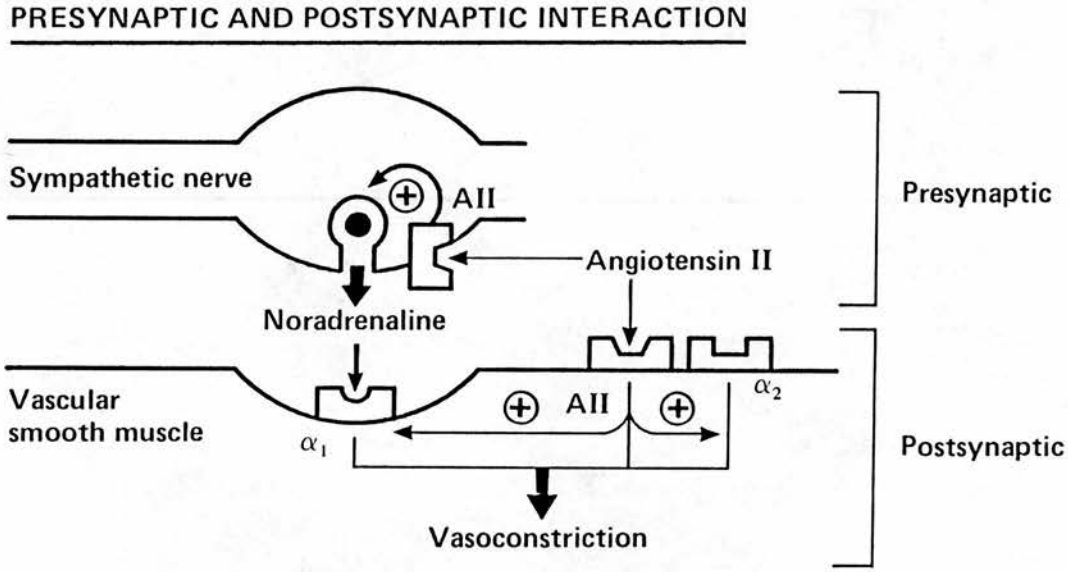
reduced AII levels. This action therefore may be relevant only in pathophysiological circumstances.

Increased noradrenaline synthesis. AII increases the biosynthesis of NA at levels which have no effect on release of NA in innervated tissues such as rat and guinea pig atria or rat vas deferens. The process appears to occur at the tyrosine hydroxylase step and may involve the synthesis of new enzyme protein. This mechanism therefore may act as a long-term homeostatic mechanism, whereas the facilitation of transmitter release is more likely to be responsible for immediate and short term actions of AII (Roth, 1972).

Summary. These findings are summarised in figure 1.3 representing the mechanisms of interaction of the RAS and SNS (after van Zweiten and de Jonge, 1986). The diagram emphasises the two interactions that appear to be of most physiological relevance on the basis of the animal experimental evidence. That is firstly the presynaptic facilitation of NA release by AII, and secondly the postsynaptic sensitisation of adrenoceptors, the $alpha_1$-receptors represented within the synaptic cleft and the $alpha_2$-recptors outwith the synaptic cleft.

The work in animals has not only revealed a number of sites of interaction but also Zimmerman et al (1984) emphasise in their review that a significant interaction between the RAS and the SNS occurred only in circumstances in which the RAS was activated either by infusion of exogenous AII, or by studying salt deplete animals or pithed rats in whom the plasma renin activity is increased (de Jonge et al, 1982). Despite this conclusion investigators who have previously sought evidence for a RAS/SNS interaction in man have usually done so in subjects with normal RAS activity and have suppressed this further by administering an ACE inhibitor or infused saralasin.

Figure 1.3



[after van Zweitan and de Jonge 1986]

Figure 1.3 legend

Schematic representation of the presynaptic and postsynaptic effects of angiotensin II on adrenergic nerve endings. The stimulation of presynaptic angiotensin II receptors enhances the release of noradrenaline from presynaptic sites. In addition postsynaptic $alpha_1$- and $alpha_2$-adrenoceptor responses are facilitated by postsynaptic angiotensin II receptors.

[after van Zwieten P.A. & de Jonge A., 1986]

The evidence in human studies

The evidence in man is conflicting. This is in part because of the limitations of experimentation in man. Information has been obtained from manipulation of the RAS and SNS either separately or simultaneously. Thus data have been obtained from the effects of AII infusion and the use of pharmacological inhibitors of the RAS and these have been coupled with the use of "physiological stimuli" of the SNS such as cold pressor testing or with pharmacological stimuli or blockade of the SNS.

Infusion of angiotensin II. Infusion of AII alone produces no change in plasma NA in healthy man (Beretta-Piccoli et al, 1980; Nicholls et al, 1981; Mendelsohn et al, 1980) and hypertensive man (Beretta-Piccoli et al, 1980). This has been interpreted as showing no interaction between the RAS and the SNS (Nicholls et al, 1981). However in studies were other pressor agents have been infused, such as phenylephrine, plasma NA falls as the pressor effect increases (Eckberg et al, 1986). Therefore the finding that during infusion of AII plasma NA levels are maintained may be the first evidence that an RAS/SNS interaction occurs in man.

Treatment with angiotensin converting enzyme inhibitors. Most other investigations in man have used ACE inhibitors and have produced further conflicting results. Treating normal volunteers with ACE inhibitors usually causes no change in plasma NA (Ajayi et al, 1985; Millar et al, 1981; Millar et al, 1982; Niarchos et al, 1982; Campbell et al,1985) but others have shown an increase (MacGregor et al, 1981; Ibsen et al, 1983). Similar findings occur in hypertensive subjects, ACE inhibition usually causing no change in plasma NA (Manhem et al, 1981; Millar et al, 1981; Morganti et al, 1980; Zanella et al, 1981; Bravo and Tarazi, 1979) or occasionally increased plasma NA (Hulthen and Hokfelt, 1978; Mohara et al, 1986).

The response of patients with heart failure has been rather more

consistent in that treatment with ACE inhibitors has uniformly been associated with reductions in plasma NA (Curtiss et al, 1978; Turini et al, 1979; Cody et al, 1982; Fitzpatrick et al, 1983; Cleland et al, 1984). However these results do not support a direct RAS/SNS interaction as ACE inhibitor treatment causes marked improvement in haemodynamic indices in patients with heart failure which in itself would produce reduction in plasma NA.

Infusion of saralasin. Infusion of saralasin, an AII antagonist, is confounded by partial agonist activity. Infusion in man causes either an increase in plasma NA (McGrath et al, 1977; Vlachakis et al, 1978) or no change in plasma NA (Carey et al, 1978).

Responsiveness to noradrenaline after ACE inhibitor treatment. Conflicting results have also been obtained when assessing the pressor responsiveness to NA infusion after ACE inhibitor treatment. In one study the responsiveness was unchanged (Vierhapper et al, 1986) and in another the pressor responsiveness was diminished, but interestingly could be restored by infusion of AII (Imai et al, 1982). Responsiveness to NA infusion after ACE inhibition is also reduced in hypertensive subjects (Fruncillo et al, 1985).

Physiological stimulation of the SNS after ACE inhibitor treatment. In studies in which the SNS has been stimulated physiologically or pharmacologically, ACE inhibitor treatment has been shown to have no effect on plasma NA in healthy man (Ibsen et al, 1983; Millar et al, 1982; Niarchos et al, 1982; Becker et al, 1986) though in one study the plasma NA response to standing from supine was increased by ACE inhibitor treatment but to none of the other manoeuvres investigated (Reid et al, 1983). In hypertensive patients the plasma NA response to physiological stimulation may be unaffected (Manhem et al, 1981) or

increased (Morganti et al, 1980; Zanella et al, 1981) by treatment with an ACE inhibitor.

There is therefore a wealth of experimental animal evidence supporting a major interaction between the RAS and the SNS. However there is much conflicting data in the literature reporting studies in man. This thesis reports studies to systematically investigate the interaction between the RAS and the SNS in healthy man.

CHAPTER TWO

CHAPTER TWO

SUBJECTS AND METHODS

SUBJECTS

All subjects were healthy normal volunteers. All had a normal medical assessment including physical examination, biochemical and haematological profile, urinalysis and electrocardiogram. None were on regular medication of any sort. Subjects were required to abstain from self medication prior to and during the course of the studies. Subjects were also required to abstain from alcohol for 36 hours before each study day and to abstain from caffeine containing drinks and smoking on the morning of the study. Sodium intake was not strictly controlled but each subject was asked to adhere to their usual diet throughout the study period and, where possible to maintain a similar pattern of meals for three days prior to each study day which was assessed by estimation of 24 hour urinary sodium excretion.

Consent

All subjects were provided with a study information sheet and after explanation gave written informed consent.

Ethical approval

All studies were performed after approval by the Medical and Dental research ethics committee of the University of Dundee, or the Medical research ethics committee of St. Georges Hospital and Medical School, London.

METHODS

The following procedures and analyses were performed by myself.

Intravenous infusion

Intravenous cannulae were placed into antecubital veins using 1% lignocaine (Antigen Ltd, Ireland) to provide local anaesthesia. Intravenous infusions were given at the indicated rate by means of constant rate infusion pumps (Perfusor E Secura, B. Braun Melsungen AG, West Germany).

Measurement of blood pressure and heart rate

Throughout each experiment the electrocardiogram and heart rate were continuously monitored by an ECG oscilloscope (Hewlett Packard, USA) and blood pressure was recorded semi-automatically (Dinamap - Vital signs monitor 1846, Critikon, Tampa, Florida, USA).

Measurement of cardiac output

Cardiac output was measured by a non-invasive acetylene rebreathing technique (Irvine et al, 1983). This technique was chosen as a reproducible non-invasive measurement that avoids the need to place pulmonary artery catheters or inject radioisotope on repeated occasions in normal volunteers, procedures that are not ethically appropriate. The intrasubject coefficient of variation (CV) for the measurement of cardiac output was 10.7%, calculated for the five repeated measures recorded on the dual placebo day by oneway analysis of variance where CV = [mean intrasubject error / global mean].

Oral hydration protocol

A standard oral hydration protocol was used (Roberts and Daneshmend, 1981). An initial water load of 15-20 mls/kg was given to drink over 10 minutes. Subsequently the subject stood to pass urine every 20 minutes until the end of the study. An aliquot of each urine sample was saved for later analysis. After voiding, a replacement volume of water equal to that of urine passed was given to drink. In this way a steady state water diuresis was established over an equilibration period of 2 hours (six 20 min urinary clearance periods).

The following excretion and clearance indices were calculated in the standard manner (Roberts and Daneshmend, 1981). Clearance (C) was calculated as UV / P, where U = urinary concentration, V = urinary flow rate and P = plasma concentration. Creatinine clearance (C_{Cr}) was used as a measure of glomerular filtration rate (GFR). Fractional excretion (FE) was calculated as absolute excretion divided by C_{Cr}. Lithium clearance (C_{Li}) was used to estimate proximal tubular outflow (Thomsen, 1984). Osmolar clearance (C_{osm}) was calculated as $U_{osm}V/P_{osm}$. Distal delivery (DD) of sodium was calculated according to the conventional formula $C_{Na}+C_{H2O}$ and fractional distal delivery (FDD) as $(C_{Na}+C_{H2O})/C_{Cr}$. Absolute resorption of sodium by the distal nephron (RD_{Na}) was calculated as $(C_{Li}-C_{Na})xP_{Na}$ and fractional reabsorption as sodium by the distal nephron (FRD_{Na}) as $(C_{Li}-C_{Na})/C_{Li}$

Lower body negative pressure

Lower body negative pressure (LBNP) was used as a method of stimulating sympathetic vasoconstriction in a number of studies described in this thesis and is discussed here in detail.

Method used: LBNP was applied using the method described by

Brown et al (1966). Subjects rested supine with a plastic-covered steel cage enclosing the lower limbs and hips and sealed above the level of the anterior superior iliac spines. Suction was applied by an industrial vacuum to produce a constant negative pressure of 20 cm of water (15 mmHg). The alteration from atmospheric pressure was both applied and relieved rapidly. This degree of LBNP was chosen as it reduces forearm blood flow without affecting systemic blood pressure or heart rate and without activating carotid baroreceptors (vide infra).

Cardiovascular responses to LBNP: The present day technique of LBNP was described in the early 1960's to study responses to gravitational change (Stevens and Lamb, 1965; Brown et al, 1966). The cardiovascular responses are well established. LBNP causes blood pooling in lower limbs, buttocks and pelvis demonstrated by pooling of plasma bound ^{131}I (Wolthuis et al, 1974). This results in a fall in central venous pressure and an increase in forearm vascular resistance at all degrees of LBNP. Increasing degrees of negative pressure (from 20 mmHg up to 80 mmHg) cause an increase in heart rate, a fall in systolic blood pressure, pulse volume and, with the higher degrees of LBNP a fall in diastolic blood pressure (Steven and Lamb, 1965; Brown et al, 1966; Ardill et al, 1967; Johnson et al, 1974; Abboud et al, 1979). Stroke volume and cardiac output fall (Stevens and Lamb, 1965; Murray et al, 1968). At sustained high degrees of LBNP most subjects experience syncope or presyncope, and some subjects will experience syncope as low as 30 mmHg sustained LBNP (Murray et al, 1968). In contrast lesser degrees of LBNP (<20 mmHg), as used in this thesis, cause marked reductions in forearm blood flow without affecting arterial blood pressure (Zoller et al, 1972; Johnson et al, 1974). These workers showed, in studies where the degree of LBNP was increased through a range from low to high degrees, that approximately 70% of

maximal reduction in forearm blood flow occurred before onset of any change in arterial pressure (Zoller et al, 1972; Johnson et al, 1974).

Evidence for reflex sympathetic forearm vasoconstriction: The evidence that the vasoconstriction seen during LBNP is mediated via the SNS is threefold. Firstly the reduction in forearm blood flow can be abolished by the local arterial administration of bretylium tosylate (Brown et al, 1966) and is attenuated by bethanidine infusion or prior sympathectomy (Ardill et al, 1967). Secondly direct recordings of human forearm muscle sympathetic nerve activity have shown increased activity during LBNP (Sundlof and Wallin, 1978). Finally there is a rise in plasma NA during sustained LBNP which correlates with the of fall in central venous pressure (Goldsmith et al, 1982; Grassi et al, 1985).

Hormonal changes during LBNP: I have commented on the change in plasma NA. Plasma renin release is also stimulated at high degrees of LBNP that cause hypotension (Baylis et al, 1978; Mark et al, 1978). This release can be blocked by propranolol and is therefore mediated via the SNS (Mark et al, 1978). The onset of symptoms of syncope is associated with a marked increase in plasma antidiuretic hormone, but this does not occur in asymptomatic individuals (Baylis et al, 1978; Goldsmith et al, 1982).

It is therefore widely thought that low levels of LBNP act by unloading "low pressure" cardiopulmonary receptors responsive to falls in central venous pressure, resulting in reflex sympathetic constriction in forearm resistance vessels. Higher levels of LBNP unload these "low pressure" receptors but by reducing arterial pressure also unload carotid baroreceptors (Wolthuis et al, 1974; Mark and Mancia, 1983).

Introduction to the alternative methodology used in chapters ten to twelve

The studies described in chapters three and seven to nine rely upon changes in arterial pressure, heart rate, stroke volume and plasma hormone levels to investigate the interaction between the RAS and the SNS in man. In chapters ten to twelve studies are described which use an alternative methodology to investigate this interaction.

The technique is that of intra-arterial infusion of hormone or placebo and concomitant measurement of forearm blood flow by strain gauge plethysmography illustrated in figure 2.1.

Subjects

Twenty healthy volunteers (19 male and 1 female: mean age 26 years, range 21-33 years) took part in one or more of the experiments described in chapters ten to twelve. All studies were performed at least one week apart. Studies were performed after volunteers had rested supine in a quiet clinical laboratory for a minimum of 30 min. The room temperature (between 25 and 28 C) was maintained within +/- 1 C for each study.

Intra-arterial infusion

A 27 standard wire gauge steel cannula (Cooper's needle Works, Aston Lane, Birmingham, UK) was inserted into the brachial artery of the non-dominant arm using 1% lignocaine hydrochloride (Antigen Ltd, Ireland) to provide local anaesthesia. Infusions were given alone (at 1.0 ml/min) or in combination (each at 0.5 ml/min), to give a continuous infusion rate of 1.0 ml/min throughout each experiment, by means of constant rate infusion pumps (Harvard 944A).

Angiotensin II (Calbiochem, USA) was prepared in saline (0.9% NaCl,

Travenol, UK) immediately prior to infusion. All other hormone infusions are described in the individual chapters.

Measurement of forearm blood flow

Forearm blood flow (FBF) was measured in both arms using venous occlusion plethysmography with temperature compensated mercury-in-silastic strain gauges (Whitney, 1953). Collecting cuff pressure was 40 mmHg and wrist cuff occlusion pressure was 200 mmHg. Flows were recorded for 10s in every 15s. The mean of the final 5 measurements of each recording period was used for analysis.

Analysis of forearm blood flow recordings.

Percentage changes in blood flow in the infused and non-infused forearm were calculated by the formula given below:

$$\frac{F_{(t)} - F_{(0)}}{F_{(0)}} \times 100\ \%$$

where $F_{(0)}$ and $F_{(t)}$ represent measured forearm blood flows (F) before (0) and at time (t) during intervention.

Where repeated measures are compared the data were transformed to the ratios of blood flow in the infused and non-infused forearm. This method of analysis was chosen to minimise the interference of variations in blood flow affecting both arms produced by extraneous factors (after Greenfield and Patterson, 1954).

In their original paper Greenfield and Patterson note that extraneous influences and/or interventions resulted in some general alterations in the circulation, and even at rest there are continual adjustments of the peripheral circulation. Assuming all general alterations act symmetrically and cause equal percentage as opposed to absolute effects on the two

forearms then the following equation holds for our experiments:

$$\frac{E_{C(t)}}{F_{NC(t)}} = \frac{F_{C(0)}}{F_{NC(0)}} \qquad \text{OR} \qquad E_{C(t)} = \frac{F_{C(0)}}{F_{NC(0)}} \times F_{NC(t)}$$

where $F_{C(0)}$ represents the resting blood flow in the cannulated (C), infused forearm and $F_{NC(0)}$ represents the resting blood flow in the non-cannulated (NC), non-infused forearm during the baseline period, and $F_{NC(t)}$ represents the observed blood flow in the non-cannulated forearm at time t during experimental intervention and $E_{C(t)}$ is the expected blood flow in the cannulated forearm at time t if alterations are due only to general factors.

The effect of experimental intervention is to make the observed blood flow $F_{C(t)}$ instead of the expected blood flow $E_{C(t)}$ in the cannulated forearm. The observed flow may be expressed as a percentage of the expected by the formula:

$$\text{Percentage} = \frac{F_{C(t)}}{E_{C(t)}} \times 100$$

$$= \frac{F_{C(t)} \cdot F_{NC(0)}}{F_{C(0)} \cdot F_{NC(t)}} \times 100$$

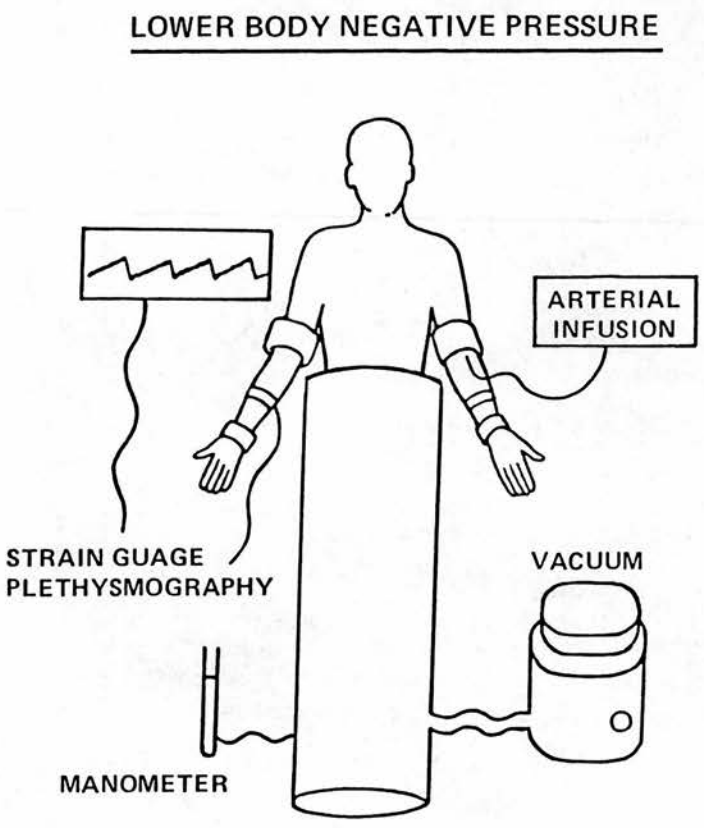
(after Greenfield and Patterson, 1954)

In the final formula it can be assumed that $F_{NC(0)}/F_{C(0)}$ approaches unity and for any given set of experimental observations is constant. Thus the formula may be reduced to:

$$\text{Percentage} = \frac{F_{C(t)}}{F_{NC(t)}} \times 100$$

In our experiments and illustrations where repeated measures are compared the data have been transformed to $F_{C(t)}/F_{NC(t)}$ ratios rather than percentages. The expected ratio is therefore unity and alterations in the ratio can be taken as due to experimental intervention rather than to extraneous general influences on the circulation.

Figure 2.1


LOWER BODY NEGATIVE PRESSURE
ARTERIAL
INFUSION
STRAIN GUAGE
PLETHYSMOGRAPHY
VACUUM
MANOMETER

Legend to figure 2.1

Experimental procedure for arterial infusion studies

Arterial infusion is performed via the left brachial artery in the resting supine subject. Forearm blood flow is measured in both forearms and compared to show the local effect of infusate.

Also illustrated is the subject within the body box which allows the rapid application of lower body negative pressure to the supine resting subject. This was used as indicated in individual chapters.

Handling of blood samples prior to analysis

Intravenous cannulae were placed (vide supra) into antecubital veins using 1% lignocaine to provide local anaesthesia. Intermittent blood samples were taken via the indwelling cannula to cause the least disturbance of the resting subject.

Aliquots (5 ml) were taken into chilled lithium heparin tubes for measurement of NA.

Aliquots (10 ml) were taken into chilled glass tubes containing a solution of 0.05 M O-phenanthroline, 2 g/l neomycin, 0.125 M EDTA disodium salt and 2% ethanol for measurement of AII.

Aliquots (5 ml) were taken into chilled potassium EDTA tubes for measurement of aldosterone.

Aliquots (10 ml) were taken into chilled tubes containing 60 mg potassium EDTA and 4000 kallikrein units of aprotinin (Trasylol, Bayer UK, UK) for measurement of atrial natriuretic peptide (ANP).

The samples for measurement of NA, AII, aldosterone and ANP were immediately centrifuged at 4C, separated and stored at -70C (NA and AII) or -20C (aldosterone and ANP) until assayed.

Aliquots were taken into plain glass tubes for measurement of creatinine, osmolality and electrolytes. The blood was allowed to clot at room temperature and serum separated and stored at -20C until assayed.

Measurement of serum and urinary electrolytes and osmolality.

Serum and urinary sodium and potassium were measured by an indirect reading ion specific electrode (Beckman System E2A). Serum and urinary osmolalities were measured by the freezing point depression method (Advanced Osmometer, Camlab).

Measurement of plasma noradrenaline

Plasma noradrenaline was measured by our double isotope radioenzymatic assay (Brown and Jenner, 1981). The intra-assay coefficient of variation for this method in our laboratory was 8.0% and the inter-assay coefficient of variation was 11.1%. [Noradrenaline analyses for the studies in chapter three and chapter eight were performed by myself, thereafter analyses were performed by laboratory staff of our Department].

The following analyses were performed by technical staff of the Department of Clinical Pharmacology and *the Department of Clinical Biochemistry, Ninewells Hospital and Medical School, Dundee .

Measurement of serum and urinary creatinine

Serum and urinary creatinine were measured on an autoanalyser by the modified Jaffe method (Cobas Bio, Roche Diagnostica, Basle, Switzerland).

Measurement of plasma AII

Plasma AII was measured, after plasma extraction, by a commercially available radioimmunoassay kit (Immuno-diagnostics Ltd, Washington, Tyne and Wear, UK). The intra-assay coefficient of variation for this method in our laboratory was 2.2% and the inter-assay coefficient of variation was 12.1%.

Measurement of plasma aldosterone

Aldosterone was also measured by radioimmunoassay using a commercially available kit (Serono Diagnostics Ltd, Woking, Surrey, UK).

The intra-assay coefficient of variation for this method in our laboratory was 6.6% and the inter-assay coefficient of variation was 14.7%.

Measurement of plasma renin activity

Plasma renin activity was measured by radioimmunoassay of angiotensin I generated during a 1.5 hour incubation at 37C with a commercially available kit (CIS, UK, Ltd, High Wycombe, Buckinghamshire, UK). The intra-assay coefficient of variation for this method in our laboratory was 1.8% and the inter-assay coefficient of variation was 10.8%

Measurement of plasma atrial natriuretic peptide

Plasma ANP levels were measured by a commercial radio-immunoassay (Amersham International Ltd, Little Chalfont, Buckinghamshire, UK) after plasma extraction (Sep-Pak cartridges, Waters Associates). The intra-assay coefficient of variation for this method in our laboratory was 8.5% and the inter-assay coefficient of variation was 7.2%.

Measurement of serum and urinary lithium concentration *

Serum and urinary lithium were measured by flame emission photometry on an atomic absorption spectrometer (Pye Unicam SP9, Phillips, Cambidge, UK).

LIST OF PHARMACOLOGICAL AGENTS:

1. **Angiotensin II:** Hypertensin Ciba [CIBA-GEIGY Ltd, Switzerland.]; val^5-hypertensin II-asp-beta-amide was diluted in 5% dextrose to final concentration of 0.25 or 0.5 mcg/ml for intravenous infusion. Angiotensin II (Calbiochem, Behring Diagnostics, USA) was prepared in 0.9% saline for intra-arterial infusion.

2. **Noradrenaline:** Levophed [Winthrop Laboratories, UK]; 1ml containing 1mg noradrenaline base as acid tartrate with 2 mg sodium metabisulphate as stabilising agent. Noradrenaline was diluted as stated in 5% dextrose alone or 0.9% saline with 1mg/ml ascorbic acid as anti-oxidant (Evans Medical Ltd, UK; ascorbic acid 500mg in 5mls stabilised with sodium metabisulphate 0.1% w/v).

3. **Isoprenaline:** Saventrine [Pharmax Ltd, UK]; 1mg/ml stabilised solution of isoprenaline hydrochloride BP for injection was diluted in 5% dextrose for intravenous infusion.

4. **Tyramine:** Tyramine [BDH Ltd, UK] was prepared as a solution (10mg/ml) for human administration to be diluted in 5% dextrose for intravenous infusion by the Pharmacy Manufacturing Unit, Ninewells Hospital and Medical School, Dundee, UK.

5. **Enalaprilat:** Enalaprilat 5mg/ml [Merck Sharp & Dohme Ltd, UK] was diluted in 0.9% saline for intra-arterial infusion.

6. **Saralasin:** [Sar^1-Val^5-Ala^8]-Angiotensin II as acetate salt [Sigma Chemical Co Ltd, UK] was diluted in 0.9% saline for intra-arterial infusion.

7. **Enalapril:** Innovace 20mg [Merck Sharp & Dohme Ltd, UK]

8. **Captopril:** Capoten Tablets 25mg [ER Squibb & Sons Ltd, UK].

9. **Frusemide:** Lasix Tablets 40mg [Hoechst UK Ltd, UK].

10. **Lithium;** Camcolit 250 [Norgine Ltd, UK]; containing 250 mg lithium carbonate BP (equivalent to 6.8 mmol).

All infusions were made up and/or diluted as indicated immediately prior to infusion.

SECTION TWO: RESULTS

CHAPTER THREE

The effect of intravenous infusion of angiotensin II alone and in combination on blood pressure, heart rate and plasma noradrenaline

Introduction

In this study evidence was sought for a postsynaptic interaction between the RAS and SNS by constructing a dose-response curve to infused exogenous NA in the presence and absence of exogenous AII.

Methods

Ten normotensive volunteers (nine male) were studied. Each volunteer attended on four separate occasions. On each occasion they had performed a 24 hour urine collection for assessment of sodium excretion. On each study day, three intravenous cannulae were inserted, two in one arm and one in the opposite arm. The forearm with two cannulae was used for the infusions while the opposite arm was used for blood sampling.

After 20 min supine rest, blood pressure (BP) and heart rate (HR) were measured and a blood sample taken for subsequent measurement of plasma NA and plasma AII levels. Thereafter, they were infused with either AII dissolved in 5% dextrose at the rate of 2 ng/kg/min on two occasions or with vehicle solution on the other two occasions. This infusion lasted 90 min. After 20 min of this infusion, measurements were made of BP and HR and blood sampled for plasma NA and AII. Thereafter, a second infusion was begun. On two days they were infused with NA (Levophed, Winthrop) in 0.9% saline containing 1 mg/ml ascorbic acid in a stepwise fashion, each step lasting 10 min. Each step consisted of NA in doses of 0, 10, 20, 35, 50, 75 and 100 ng/kg/min. On two other days,

vehicle solution was infused in an identical manner. Both the first AII/dextrose infusion and the second NA/saline infusion were terminated at the same time. The four study days were administered in a randomised single-blind fashion. Overall therefore, the volunteers received dextrose followed by saline, AII followed by saline, dextrose followed by NA and AII followed by NA. The study protocol is illustrated in figure 3.1.

Blood pressure was measured by a semi-automatic sphygmomanometer placed on the arm with the blood sampling intravenous cannula. Blood samples were taken immediately before cuff inflation for BP measurement. Heart rate was recorded from the ECG monitor. Blood samples for measurement of plasma NA and AII were taken during the last minute of each infusion increment.

All data were analysed by two way analysis of variance (ANOVA) to look for a significant effect of NA, a significant effect of AII and a significant synergistic interaction between NA and AII.

Results

The 24 hour urinary sodium excretion (mmol/24 h) was similar on all four study days, i.e. 173+/-79 on the dextrose/saline day, 144+/-31 on the AII/saline day, 186+/-66 on the dextrose/NA day and 168+/-13 on the AII/NA day.

Figure 3.2 shows the systolic BP (SBP) data. Despite 20 min initial supine rest, SBP still fell throughout the 90-min study period. In the presence of AII alone, SBP rose at the end of the study period but overall AII had no statistically significant effect on SBP (ANOVA). On the other hand, the stepwise infusion of NA did significantly ($P<0.05$, ANOVA) increase SBP. Of major interest, however, is that there was a statistically significant ($P<0.05$, ANOVA) synergistic interaction between AII and NA in

the SBP data.

Figure 3.3 shows the diastolic BP (DBP) data. This differs markedly from the SBP data. AII alone did significantly ($P<0.05$, ANOVA) increase DBP. The stepwise infusion of NA also increased DBP significantly. However, there was no statistically significant interaction between AII and NA in the DBP data. Indeed it is clear from fig. 3.3 that the DBP response to AII/NA was merely additive, i.e. the response to AII and NA together could be predicted by summing the response to NA and the response to AII.

Figure 3.4 shows the HR data. AII had no statistically significant effect on HR while the stepwise infusion of NA produced a statistically significant ($P<0.05$, ANOVA) reflex bradycardia. ANOVA showed that, as with SBP, there was a statistically significant ($P<0.05$, ANOVA) synergistic interaction when NA and AII were infused together.

Figure 3.5 shows the mean BP data. Both AII and NA increased mean BP significantly. There was also a significant synergistic interaction with AII/NA.

Linear regression was performed between the HR response and the mean BP response to NA for each individual on each study day. Overall the slope of the HR/mean BP regression line was -0.30+/-0.14 in the absence of AII and was -0.27+/-0.13 in the presence of AII. This difference was not statistically significant by paired t-test.

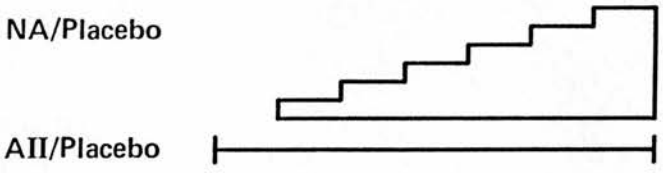
Figure 3.6 shows the plasma NA data. Plasma NA did not change on the placebo or the AII only study day. Plasma NA increased in the expected stepwise fashion during the incremental infusion and there was no significant difference in the plasma NA response whether AII was infused or not. The third infusion rate in this study produced the kind of plasma NA levels which are found in normal volunteers performing bicycle exercise

(Struthers et al, 1986).

Table 3.1 shows the plasma AII results. Clearly plasma AII levels increased during the infusion of AII and these levels were not affected by the coincidental infusion of NA. The AII levels produced are within the physiological range in that salt-deplete individuals usually have higher plasma AII levels than were generated by these AII infusions.

Figure 3.1

PROTOCOL : POSTSYNAPTIC STUDY



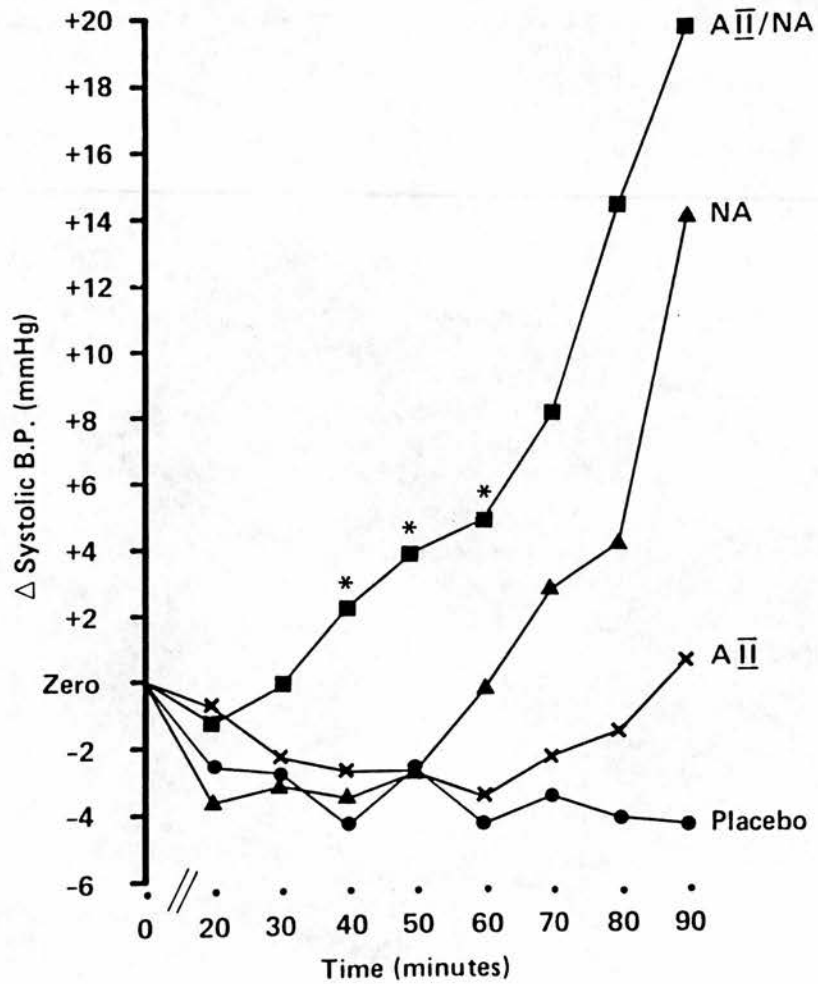
1. Placebo/Placebo
2. AII/Placebo
3. Placebo/NA
4. AII/NA

Legend to figure 3.1

Each volunteer received two concomitant infusions of an incremental noradrenaline infusion (or placebo) and a constant rate infusion of angiotensin II (or placebo).

There were therefore four separate study days with the listed combinations of infusion being given in a single blind randomised fashion.

Figure 3.2

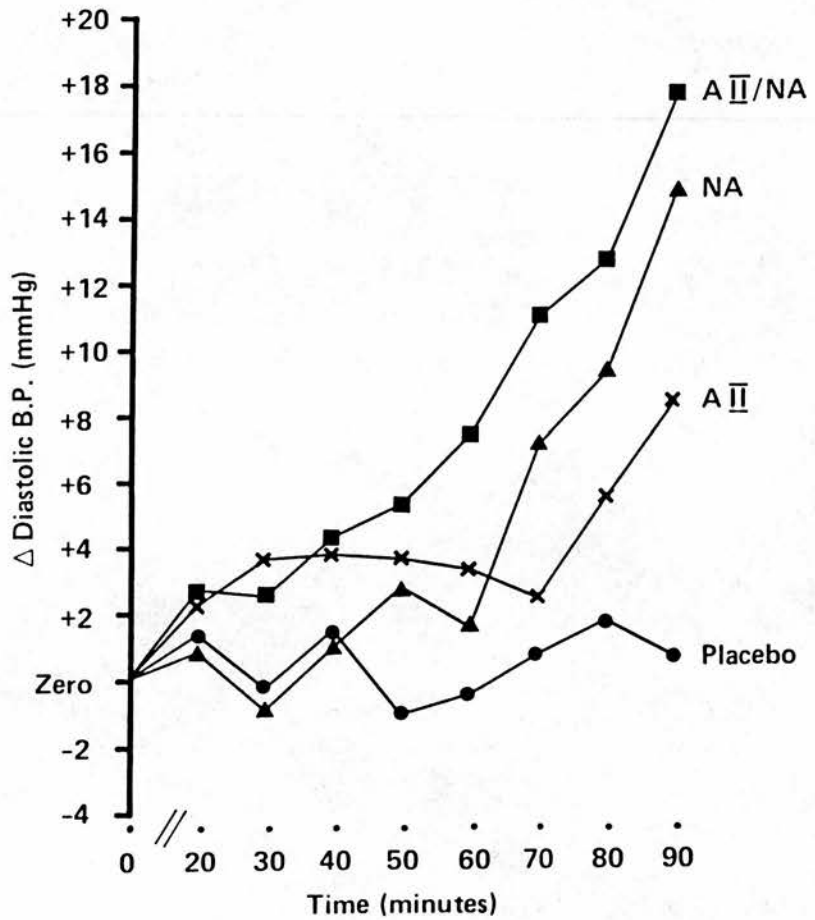

+20
+18
+16
+14
+12
+10
+8
+6
+4
+2
Zero
-2
-4
-6
Δ Systolic B.P. (mmHg)
AII/NA
NA
AII
Placebo
*
*
*
0
20
30
40
50
60
70
80
90
Time (minutes)

Legend to figure 3.2

Change in systolic blood pressure (BP, mmHg) in 10 normotensive volunteers during the infusion of placebo, angiotensin II (AII), noradrenaline (NA) or AII/NA. See text for details. Two way ANOVA showed that AII had no significant effect while NA did significantly increase systolic BP. There was also a significant synergistic interaction with AII/NA (ANOVA).

* $P<0.05$ paired t-test between AII/NA and NA at equivalent time points.

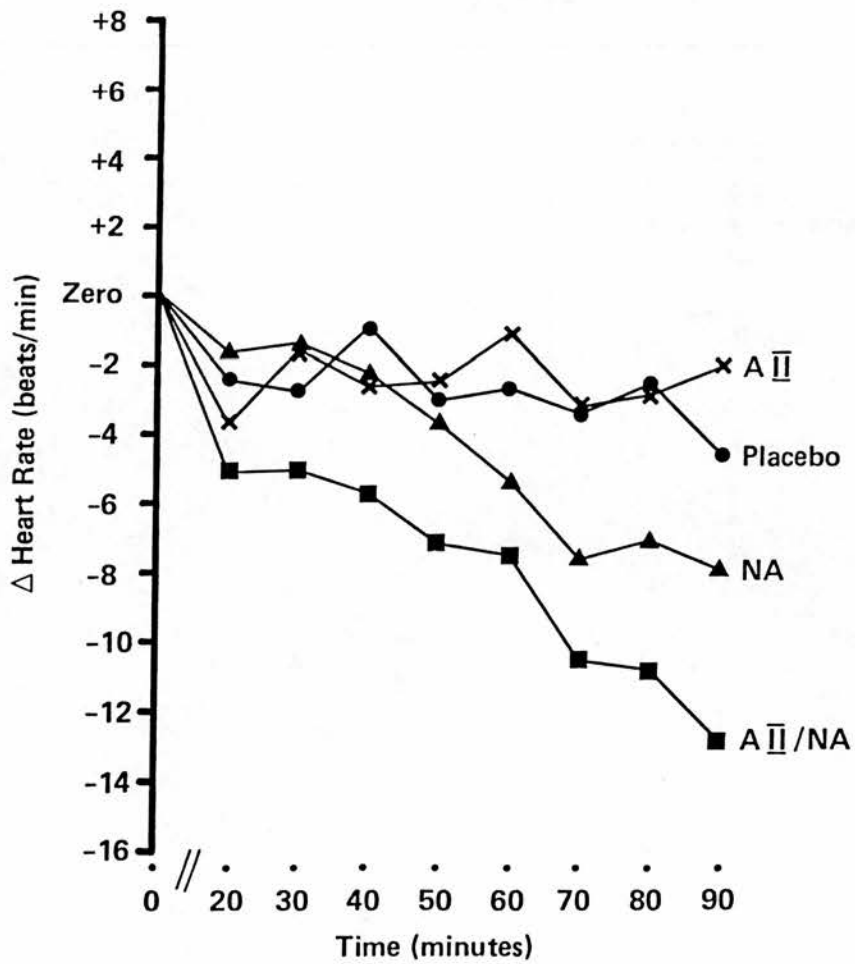
Figure 3.3


+20
+18
+16
+14
+12
+10
+8
+6
+4
+2
Zero
-2
-4
△ Diastolic B.P. (mmHg)
A II/NA
NA
A II
Placebo
0
20
30
40
50
60
70
80
90
Time (minutes)

Legend to figure 3.3

Change in diastolic blood pressure (BP, mmHg) in 10 normotensive volunteers during the infusion of placebo, angiotensin II (AII), noradrenaline (NA) or AII/NA. See text for details. Two way ANOVA showed that both AII and NA increased diastolic BP significantly while there was no synergistic interaction with AII/NA.

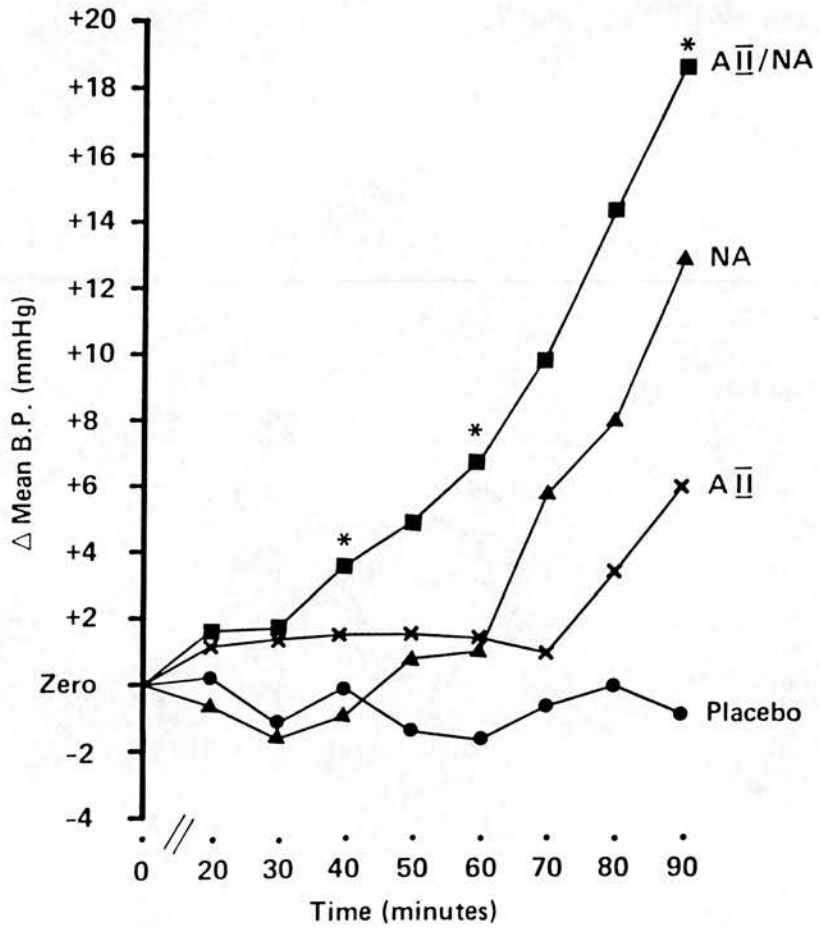
Figure 3.4


+8
+6
+4
+2
Zero
-2
-4
-6
-8
-10
-12
-14
-16
Δ Heart Rate (beats/min)
A II
Placebo
NA
A II /NA
0
20
30
40
50
60
70
80
90
Time (minutes)

Legend to figure 3.4

Change in heart rate (beats/min) in normotensive 10 volunteers during the infusion of placebo, angiotensin II (AII), noradrenaline (NA) or AII/NA. See text for details. Two way ANOVA showed that AII had no significant effect while NA did significantly decrease heart rate. There was also a significant synergistic interaction with AII/NA (ANOVA). No individual points were significantly different by paired t-testing between AII/NA at equivalent time points.

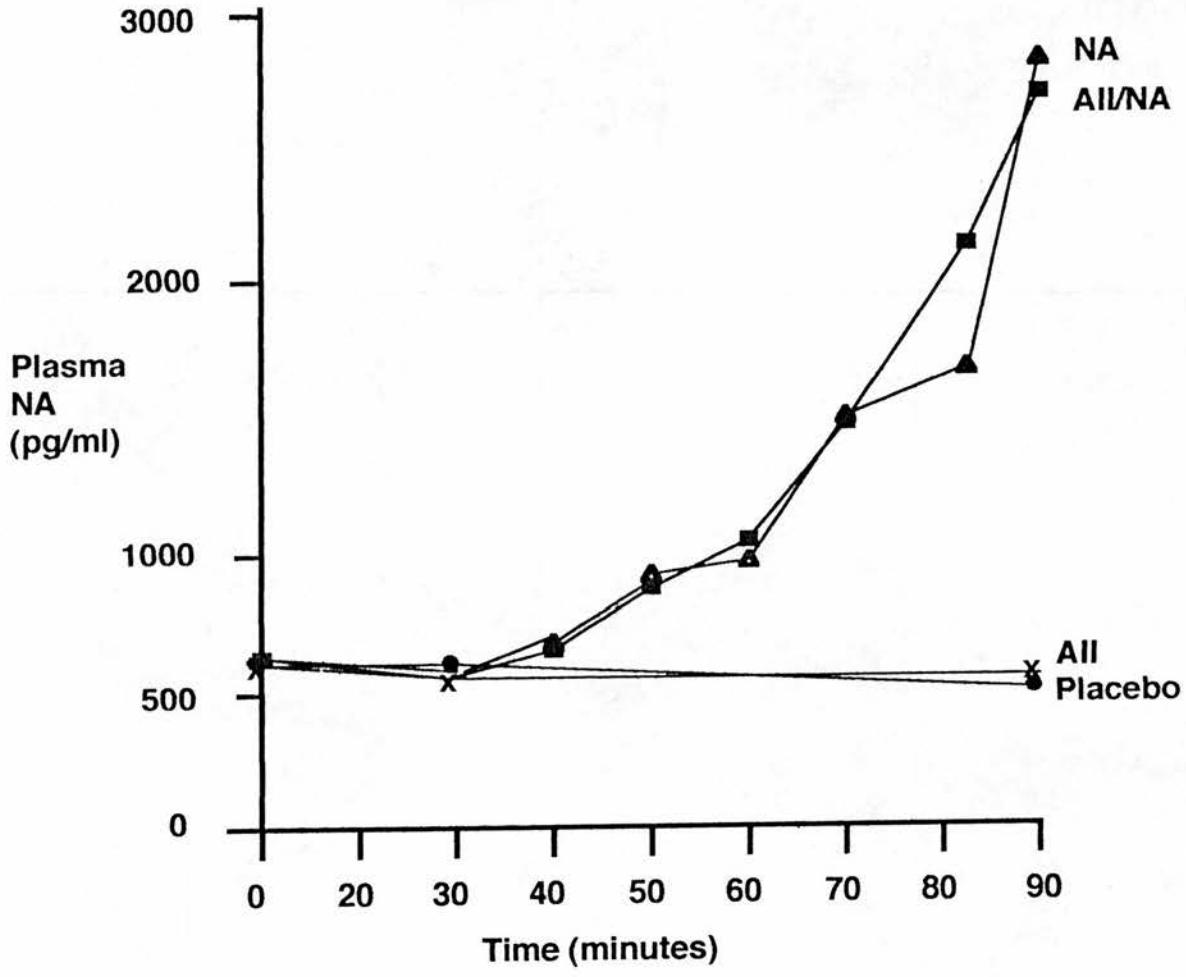
Figure 3.5


+20
+18
+16
+14
+12
+10
+8
+6
+4
+2
Zero
-2
-4
Δ Mean B.P. (mmHg)
AII/NA
NA
AII
Placebo
*
*
*
0
20
30
40
50
60
70
80
90
Time (minutes)

Legend to figure 3.5

Change in mean blood pressure (BP, mmHg) in 10 normotensive volunteers during the infusion of placebo, angiotensin II (AII), noradrenaline (NA) or AII/NA. See text for details. Two way ANOVA showed that both AII and NA increased mean BP significantly. In addition, there was a significant synergistic interaction with AII/NA (ANOVA). *P<0.05 paired t-test between AII and NA at equivalent time points.

Figure 3.6


3000
2000
1000
500
0
Plasma
NA
(pg/ml)
NA
AII/NA
AII
Placebo
0
20
30
40
50
60
70
80
90
Time (minutes)

Legend to figure 3.6

Plasma noradrenaline (NA, pg/ml) in 10 normotensive volunteers during the infusion of placebo, angiotensin II (AII), noradrenaline (NA) or AII/NA. See text for details.

TABLE 3.1

Plasma AII levels (pg/ml) [means+/-s.d.] at baseline, at end of first infusion alone (i.e. dextrose or AII), during both infusions (i.e. dextrose or AII plus saline or NA) or at the end of both infusions.

	5%D/saline	AII/saline	5%D/NA	AII/NA
Baseline	12.9+/-10.4	13.4+/-10.6	9.6+/-3.9	10.9+/-6.7
End of first infusion alone	14.2+/-5.0	26.8+/-12.4	9.2+/-4.3	31.3+/-13.4
During both infusions	-	-	7.2+/-3.4	28.2+/-8.6
End of both infusions	10.4+/-5.1	39.9+/-36.8	9.2+/-4.1	35.7+/-22.6

AII, angiotensin II; NA, noradrenaline; 5%D, 5% dextrose;

Summary

In this study evidence was sought for a postsynaptic interaction between AII and NA. Ten normotensive volunteers were infused with dextrose/saline, AII/saline, dextrose/NA or AII/NA in a randomised single blind fashion. The respective increases in SBP were -4+/-6, -2+/-9, 4+/-6 and 14+/-16 mmHg on respective study days and at comparative time intervals while the corresponding increases in DBP were 2+/-4, 6+/-7, 9+/-6 and 14+/-8 mmHg. ANOVA confirmed that AII and NA had a synergistic interaction ($P<0.05$) in elevating SBP while there was merely an additive effect in elevating DBP. Plasma NA and AII levels were unchanged by the coincidental presence of AII and NA, respectively, which excludes a generalised pharmacokinetic interaction between AII and NA. This study therefore provides support for a postsynaptic AII/NA interaction with regard to SBP but not DBP although the precise location of this interaction remains uncertain.

[Discussion: chapter 13.1]

CHAPTER FOUR

CHAPTER FOUR

The effect of angiotensin II on renal sodium excretion in man

Introduction

The RAS is crucial for the maintenance of sodium balance (Laragh and Sealey, 1973). The role of aldosterone in enhancing renal sodium reabsorption is well recognised (Davis, 1974). Less well established is the direct effect of AII itself on renal sodium handling in man. In experimental animals, however, there is now a considerable body of evidence to suggest that physiological levels of AII exert a direct antinatriuretic effect, mainly at the proximal tubule (Johnson and Malvin, 1977; Olsen et al, 1985). A few studies, using mainly pharmacological doses, have shown that AII also reduces sodium excretion in man (Finnerty et al, 1961; Hollenberg et al, 1976; Usberti et al, 1985). The usual approach to assessing the renal physiological significance of AII in man, however, has been to employ angiotensin converting enzyme inhibitors. The interpretation of such studies is difficult because of the enhanced production of other renally active substances such as prostaglandins and kinins by these drugs (Usberti et al, 1985; McCaa, 1979; Shoback et al, 1983; Swartz and Williams, 1982; Brunner et al, 1981). ACE inhibitors also reduce aldosterone secretion which may in itself induce a natriuresis (Shoback et al, 1983). A further complication in the study of renal sodium handling is the significant hypotensive response that can be induced by these drugs. Finally, the renal tubular site of action of AII in man, unlike animals, is still not known. In this study we have therefore investigated the effects of low-dose AII infusion on renal sodium handling in man. The lithium clearance technique was used as lithium may act as a specific marker for proximal tubular

sodium handling. In addition, conventional clearance parameters based on the generation of 'solute free water', were estimated, in parallel, to independently corroborate the lithium method.

List of abbreviations

These abbreviations are used throughout chapters 4-6 and section three (discussion):

C_{Cr}, C_{H2O}, C_{Li}, C_{Na} and C_{osm}, creatinine, free water, lithium, sodium and osmolar clearances respectively; FDD, fractional distal delivery; FE_{Na} and FE_{Li}, fractional excretion of sodium and lithium, respectively; FRD_{Na}, fractional reabsorption of sodium by the distal nephron; GFR, glomerular filtration rate; RD_{Na}, absolute reabsorption of sodium by the distal nephron; U_{osm}, urinary osmolality.

Methods

Six salt-replete male normal volunteers aged 20-37 years (mean 24 years) were studied on two separate occasions at least one week apart. Volunteers attended the clinical laboratory at 08.30 hours on the morning of the study. All had fasted (food and drink) from 22.00 hours the previous evening. Each volunteer ingested 500mg of lithium carbonate at 22.00 hours at the beginning of their fast before the study day.

Subjects were seated and an intravenous cannula was placed in each antecubital fossa at the start of the experiment. A steady state water diuresis was established over a period of 2 h (six 20 min urinary clearance periods, as described in general methods). After a further two clearance periods (clearance periods A and B), an intravenous infusion of either carrier [30 ml of 5% dextrose](placebo day) or AII (1 ng/kg/min), in 30 ml of carrier, was commenced (i.e. at the start of clearance period C) and

continued for 20 min. Urine was collected at the end of clearance periods A and B, at the end of the infusion period (C), and for two further periods (D and E). The order of the infusions was random and single blind.

Venous blood (35 ml) was collected 12 min into each of the two clearance periods before infusion (periods A and B), during the infusion period (period C) and during the two clearance periods after the infusion (periods D and E). Samples were aliquoted for measurement of plasma AII, plasma aldosterone and creatinine, osmolality and electrolytes. Two 20 ml urine aliquots were taken at the end of each urinary clearance period. Osmolality was measured on the experimental day and the remaining aliquot was preserved at -20 C for later electrolyte and creatinine measurement.

Analysis

All parameters after infusion (clearance period C) were compared with the pre-infusion baseline period (period B) by Student's t-test for paired samples. A P value of <0.05 was considered to represent a statistically significant difference. Data are presented as means+/-sem.

Results

Twenty-four hour sodium excretion before the placebo day was 187+/-28 mmol and 177+/-17 mmol on the AII day. The serum lithium levels on the two experimental days were 0.19+/-0.01 and 0.20+/-0.01 mmol/l, respectively. Sitting blood pressure did not change on either study day (table 4.1).

U_{osm} was <65 mosmol/kg in all subjects on both study days and did not change significantly during either experiment. Urinary flow rate and sodium excretion on the two experimental days are shown in fig. 4.1. FDD

and FE_{Li} are shown in fig. 4.2 Urinary potassium excretion (U_KV), C_{Cr}, RD_{Na}, FRD_{Na} and FE_{Na} are shown in table 4.1.

There was no significant change in any parameter on the placebo day. Urinary flow rate and absolute and fractional sodium excretion fell significantly after infusion of AII. Potassium excretion declined though this decrease just failed to reach statistical significance. Both parameters of proximal tubule function investigated, FE_{Li} and FDD, also declined sharply during infusion of AII, suggesting that AII enhanced proximal tubular sodium reabsorption. RD_{Na} fell significantly during infusion of AII, although FRD_{Na} was unchanged (Table 4.1). For all secretory parameters the onset of action was rapid and all values rose quickly back towards baseline after cessation of the AII infusion. AII and aldosterone levels on each of the 2 study days are shown in Table 4.1. Basal levels of both hormones were higher than most quoted ranges for normal resting subjects in the supine position but our volunteers were studied sitting and stood every 20 min to void, both of which will tend to increase AII and aldosterone levels. AII levels rose in all subjects during infusion though the peak value remained well within the physiological range. Aldosterone levels rose during the infusion of AII but did not reach a peak until the post infusion period D.

TABLE 4.1

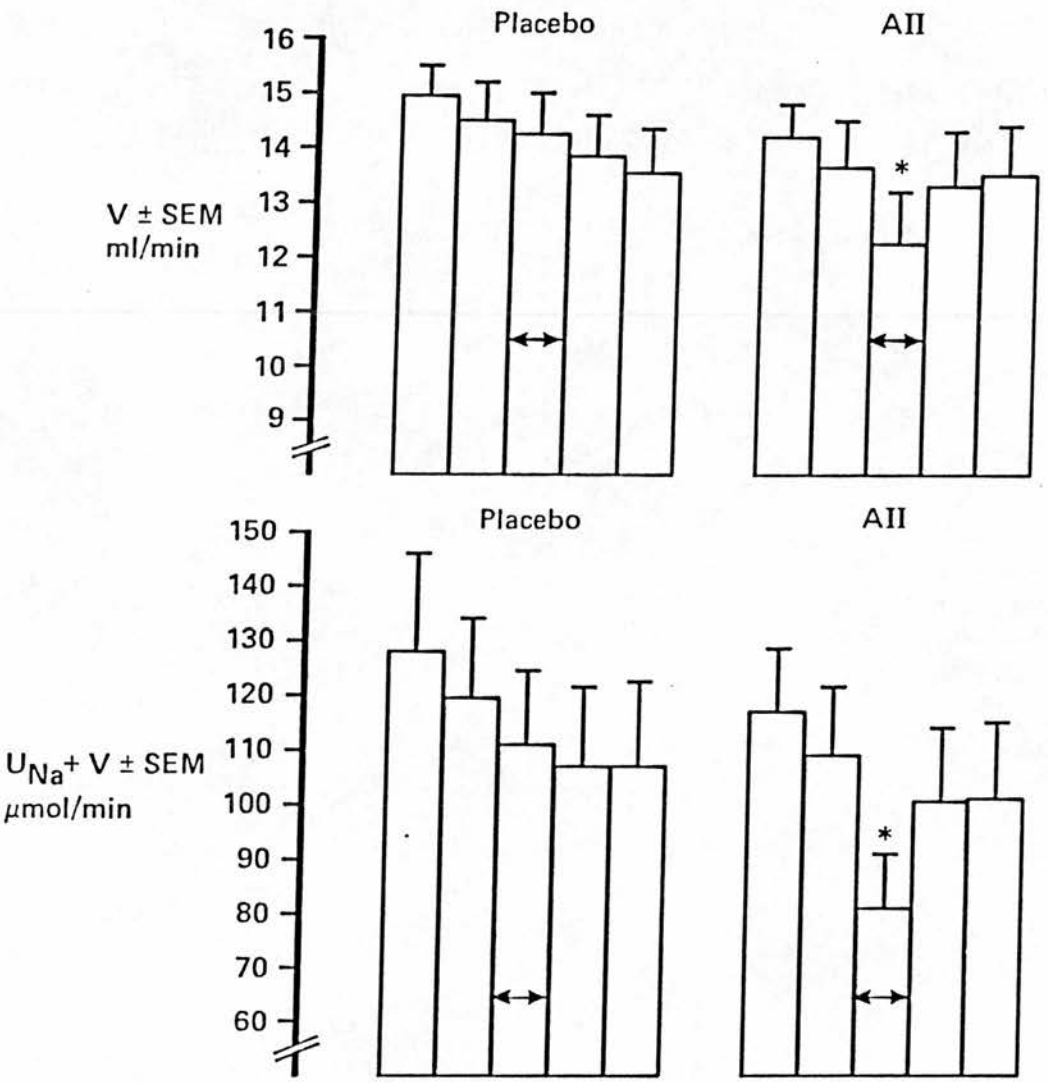
Mean arterial pressure, urinary potassium excretion (U_KV), C_{Cr}, FE_{Na}, RD_{Na}, FRD_{Na}, aldosterone levels and AII levels on each of the two study days.

Urinary clearance periods A and B represent baseline periods, period C is the period of the infusion and periods D and E are recovery periods.

	Urinary clearance period				
Study day	A	B	C	D	E
MAP (mmHg)					
Placebo	88+/-4	86+/-4	84+/-4	84+/-4	89+/-4
AII	82+/-3	87+/-3	87+/-4	85+/-2	87+/-3
U_KV (umol/min)					
Placebo	45+/-9	46+/-8	47+/-6	48+/-5	49+/-5
AII	43+/-5	44+/-5	40+/-5	44+/-7	46+/-6
C_{Cr} (ml/min)					
Placebo	114+/-5	109+/-4	113+/-3	111+/-3	108+/-3
AII	103+/-3	111+/-3	113+/-3	112+/-3	110+/-4
FE_{Na} (%)					
Placebo	0.85+/-0.33	0.83+/-0.30	0.75+/-0.24	0.72+/-0.23	0.75+/-0.29
AII	0.88+/-0.20	0.74+/-0.20	0.56+/-0.17**	0.69+/-0.24	0.72+/-0.25
RD_{Na} (mmol/min)					
Placebo	4.48+/-0.55	4.48+/-0.29	4.53+/-0.35	5.14+/-0.76	4.38+/-0.37
AII	3.86+/-0.35	3.86+/-0.38	3.25+/-0.39*	3.88+/-0.49	3.83+/-0.25
FRD_{Na} (%)					
Placebo	96.7+/-0.5	97.3+/-0.4	97.5+/-0.3	97.8+/-0.4	97.4+/-0.3
AII	96.7+/-0.5	97.1+/-0.3	97.5+/-0.3	97.4+/-0.3	97.6+/-0.2
Aldo (pg/ml)					
Placebo	158+/-25	166+/-25	187+/-29	169+/-29	-
AII	194+/-32	238+/-32	281+/-49	292+/-32**	-
AII (pg/ml)					
Placebo	29+/-5	30+/-3	30+/-4	28+/-5	-
AII	24+/-3	30+/-6	48+/-9**	31+/-4	-

MAP, mean arterial pressure; Aldo, aldosterone. All values are means+/-sem. Statistical significance: *P<0.05, **P<0.01 vs period B.

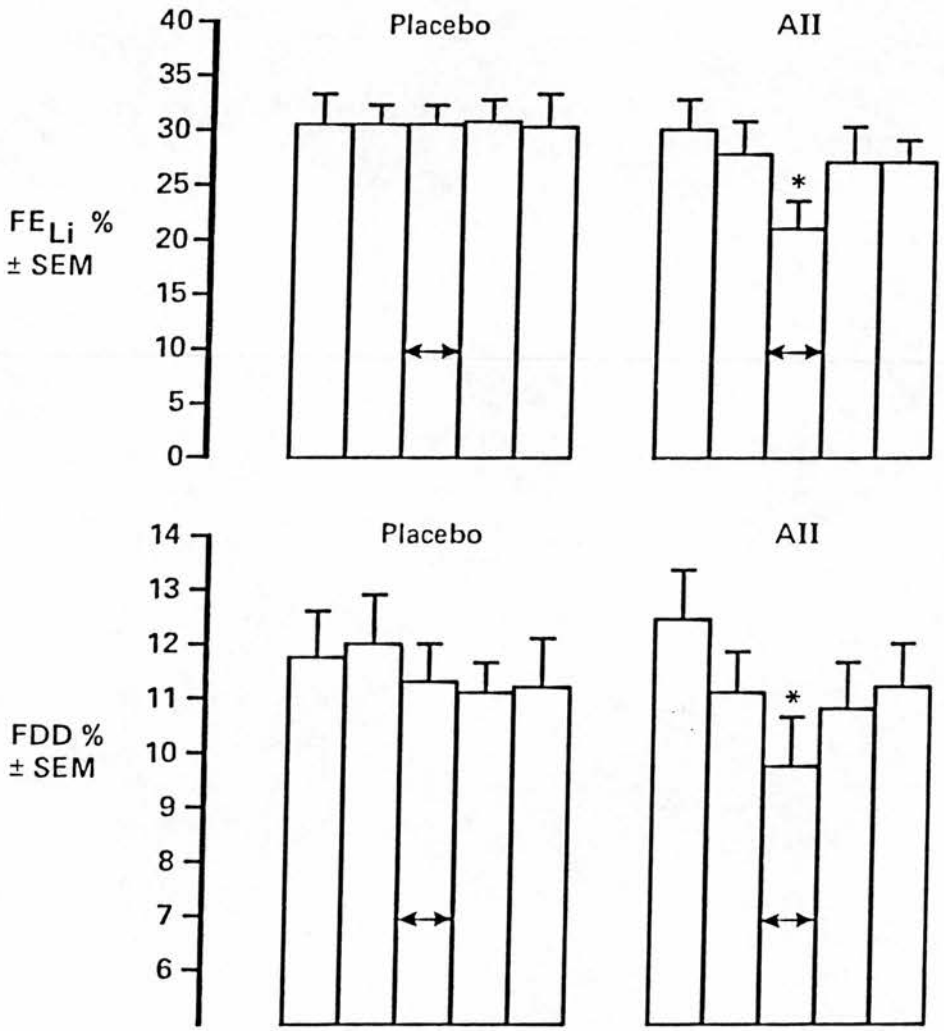
Figure 4.1


Placebo
AII
16
15
14
13
12
11
10
9
V ± SEM
ml/min
*
Placebo
AII
150
140
130
120
110
100
90
80
70
60
U_{Na^+} V ± SEM
μmol/min
*

Legend to figure 4.1

Urinary flow rate (V) and urinary sodium excretion ($U_{Na}V$) on each of the two study days. Each bar represents a 20 min clearance period. The horizontal arrow indicates the duration of the infusion period. Results are mean+/-sem. Statistical significance: * $P<0.01$ vs period B. Placebo and AII indicate placebo or angiotensin II infusion days respectively.

Figure 4.2


Placebo
AII
FE_{Li} %
± SEM
40
35
30
25
20
15
10
5
0
*
Placebo
AII
FDD %
± SEM
14
13
12
11
10
9
8
7
6
*

Legend to figure 4.2

FE_{Li} and FDD on each of the two study days. Each bar represents a 20 min clearance period. The horizontal arrow indicates the duration of the infusion period. Results are mean+/-sem. Statistical significance: * P<0.01 vs period B. Placebo and AII indicate placebo or angiotensin II infusion days respectively.

Summary

Angiotensin II (AII; 1 ng/kg/min) or 5% dextrose (placebo) was infused in six normal male volunteers, pretreated with 500 mg of lithium carbonate, who were undergoing maximal water diuresis. This dose of AII caused a circulating increment in plasma AII within the physiological range (27+/-4 to 48+/-9 pg/ml).

Compared with placebo, AII caused a significant fall in urinary sodium excretion (113+/-13 to 82+/-10 umol/min). This antinatriuretic effect occurred without a fall in creatinine clearance (107+/-3 versus 113+/-3 ml/min).

AII caused a significant fall in fractional lithium clearance (28+/-2 to 23+/-1 %). This may indicate a proximal tubular effect of AII. AII also reduced fractional distal delivery [(sodium clearance plus free water clearance) divided by creatinine clearance], another measure of proximal tubular outflow. A parallel change in these two separate markers of proximal function supports an action of AII at this nephron segment.

Furthermore, the antinatriuretic effect of AII was unlikely to be due to stimulation of aldosterone secretion because (a) the fall in sodium excretion was temporally dissociated from the rise in aldosterone secretion, (b) potassium excretion also tended to fall during AII infusion and (c) aldosterone has a distal nephron effect, while, in this study, proximal nephron fractional reabsorption of sodium increased and distal nephron fractional reabsorption of sodium was unchanged.

These observations suggest that physiological increments in AII can have an antinatriuretic effect in man, which, at least initially, results from increased proximal tubular sodium reabsorption and is independent of the effect of aldosterone.

[Discussion: chapter 13.2]

CHAPTER FIVE

CHAPTER FIVE

The effect of noradrenaline on renal sodium excretion in man

Introduction

In recent years evidence has accumulated to suggest that the renal SNS is an important regulator of sodium excretion (DiBona, 1977; Gill, 1979). SNS activity consists of two components, localised synaptic NA release and spillover into the general circulation. Lately it has been suggested that small increments in circulating NA are capable of a systemic haemodynamic effect. Two studies have shown that infusion of physiological doses of NA increases blood pressure and reduces heart rate in supine resting subjects (Izzo, 1983; Scriven et al, 1983). This study examines the question of whether similar increments in circulating NA influence renal sodium handling in man.

Methods

Nine normal volunteers (aged 19-37 years, mean 25 years) were examined on two separate occasions at least one week apart. Volunteers attended the clinical laboratory at 08.30 hours on the morning of the study. All volunteers were salt-replete as assessed by timed urine collections (mean +/-sem 24-h sodium excretion 185+/-21 mmol). All had fasted (food and drink) from 22.00 hours the previous evening. Seven of the volunteers ingested 500mg of lithium carbonate (Camcolit, Norgine, Oxford,UK) at 22.00 hours, the beginning of their fast before the study day (the other two volunteers had no lithium on either study day).

Subjects were seated and an intravenous cannula was placed in each antecubital fossa at the start of the experiment. A steady state water

diuresis was established over a period of 2 h (six 20 min urinary clearance periods, as described in general methods). A further 20 min period was taken as baseline (period A) after which an infusion of NA at 25 ng/kg /min in 5% dextrose vehicle, or vehicle solution only (placebo), was commenced and continued for another four urinary clearance periods, B-E (i.e. until the end of the experiment). The order of these infusions was random and their administration single blind.

Blood samples for measurement of plasma AII, NA, aldosterone and serum electrolytes and creatinine, were taken 12 min into the baseline period (period A), during the first infusion period (B) and during the third infusion period (D). An aliquot of urine was collected for measurement of creatinine and electrolyte.

Analysis

The haemodynamic and urinary data (the latter logarithmically transformed) were analysed by repeated measures analysis of variance (SPSS). This analysis examined the effect of time (periods A-E), treatment (placebo, NA) and treatment over time. Where a significant ($P<0.05$) treatment/time interaction was identified individual time points were compared by paired t-test. Plasma NA, AII and aldosterone levels were compared to basal levels on each of the 2 study days by paired t-test.

Results

Baseline sodium excretion (+/-sem) on the placebo and NA study days was not significantly different (116+/-13 and 142+/-19 µmol/min, respectively). Creatinine clearance was also similar on the two study days (Table 5.1). Lithium clearance was 25.8+/-2.6 ml/min on the placebo day and 28.1+/-2.6 ml/min on the NA day (not significant). Heart rate, blood

pressure, plasma NA, AII and aldosterone levels for the 2 study days are shown in Table 5.1. There was no significant haemodynamic changes during the infusion of NA. The infusion increased plasma levels of NA ($P<0.01$) but there was no significant changes in circulating aldosterone or AII.

The change in sodium excretion from baseline during infusion of placebo, or NA, is shown in fig. 5.1. Sodium excretion declined with time but there was also a significant treatment/time interaction (ANOVA, $P<0.01$). Paired t-tests showed a significant ($P<0.01$) difference between placebo and NA for periods D and E. Creatinine clearance did not change significantly with time and there was no difference between treatment days. Lithium clearance declined on the NA day compared to the placebo day (ANOVA, $0.01<P<0.02$). Paired t-tests showed that this difference was significant ($P<0.01$) for periods D and E, as with sodium excretion (fig. 5.1).

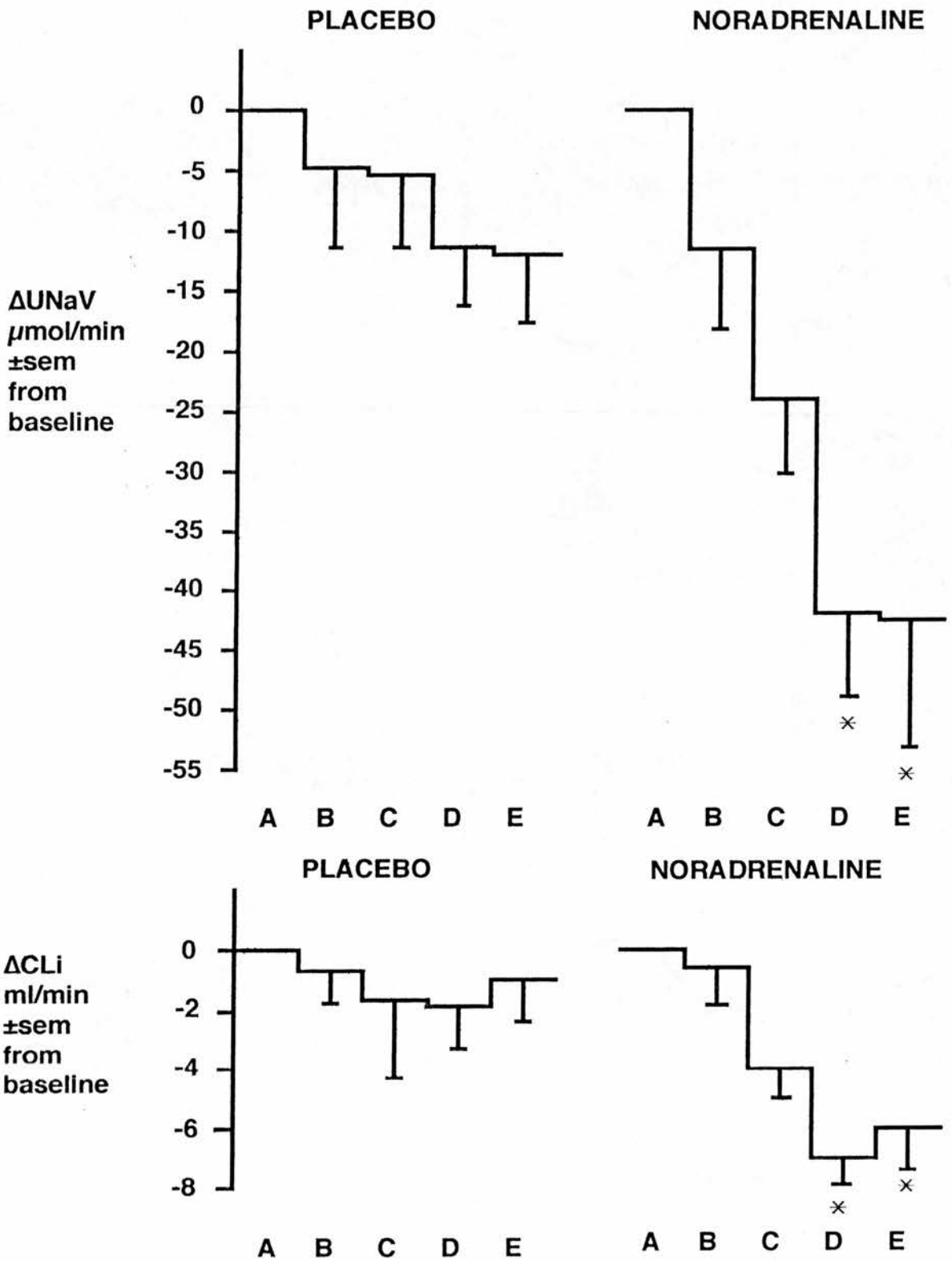
TABLE 5.1

Sitting haemodynamic, hormonal and creatinine clearance measurements for each of the two study days.

	Urinary clearance period				
Infusion	A	B	C	D	E
HR (Beats/min)					
P	67+/-3	67+/-4	69+/-3	68+/-2	69+/-3
NA	61+/-2	63+/-2	62+/-2	64+/-2	63+/-3
DBP (mmHg)					
P	66+/-2	69+/-4	67+/-4	63+/-3	69+/-2
NA	68+/-2	68+/-3	69+/-3	68+/-2	68+/-3
SBP (mmHg)					
P	118+/-3	121+/-5	115+/-3	117+/-4	121+/-3
NA	124+/-3	120+/-4	124+/-4	121+/-2	118+/-3
C_{Cr} (ml/min)					
P	111+/-5	108+/-4	112+/-5	108+/-5	110+/-3
NA	110+/-4	109+/-4	109+/-4	103+/-4	108+/-3
NA (pg/ml)					
P	571+/-69	607+/-76	-	673+/-91	-
NA	597+/-25	894+/-71**	-	865+/-46**	-
ALDO (pg/ml)					
P	148+/-25	155+/-25	-	159+/-22	-
NA	122+/-29	148+/-29	-	177+/-32	-
AII (pg/ml)					
P	32+/-4	31+/-3	-	34+/-4	-
NA	35+/-5	31+/-4	-	31+/-5	-

Means +/- sem. SBP= systolic blood pressure; DBP= diastolic blood pressure; HR= heart rate; C_{Cr}= creatinine clearance; NA= noradrenaline; P= placebo; AII= angiotensin; ALDO= aldosterone.
A= baseline and B-E= periods of measurement.
** $P<0.01$ versus baseline (paired t-test).

Figure 5.1


PLACEBO
NORADRENALINE
ΔUNaV
μmol/min
±sem
from
baseline
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
A B C D E
A B C D E
PLACEBO
NORADRENALINE
ΔCLi
ml/min
±sem
from
baseline
0
-2
-4
-6
-8
A B C D E
A B C D E

Legend to figure 5.1

Change in sodium excretion ($U_{Na}V$) from baseline (A) on placebo day and NA day for periods B-E. The change in lithium clearance (C_{Li}) from baseline is shown in the same format on the lower part of the figure. Bars represent 1 s.e.m. * $P<0.01$ versus placebo day (paired t-test after ANOVA).

Summary

Low-dose (25 ng/kg/min) NA infusion, resulting in a physiological plasma increment (280 pg/ml), was antinatriuretic in normal salt-replete male subjects. The reduction in sodium excretion (-20%, $P<0.01$) occurred without any change in the glomerular filtration rate but was associated with a significant ($P<0.02$) decline in lithium clearance. These results suggest that changes in circulating NA, within the physiological range, can decrease sodium excretion in man by enhancing proximal tubular reabsorption. These findings extend previous investigations in man which used pharmacological doses of NA and are in agreement with animal evidence for a tubular antinatriuretic effect of the sympathetic nervous system.

[Discussion: chapter 13.3]

CHAPTER SIX

CHAPTER SIX

The effect of angiotensin II and noradrenaline alone and in combination on renal sodium excretion in man.

Introduction

The independent role of the renal sympathetic nervous system (SNS) on sodium excretion has also been established (DiBona, 1982) and studied further in chapter five. However, AII/NA facilitation has been clearly demonstrated for the vascular tree in animals but experimental work also suggests that AII can modulate the effect of the SNS on renal function. Investigation of a possible interaction between the RAS and SNS on sodium excretion in rats showed that renal nerve induced antinatriuresis and antidiuresis required the presence of AII, suggesting it may perform a facilitatory role at the level of the renal tubules (Handa and Johns, 1987; Johns, 1987a). AII may also modulate the effect of renal nerve stimulation at the glomerulus (Pelayo et al, 1984). Furthermore the effect of renal sympathetic activity in experimentally induced heart failure may in part be due to an interaction with the RAS (Kon et al, 1985; Ichikawa et al, 1987). Such an interaction may be of importance in the altered renal function of cardiac failure in man, in which both the RAS and the SNS are activated (Curtiss et al, 1978; Dzau et al, 1981). We studied a possible interaction between the RAS and the SNS on sodium excretion in man by the infusion of low doses of AII and NA alone and in combination.

Methods

Seven male normal volunteers (mean age 25, range 20-37) were

studied on four separate occasions 5-8 days apart. Volunteers attended the clinical laboratory at 08.30 hours on the morning of the study. All had fasted (food and drink) from 22.00 hours the previous evening. They were seated and an intravenous cannulae placed in veins in both forearms. A steady state water diuresis was established over an equilibration period of 2 hours (six 20 min clearance periods, as described in general methods). After a further clearance period (clearance period A) an intravenous infusion of either placebo (P - 5% dextrose) or NA (25 ng/kg/min in 5% dextrose) was commenced and infused in a volume of 1.0 ml/min throughout the rest of the study (clearance periods B-E). After a further clearance period (period B) a second infusion of either placebo (P - 5% dextrose) or AII (1 ng/kg/min) was given for one 20 minute clearance period (period C) in a volume of 2.0 mls/min, after which it was discontinued. The infusions were given in randomised single blind fashion.

Urine samples were obtained at the end of all clearance periods (A-E). Venous blood (35 mls) was sampled during the 12th minute of each collection period A-E. Each sample was aliquoted into four for measurement of plasma NA, AII, aldosterone and finally creatinine, osmolality and electrolytes.

Analysis

The results for change over time and effect of NA were analysed by linear regression analysis (Wallenstein et al, 1980). This analysis takes account of the development of an effect of NA over time which would bias any estimate of the effect of AII calculated as the difference between the value in the AII infusion period and either the previous or succeeding period. To minimise such bias, regression analysis was used to

fit a time trend for each subject, using the data from the two periods prior to AII infusion and the two periods after infusion. The value corresponding to the AII infusion period was not used in the estimation of the time trend. The effect of AII for each subject was taken to be the proportionate reduction from the value predicted by the time trend for the AII infusion period, using the actual value at the time of AII infusion to estimate the proportionate reduction. Similarly the placebo arms of the study were analysed by linear regression analysis to take account of change over time and allow a valid assessment of the time trend caused by the administration of NA by comparison with the time trends when NA was not administered. The effect of AII is expressed as a percentage change with confidence intervals for the percentage changes coincident with the period of AII infusion. Blood pressure, heart rate, creatinine clearance and plasma hormone levels were analysed by repeated measures analysis of variance (MANOVA) and subsequent paired t-testing where significant differences were indicated by MANOVA (Nie et al, 1975).

Results

Twenty four hour sodium excretion and baseline sodium excretion (Period A) were not significantly different for any of the four study days (155+/-19; 148+/-19; 167+/-22 and 192+/-41 mmol/24hours for P/P; P/AII; NA/AII and NA/P days respectively). Urinary osmolality was below 64 mosm/kg in all subjects on all study days. This confirms suppression of endogenous vasopressin. Creatinine clearance was similar on all study days and was not changed by infusion of AII, NA or the combination of NA/AII (table 6.1). Heart rate and mean blood pressure also did not change on any study day (table 6.1). Plasma AII, aldosterone and NA levels are shown in

table 6.2. Plasma AII rose significantly during exogenous AII infusion on both the P/AII study day and on the NA/AII study day. There was no significant difference in the plasma AII data between the P/AII and the NA/AII days. Plasma NA rose significantly during NA infusion, with no significant difference between the NA/P and the NA/AII days. Plasma NA did not change significantly on P/P or P/AII days. Plasma aldosterone rose significantly on P/AII and NA/AII days, reaching highest levels in period D, after AII infusion had finished (period C). There was no significant difference between P/AII and NA/AII days and no significant change on P/P and NA/P days.

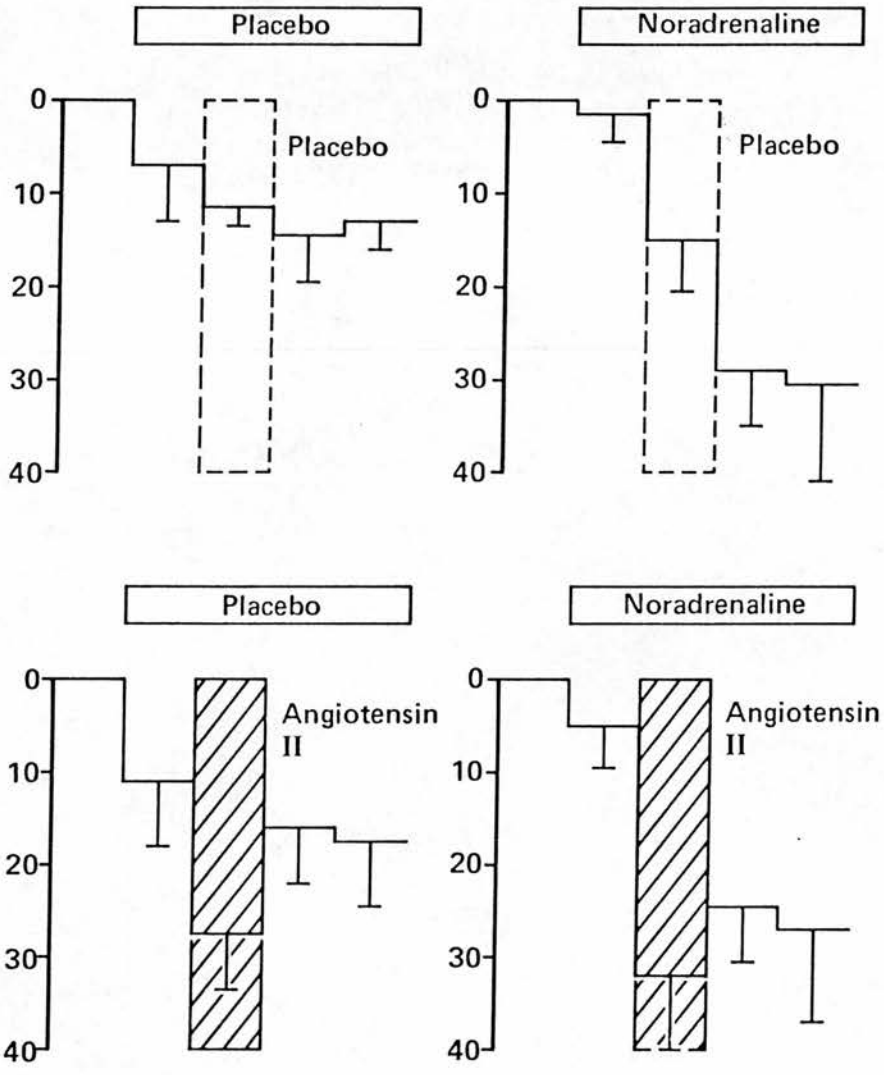
Changes in absolute sodium excretion ($U_{Na}V$) are shown in figure 6.1. The results of time trend analysis for change over time (placebo day) and effect of NA over time with respect to $U_{Na}V$, urinary flow rate (UV), fractional sodium excretion (FE_{Na}) and absolute potassium excretion (U_KV) are shown in table 6.3(a). The changes in $U_{Na}V$, UV, FE_{Na} and U_KV caused by AII infusion are shown in table 6.3(b).

There was a small fall in UV with time on the P/P study day ($p<0.02$). $U_{Na}V$, FE_{Na} and U_KV did not change significantly on the P/P study day. NA infusion (P/NA study day) caused reductions in $U_{Na}V$ ($p<0.02$), FE_{Na} ($p<0.01$), UV ($p<0.02$) and a small but non significant fall in U_KV. AII caused a 37% reduction in $U_{Na}V$ on the P/AII study day and 32% reduction during concomitant NA infusion (NA/AII day). AII also caused significant falls in UV and FE_{Na} on both study days with no difference in the presence of NA infusion. There was no significant effect of AII on U_KV on either study day.

Figure 6.1

Figure 1

CHANGE IN ABSOLUTE SODIUM EXCRETION



Legend to figure 6.1

Mean change in absolute sodium excretion +/- s.e.m. (µmol/min).
(a) Placebo/Placebo (b) Placebo/Noradrenaline (c) Placebo/Angiotensin II and (d) Noradrenaline/Angiotensin II, where placebo = 5% dextrose infusion, Angiotensin II = infusion at 1ng/kg/min and Noradrenaline = infusion at 25ng/kg/min. The horizontal bar represents the period over which placebo or noradrenaline was infused. The vertical broken-line box the period in which placebo was given and the hatched vertical box represents the period in which AII was given, as the second infusion.

TABLE 6.1

	INFUSION	URINARY COLLECTION PERIOD				
	COMBINATION	A	B	C	D	E
MEAN BP	P / P	88+/-4	86+/-4	86+/-4	84+/-4	89+/-4
(mmHg)	NA/ P	88+/-3	87+/-4	86+/-3	89+/-3	85+/-4
	P /AII	82+/-3	87+/-3	87+/-4	85+/-2	87+/-3
	NA/AII	86+/-3	91+/-5	91+/-3	90+/-3	94+/-3
HEART RATE	P / P	65+/-3	64+/-3	66+/-4	68+/-3	69+/-4
(beats/min)	NA/ P	61+/-3	61+/-3	61+/-2	62+/-2	62+/-3
	P /AII	64+/-4	62+/-4	63+/-4	68+/-5	65+/-6
	NA/AII	65+/-4	64+/-2	62+/-2	64+/-2	66+/-3
CREATININE	P / P	113+/-6	109+/-4	113+/-3	108+/-6	109+/-3
CLEARANCE	NA/ P	112+/-5	111+/-4	111+/-3	108+/-4	109+/-3
(mls/min)	P /AII	101+/-3	107+/-3	110+/-4	107+/-4	107+/-4
	NA/AII	109+/-5	109+/-2	112+/-4	106+/-3	109+/-7

Table legend

Mean blood pressure (BP), heart rate and creatinine clearance for consecutive collection periods A-E. All results are expressed as mean +/- standard error. P = Placebo infusion (5% dextrose), AII = Angiotensin II infusion (1 ng/kg/min) and NA = noradrenaline infusion (25 ng/kg/min).

TABLE 6.2

	INFUSION COMBINATION	URINARY COLLECTION PERIOD A	B	C	D
AII	P / P	31+/-3	31+/-3	31+/-3	35+/-3
(pg/ml)	NA/ P	34+/-5	31+/-4	29+/-3	30+/-4
	P /AII	28+/-5	34+/-6	51+/-10*	36+/-5
	NA/AII	34+/-4	33+/-4	49+/-5*	30+/-3
Aldosterone	P / P	146+/-27	146+/-27	163+/-29	148+/-25
(pg/ml)	NA/ P	101+/-30	129+/-26	134+/-28	159+/-32
	P /AII	139+/-28	177+/-30**	217+/-34++	233+/-31++
	NA/AII	112+/-22	142+/-30**	178+/-24++	199+/-29++
Noradrenaline	P / P	495+/-71	533+/-84	530+/-64	577+/-109
(pg/ml)	NA/ P	552+/-27	821+/-77++	616+/-59+	826+/-52++
	P /AII	469+/-66	491+/-74	482+/-52	529+/-44
	NA/AII	536+/-63	898+/-106++	845+/-121+	845+/-109++

Table legend

Plasma angiotensin II, aldosterone and noradrenaline levels during urinary collection periods A-D. All results are expressed as mean +/- standard error. P = Placebo infusion (5% dextrose), AII = Angiotensin II infusion (1 ng/kg/min) and NA = noradrenaline infusion (25 ng/kg/min). * = $p<0.05$, ** = $p<0.01$, + = $P<0.005$ and ++ = $p<0.001$ (by paired t-testing with period A for any given study day in which a significant treatment by time effect was shown by MANOVA)

TABLE 6.3(a) Results of time trend analysis.

	$U_{Na}V$		UV		FE_{Na}		U_KV	
	Slope	P<	Slope	P<	Slope	P<	Slope	P<
P / P	-3.40	NS	-0.29	0.02	-0.12	NS	+0.37	NS
NA/ P	-8.74	0.02	-0.54	0.01	-0.54	0.02	-2.36	NS

Mean slope of regression analysis (see Methods for description of time trend analysis).

TABLE 6.3(b) Effect of Angiotensin II

	$U_{Na}V$	UV	FE_{Na}	U_KV
P /AII	-37	-10	-20	-11
	(-24 to-50)	(-2 to -18)	(-2 to -38)	(0 to -21)
NA/AII	-32	-9	-17	-6
	(-23 to-41)	(-3 to -15)	(-5 to -30)	(+5 to -17)

Mean change as percentage and 95% confidence interval

Table legend: (a) Results of regression analysis and (b) effect of Angiotensin II. P = Placebo infusion (5% dextrose), AII = Angiotensin II infusion (1 ng/kg/min) and NA = noradrenaline infusion (25 ng/kg/min), $U_{Na}V$= absolute sodium excretion, UV= urinary flow rate, FE_{Na}= fractional sodium excretion and U_KV= absolute potassium excretion. NS = non-significant.

Summary

This study sought to investigate the possible interaction of physiological doses of AII and NA on sodium excretion in man. Seven normal volunteers were studied on four occasions during maximum water diuresis sustained by oral hydration. Samples were obtained during a baseline and four subsequent 20 minute periods (A-E). Placebo or NA was infused over periods B-E, and placebo or AII infused over period C.

There was no change in systemic blood pressure, heart rate or creatinine clearance caused by infusion of either AII, NA or both in combination. Noradrenaline alone caused a significant fall in absolute and fractional sodium excretion. AII when infused with placebo caused a 37% fall in absolute sodium excretion and a 32% fall when infused with NA (no significant difference between the two days). Similar changes were seen for urinary flow and fractional sodium excretion.

We have therefore found no evidence to support a postsynaptic interaction of low doses of AII and NA on renal sodium excretion in man.

[Discussion: chapter 13.4]

CHAPTER SEVEN

CHAPTER SEVEN

The effect of angiotensin II on the stroke volume response to beta-agonism in man

Introduction

The data described in chapter three show that when NA and AII are infused coincidentally into normal man, there is a synergistic augmentation of the systolic blood pressure response but this did not occur with diastolic blood pressure. The fact that this was limited to systolic pressure suggests that this NA/AII interaction occurs at an intrathoracic or myocardial level rather than in the peripheral vasculature.

Recently Stokland et al (1986) have described an animal experiment which may explain the mechanism for this effect. In dogs, during isoprenaline (ISO) infusion AII was found to relocate venous blood from the liver and spleen to the heart hence increasing filling pressure and therefore cardiac output (CO). We were interested to perform the analogous human experiment including a non-invasive measure of stroke volume to seek evidence for a similar effect in man.

Methods

Ten normal volunteers (8 male and 2 female, mean age 24 years, range 20-33 and mean weight 70 kg, range 56-87.5 kg) were studied on four separate occasions a minimum of 5 days apart. Subjects attended the clinical laboratory at 08.30 hours or 13.00 hours and were studied at the same time on all four days. They were positioned supine and intravenous cannulae placed in veins in both forearms. After 30 minutes of supine rest an infusion of either AII (2ng/kg/min) or placebo (PL, 5% dextrose) was

commenced and infused in a volume of 0.5 mls/min throughout the rest of the study. After a further 15 minutes an incremental infusion of placebo (PL, 5% dextrose) or ISO (Saventrine, Pharmax Ltd., Kent, UK.) was commenced at 0.25 mcg/min and increased at 10 minute intervals to 0.5, 1.0 and 2.0 mcg/min. The infusions were given in randomised single blind fashion. Blood pressure and heart rate were recorded semi-automatically every five minutes throughout each experiment. The values recorded at the end of the rest period, at the end of the AII or placebo alone period and at the end of each ISO infusion period were used for statistical analysis. The ECG was monitored continuously on an ECG oscilloscope. Cardiac output was measured at the end of the rest period, at the end of the AII or placebo alone period and at the end of each ISO infusion period by a non-invasive acetylene rebreathing technique. Blood samples were taken and divided into three aliquots for later analysis of plasma AII, plasma NA and plasma atrial natriuretic peptide (ANP).

Results were analysed by repeated measures analysis of variance (MANOVA, SPSS/PC+) to examine the effect of dual placebo infusion, treatment (dual placebo vs. AII and dual placebo vs. ISO) and for an interaction between treatments. In considering the interaction term the change due to ISO in the presence of PL or AII infusion was obtained by using each subjects responses on the dual PL and PL/AII days respectively as control. The data were thus considered as change produced by ISO (a) in the presence of placebo as [ISO/PL - PL/PL] and (b) in the presence of AII as [AII/ISO - AII/PL] for all parameters in each individual. These two changes (a) and (b) were then compared by ANOVA for repeated measures. A p-value of <0.05 was considered significant.

All subjects noticed tachycardia during ISO infusion regardless of whether PL or AII was being infused simultaneously. No subject reported

any unpleasant side effect during any infusion or combination of infusions. One subject developed asymptomatic ventricular premature beats at the highest dose of ISO on the PL/ISO day.

Results

The data for SV are shown in figure 7.1 and Table 7.1. There was no significant change in SV over the dual placebo day nor with AII infusion alone (ANOVA). ISO caused an 11-20% rise in SV which did not reach significance. But of major interest is that the combination of AII and ISO caused a significant 31-55% rise in SV ($p<0.01$), and considering the interaction term (see methods) this rise in SV was significantly greater than with ISO confirming an AII/ISO interaction with regard to increase in SV ($p<0.02$).

Figure 7.2 shows the data for TPR. This contrasts with the SV data. There was no effect of infusion with dual placebo, but AII alone caused a significant rise in TPR ($p<0.05$, figure 7.2 and table 7.2). ISO alone caused a dose dependent fall in TPR ($p<0.001$). Of interest, however, with the combination of AII/ISO is that the expected dose dependent fall in TPR with ISO was unaffected by concomitant AII infusion. Considering the interaction term (see methods) the difference in response to ISO and the AII/ISO combination (illustrated in figure 7.2) was highly significant ($p<0.01$).

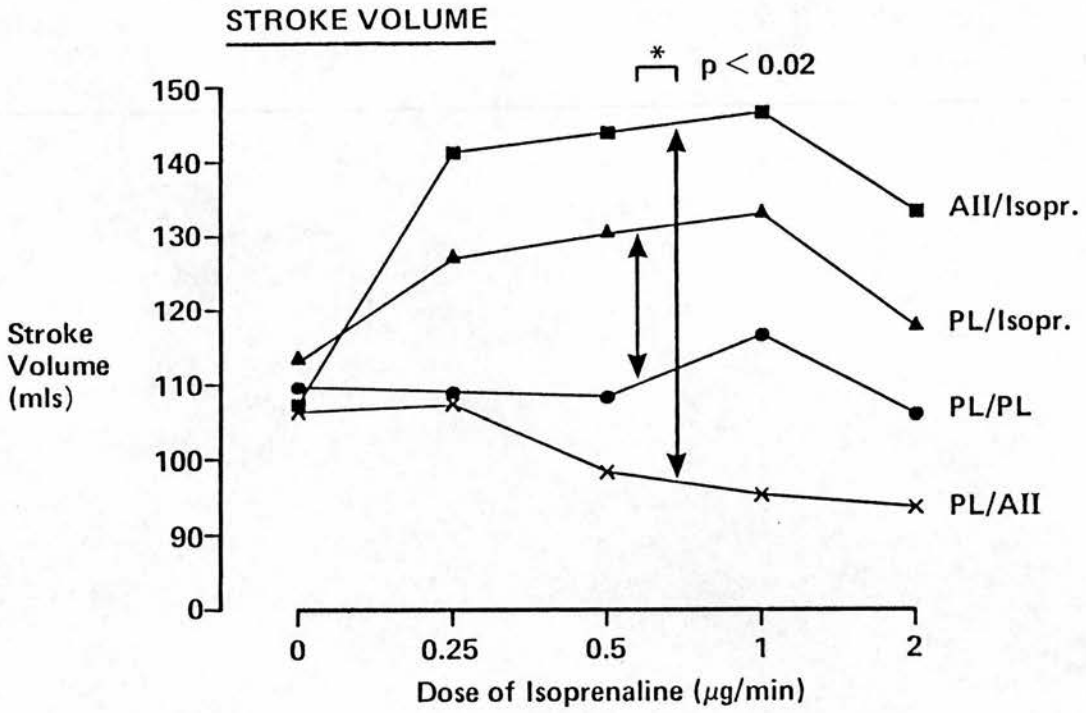
The CO data are given in Table 7.1. Neither dual placebo nor AII alone caused significant change in CO. ISO produced a dose dependent increase in CO ($p<0.001$), and the combination of AII and ISO produced a further increase in CO that just failed to reach significance ($p=0.065$). The HR data is given in table 7.1. Neither dual placebo nor AII alone caused significant change in HR. ISO produced a dose dependent increase in HR ($p<0.001$), but there was no significant interaction between AII and ISO.

The blood pressure data are given in Table 7.2. There was no effect

of dual placebo infusion, but AII alone produced a significant rise in SBP ($p<0.01$) which did not change with time. ISO produced a significant dose dependent rise in SBP ($p<0.001$). However there was no interaction between AII and ISO in the SBP data. There was no effect of dual placebo infusion on DBP, but AII alone caused a significant rise in DBP ($P<0.002$). ISO caused a significant fall in DBP ($p<0.001$). There was, however, no interaction between AII and ISO in the DBP data.

The results of plasma AII analysis are given in Table 7.3(a). There was no significant change in plasma AII with time on the dual placebo day. Infusion of AII caused a significant rise in plasma AII ($p<0.001$). ISO alone had no significant effect on plasma AII and there was no interaction between AII and ISO in the plasma AII data. The results of plasma NA analysis are given in Table 7.3(b). There was no significant change on the dual placebo day. The mean resting supine plasma NA levels for the four study days varied between 395 to 440 pg/ml which is in the normal range for our assay and are the same as in many previous studies with this assay (chapter three; Brown et al, 1985; Becker et al, 1986; Struthers et al, 1986). AII infusion did not affect plasma NA levels. ISO produced a small rise in plasma NA levels, but there was no significant interaction between AII and ISO in the NA data. ISO induced increases in plasma NA have been reported before and are thought to be due either to baroreflex activation or to ISO stimulating presynaptic beta-adrenoceptors (Vincent et al, 1982). The results of plasma ANP analysis are given in table 7.3(c). ANP levels did not change on the dual placebo day, nor during infusion of AII alone or ISO alone. There was a rise in plasma ANP when both AII and ISO were infused together but this did not reach significance.

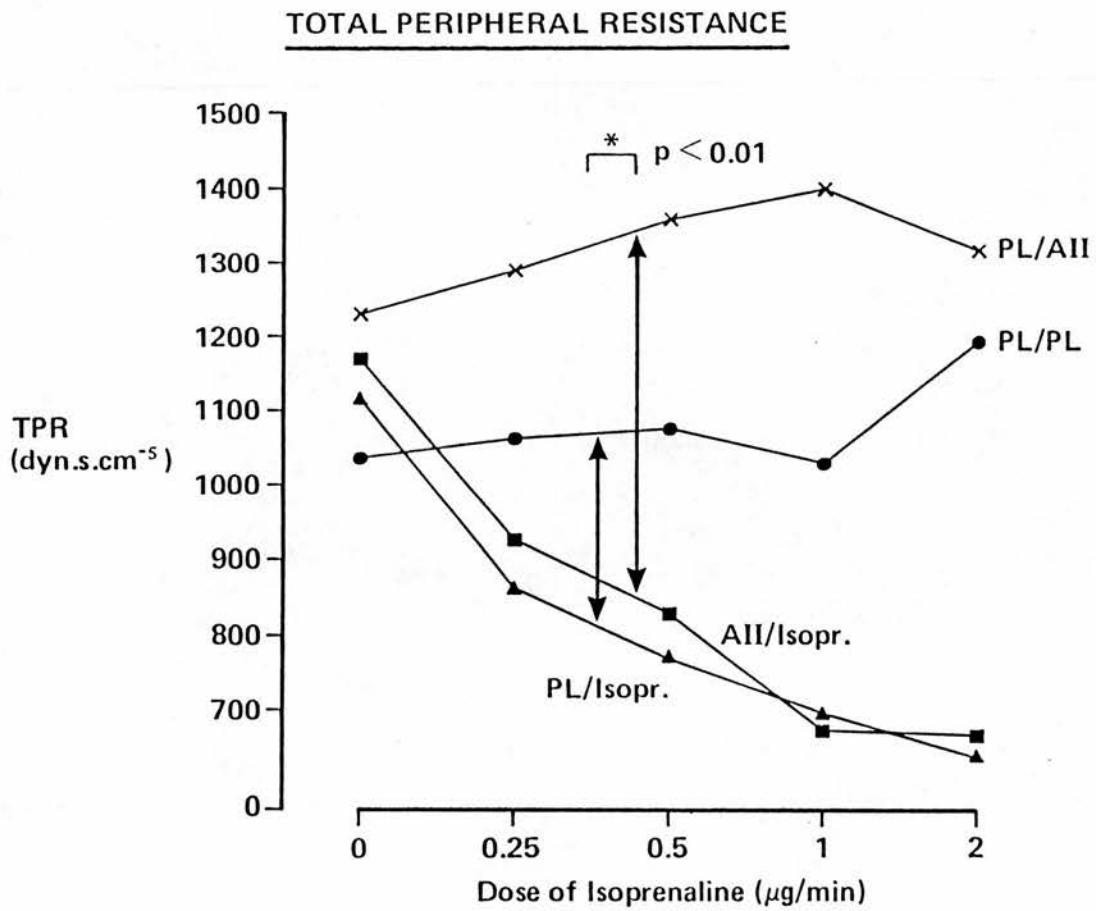
Figure 7.1


STROKE VOLUME
* p < 0.02
150
140
130
120
110
100
90
0
Stroke Volume (mls)
AII/Isopr.
PL/Isopr.
PL/PL
PL/AII
0
0.25
0.5
1
2
Dose of Isoprenaline (μg/min)

Legend to figure 7.1

Stroke volume in mls. AII = angiotensin II (2ng/kg/min), PL = placebo (5% dextrose) and ISO = isoprenaline (0.25-2 mcg/min). Horizontal axis represents dose of isoprenaline or placebo as indicated. Results expressed as mean (n=10). See text for details of analysis.

Figure 7.2


TOTAL PERIPHERAL RESISTANCE
TPR
(dyn.s.cm⁻⁵)
1500
1400
1300
1200
1100
1000
900
800
700
0
* p < 0.01
PL/AII
PL/PL
AII/Isopr.
PL/Isopr.
0
0.25
0.5
1
2
Dose of Isoprenaline (μg/min)

Legend to figure 7.2

Total peripheral resistance in dyne.sec.cm^{-5}. AII = angiotensin II (2ng/kg/min), PL = placebo (5% dextrose) and ISO = isoprenaline (0.25-2 mcg/min). Horizontal axis represents dose of isoprenaline or placebo as indicated. Results expressed as mean (n=10). See text for details of analysis.

Table 7.1

HAEMODYNAMIC RESPONSES TO ISOPRENALINE INFUSION IN THE PRESENCE OF ANGIOTENSIN II OR PLACEBO INFUSION

		Dose of Isoprenaline (mcg/min)					Sign. by
	Resting	0	0.25	0.5	1	2	ANOVA
			Stroke Volume (mls)				
AII/ISO	94+/-10	108+/-11	141+/-18	144+/-19	147+/-14	134+/-16	p<0.01
AII/PL	106+/-11	107+/-11	107+/-11	98+/-10	95+/-10	94+/-7	NS
PL/ISO	108+/-12	113+/-11	127+/-9	130+/-11	133+/-10	118+/-11	NS
PL/PL	109+/-9	110+/-8	109+/-8	108+/-8	117+/-10	106+/-11	NS
			Cardiac Output (l/min)				
AII/ISO	6.0+/-0.5	6.1+/-0.4	8.3+/-0.8	9.0+/-0.9	10.6+/-0.7	11.3+/-0.8	p<0.001
AII/PL	6.1+/-0.7	6.1+/-0.5	5.9+/-0.5	5.6+/-0.4	5.3+/-0.3	5.5+/-0.3	NS
PL/ISO	6.3+/-0.7	6.4+/-0.6	7.9+/-0.5	8.9+/-0.6	10.1+/-0.8	10.9+/-0.8	p<0.001
PL/PL	6.5+/-0.6	6.6+/-0.5	6.5+/-0.5	6.4+/-0.5	6.5+/-0.5	6.2+/-0.7	NS
			Heart Rate (beats/min)				
AII/ISO	59+/-2	59+/-3	62+/-3	65+/-3	74+/-3	89+/-5	p<0.001
AII/PL	58+/-2	58+/-2	56+/-2	59+/-3	57+/-2	60+/-3	NS
PL/ISO	60+/-3	58+/-2	63+/-2	69+/-3	77+/-3	94+/-3	p<0.001
PL/PL	60+/-3	60+/-2	60+/-2	59+/-2	56+/-2	58+/-2	NS

AII = angiotensin II infusion (2ng/kg/min), ISO = isoprenaline infusion,
PL = placebo infusion (5% dextrose).
All results are expressed as mean +/- s.e.m. NS = not significant.
Sign. by ANOVA = significance by ANOVA for repeated measures within study day for dual placebo (PL/PL) and for each other study day vs. dual placebo (see Methods)

Table 7.2

HAEMODYNAMIC RESPONSES TO ISOPRENALINE INFUSION IN THE PRESENCE OF ANGIOTENSIN II OR PLACEBO INFUSION

		Dose of Isoprenaline (mcg/min)					Sign. by
	Resting	0	0.25	0.5	1	2	ANOVA
			Systolic Blood Pressure (mmHg)				
AII/ISO	119+/-2	120+/-3	126+/-3	130+/-3	136+/-4	139+/-2	p<0.001
AII/PL	121+/-2	126+/-2	124+/-3	128+/-3	126+/-3	126+/-2	p<0.01
PL/ISO	120+/-3	118+/-3	121+/-2	127+/-3	134+/-3	139+/-4	p<0.001
PL/PL	121+/-2	120+/-1	118+/-2	119+/-2	116+/-2	119+/-2	NS
			Diastolic Blood Pressure (mmHg)				
AII/ISO	64+/-2	70+/-2	66+/-2	65+/-2	61+/-2	61+/-6	p<0.001
AII/PL	67+/-2	71+/-1	72+/-2	73+/-1	70+/-1	70+/-2	p<0.002
PL/ISO	64+/-2	65+/-2	63+/-3	60+/-2	60+/-2	57+/-3	p<0.001
PL/PL	66+/-1	64+/-2	63+/-2	63+/-2	63+/-2	67+/-2	NS
			Total Peripheral Resistance (dyn.s/cm^5)				
AII/ISO	1179+/-93	1170+/-70	925+/-120	834+/-88	679+/-57	665+/-57	p<0.001
AII/PL	1191+/-101	1231+/-85	1293+/-109	1361+/-97	1400+/-87	1317+/-76	p<0.05
PL/ISO	1161+/-126	1115+/-108	861+/-57	770+/-56	697+/-54	647+/-58	p<0.001
PL/PL	1109+/-94	1040+/-72	1063+/-79	1082+/-81	1034+/-71	1195+/-118	NS

AII = angiotensin II infusion (2ng/kg/min), ISO = isoprenaline infusion,
PL = placebo infusion (5% dextrose).
All results are expressed as mean +/- s.e.m. NS = not significant.
Sign. by ANOVA = significance by ANOVA for repeated measures within study day for dual placebo (PL/PL) and for each other study day vs. dual placebo (see Methods)

Table 7.3(a) **PLASMA ANGIOTENSIN II (pg/ml)**

Study day		Dose of isoprenaline (mcg/ml)		
	Baseline	0	0.5	2
AII/ISO	17+/-3	64+/-6	50+/-7	50+/-7
AII/PL	19+/-2	78+/-8	68+/-7	50+/-7
PL/ISO	18+/-1	19+/-2	18+/-2	27+/-2
PL/PL	19+/-4	18+/-2	18+/-2	20+/-2

Table 7.3(b) **PLASMA NORADRENALINE (pg/ml)**

Study day		Dose of isoprenaline (mcg/ml)		
	Baseline	0	1.0	2.0
AII/ISO	429+/-28	432+/-23	487+/-34*	480+/-35
AII/PL	398+/-38	ND	ND	397+/-26
PL/ISO	395+/-32	408+/-23	408+/-37	520+/-48**
PL/PL	440+/-21	ND	ND	430+/-20

Table 7.3(c) **ATRIAL NATRIURETIC PEPTIDE (pmol/l)**

Study day		Dose of isoprenaline (mcg/ml)		
	Baseline	0	0.5	2.0
AII/ISO	14+/-2	15+/-2	17+/-4	16+/-4
AII/PL	15+/-2	14+/-2	14+/-2	12+/-2
PL/ISO	14+/-1	13+/-2	13+/-2	13+/-2
PL/PL	14+/-2	15+/-2	14+/-2	14+/-2

AII = angiotensin II infusion (2ng/kg/min), ISO = isoprenaline infusion, PL = placebo infusion (5% dextrose). ND = not done.

All results are expressed as mean +/- s.e.m.

* = $p<0.05$ and ** = $p<0.01$ by paired t-testing after MANOVA revealed a significant effect of ISO ($p<0.001$, MANOVA).

Summary

In this study evidence was sought for an RAS/SNS interaction using AII and beta-adrenoceptor stimulation with ISO. Ten normal volunteers were infused with placebo/placebo, placebo/AII, placebo/ISO and AII/ISO in a randomised single-blind fashion. ISO alone caused a non-significant 11-20% rise in SV. AII alone caused no significant change in SV. However the combination of AII/ISO caused a significant increase in SV of 31-55% ($p<0.01$), and this increase was significantly greater than with ISO alone ($p<0.02$ by repeated measures ANOVA). This occurred with no difference in change of heart rate. ISO significantly reduced total peripheral resistance and this reduction was not affected by concomitant infusion of AII. This study provides evidence that a physiological dose of AII can synergistically augment the stroke volume effect of beta-agonism in man.

[Discussion: chapter 13.7]

CHAPTER EIGHT

CHAPTER EIGHT

The effect of angiotensin II on endogenous noradrenaline release in man

Introduction

In chapter three data was presented supporting a possible post-synaptic AII/NA interaction in that AII synergistically augments the systolic blood pressure response to NA. In this study we have now sought evidence for a presynaptic AII/NA interaction by examining whether exogenous AII is able to enhance the presynaptic release of endogenous NA in response to physiological stimuli in man. The stimuli were chosen to produce a wide range of different levels of sympathetic activation.

Methods

Nine normotensive volunteer males were studied (mean age 26 years, range 19-38 years and mean weight 80 kg, range 67-109 kg). They were studied on three occasions, the first a pre-study assessment as described below and two formal study days at least one week apart. The subjects attended at 09.30 hours after a light breakfast or at 13.30 hours after a light luncheon. Each individual was investigated at the same time of day on both study days.

Pre-study assessment: An initial assessment of bicycle workload and hand dynomanometry was made 7 days before the first study day. Each subject commenced cycling on a bicycle ergometer (Tunturi, Finland) at a load of 50 kilopunds and the workload was then increased at one minute intervals until a heart rate of 130-140 beats per minute was achieved as recorded by ECG monitor. After a period of 15 minutes rest maximal

voluntary contraction of handgrip was measured using a hand dynomanometer.

Formal study days: At the start of the study intravenous cannulae were inserted into the antecubital vein of each arm, one for infusion and the other for blood sampling. After 20 minutes supine rest, baseline recordings of heart rate and blood pressure and blood samples were taken.

Infusion was commenced with either a subpressor dose of AII (1.5 ng/kg/min) or placebo (5% dextrose) in a randomised single-blind fashion. After a further 15 minutes supine rest blood pressure and heart rate recordings and blood sampling were repeated. Thereafter each subject underwent a series of standard tests to endogenously stimulate the sympathetic nervous system. These tests were always administered in the same order as described below and illustrated in figure 8.1.

Cold pressor test: The subject immersed one foot to the ankle in melting ice for two minutes. Blood pressure and heart rate were recorded at 0, 1 and 2 minutes and a blood sample taken at 2 minutes. The subject then rested in the supine position for a further 15 minutes during which blood pressure and heart rate were measured every 5 minutes and at the end of which a blood sample was taken.

Response to standing: Blood pressure and heart rate were recorded and blood samples taken after two minutes standing erect.

Bicycle exercise: The subject then sat on a bicycle ergometer and after baseline haemodynamic recordings performed five minutes exercise at a workload predetermined in each individual at the pre-study assessment to cause a rise in heart rate to 130-140 beats per minute. The same workload was used on both study days. Blood pressure and heart rate were recorded at 2 and 5 minutes of exercise and 1, 2 and 3 minutes after

exercise. Blood samples were taken during the fifth minute of exercise.

Forearm isometric exercise: Following a further rest period of 15 minutes, baseline haemodynamic recordings and blood samples were taken. The subject then undertook 3 minutes of forearm isometric exercise when they maintained 30% of their maximal voluntary handgrip which had previously been determined at the pre-study assessment. The same workload was used on both study days. Blood pressure and heart rate were recorded at one minute intervals during handgrip and blood samples taken after three minutes.

The results were analysed by two way analysis of variance comparing responses during placebo infusion with those during AII infusion (Statistical package for the social sciences - Nie et al, 1975). The Duncan procedure was then used to compare each stimulus pair wise with every other stimulus (SPSS - Nie et al, 1975).

Results

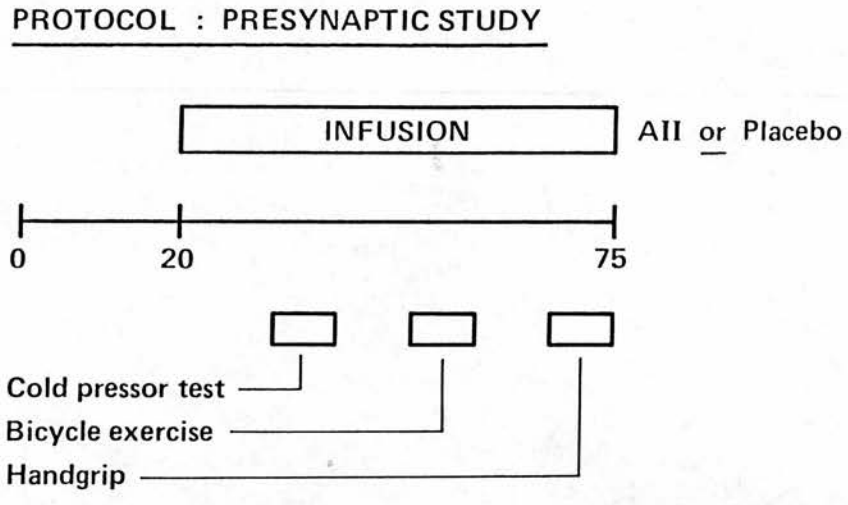
Table 8.1 shows the initial recordings of blood pressure, heart rate and plasma NA before and 15 minutes after the start of an AII (1.5 ng/kg/min) or a placebo infusion. There were no changes in any of these observations in response to AII.

The haemodynamic and plasma NA responses to two minutes cold pressor testing, after two minutes standing from lying, at five minutes bicycle exercise and at three minutes isometric handgrip are shown in figure 8.1 and figure 8.2. Two way analysis of variance showed that there was no overall effect of infusion of AII compared with a placebo infusion on haemodynamic or plasma NA responses to the physiological stimuli investigated.

Since the AII study day was not significantly different from the placebo study day, the Duncan procedure was applied to the data from both study days to compare the results of each test. The results of this analysis of haemodynamic and plasma NA responses are shown in Table 8.2. The haemodynamic data show a significant pressor response to cold pressor testing, bicycle exercise and forearm isometric exercise and significant rises in heart rate during standing, bicycle exercise and forearm isometric exercise. There were significant rises in plasma NA after cold pressor testing, standing and bicycle exercise. The changes in plasma NA demonstrated in our study are similar to those shown by other groups (Robertson et al, 1979; Struthers et al, 1986).

The mean +/- s.e.m. 24 hour urinary sodium excretion (mmol/24 hr) was 174+/-16 prior to the placebo study day and 201+/-20 prior to the AII infusion study day, which was not significantly different by paired t-testing. The plasma AII levels (Table 8.3) show that there was no significant difference between the resting levels on the two study days and no change during placebo infusion but there was a 312% rise in plasma AII levels during the AII infusion.

Figure 8.1

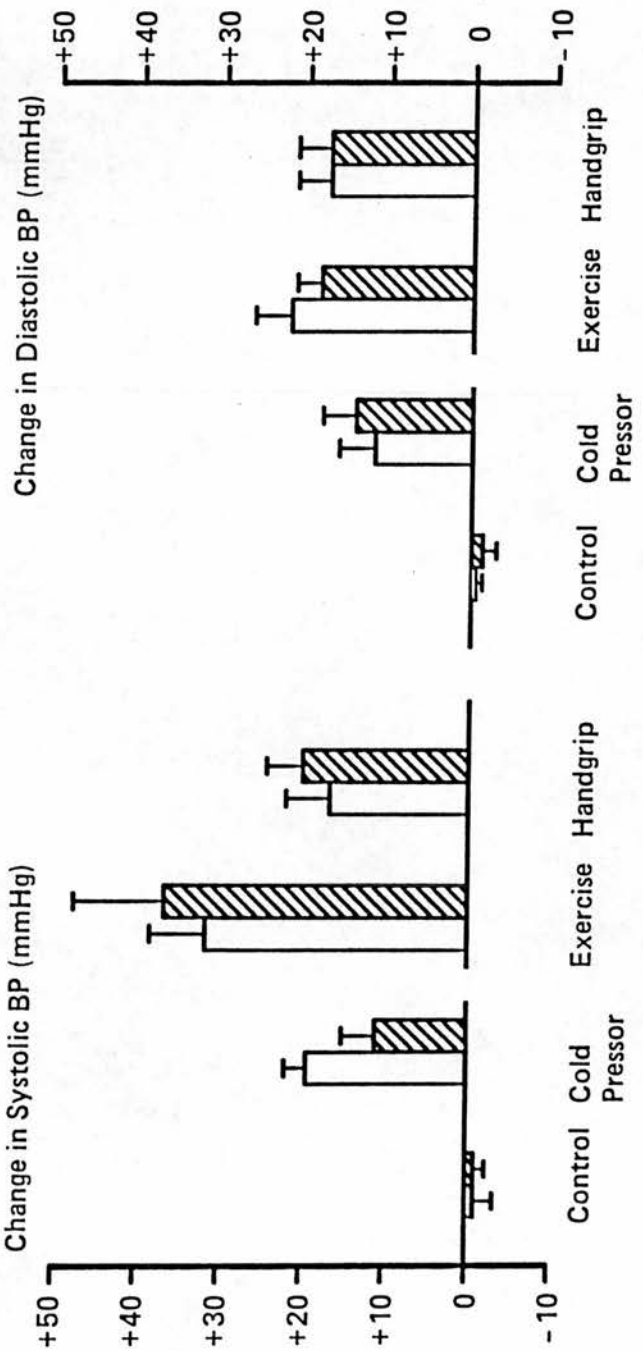

PROTOCOL : PRESYNAPTIC STUDY
INFUSION
AII or Placebo
0
20
75
Cold pressor test
Bicycle exercise
Handgrip

Legend to figure 8.1

Subjects received an infusion of either a subpressor dose of AII (1.5 ng/kg/min) or placebo (5% dextrose) in a randomised single-blind fashion on either of two separate study days. After a period of rest the subjects performed a series of standard tests in the following order:

1. Cold pressor test
2. Response to standing
3. Bicycle exercise
4. Forearm isometric exercise

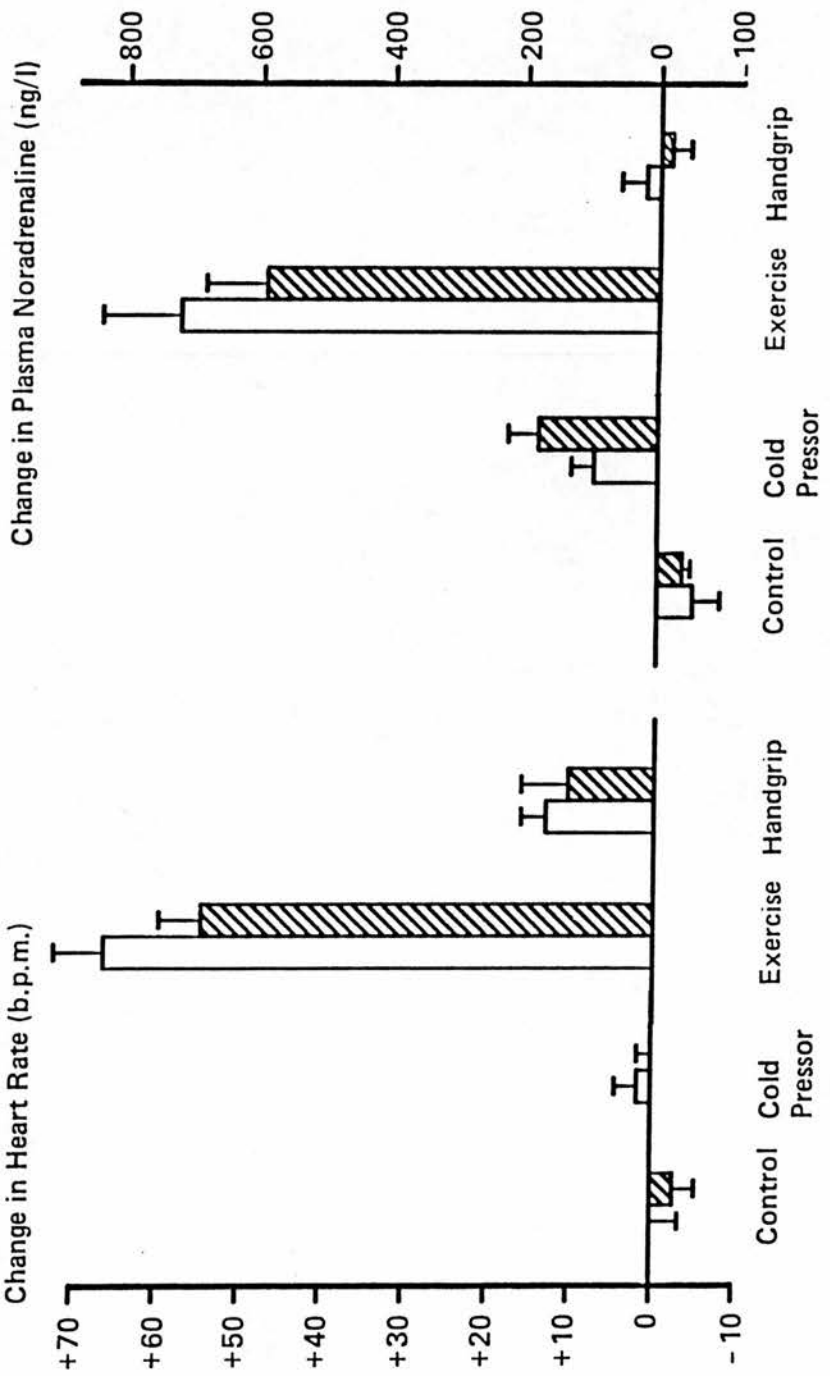
Figure 8.2


Change in Systolic BP (mmHg)
+50
+40
+30
+20
+10
0
-10
Control
Cold Pressor
Exercise Handgrip
Change in Diastolic BP (mmHg)
+50
+40
+30
+20
+10
0
-10
Control
Cold Pressor
Exercise Handgrip

Legend to figure 8.2

Systolic and diastolic blood pressure responses to physiological stimuli in the presence of angiotensin II infusion (hatched bars) or dextrose infusion (plain bars).

Figure 8.3


Change in Plasma Noradrenaline (ng/l)
800
600
400
200
0
-100
Control
Cold Pressor
Exercise Handgrip
Change in Heart Rate (b.p.m.)
+70
+60
+50
+40
+30
+20
+10
0
-10
Control
Cold Pressor
Exercise Handgrip

Legend to figure 8.3

Heart rate and plasma noradrenaline responses to physiological stimuli in the presence of angiotensin II infusion (hatched bars) or dextrose infusion (plain bars).

Table 8.1

Systolic and diastolic pressure, heart rate and plasma noradrenaline before and 15 minutes after angiotensin II (1.5 ng/Kg/min) or placebo infusion.

Results are expressed as mean +/- s.e.m. (n=9).

	SBP(mmHg)		DBP(mmHg)		HR(beats/min)		NA(pg/ml)	
	PL	AII	PL	AII	PL	AII	PL	AII
Before	125+/-4	127+/-4	65+/-3	67+/-3	64+/-3	65+/-4	560+/-60	510+/-20
After	124+/-4	126+/-4	66+/-3	66+/-3	64+/-4	63+/-4	510+/-50	480+/-20

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, NA = Noradrenaline. None of the above data are significantly different between placebo and angiotensin II by ANOVA.

Table 8.2

Haemodynamic and plasma noradrenaline response to physiological stimuli.

(Mean data only are displayed for clarity. This table includes AII pretreatment and placebo pretreatment days as they were not significantly different from each other).

	SBP(mmHg)	DBP(mmHg)	HR(beats/min)	NA(ng/l)
Control	125	66	64	494
Cold pressor	140 *	79 **	67	674 *
Standing	124	73	83 **	654 *
Exercise	159 **	86 **	130 **	1196 **
Handgrip	145 **	84 **	77 **	537

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, NA = Noradrenaline. None of the above data are significantly different between placebo and angiotensin II.

* $p < 0.05$ }
} represent significant differences from control
** $p < 0.01$ } values as obtained by the Duncan procedure.

Table 8.3

Plasma angiotensin II levels (pg/ml) before and after 15 minutes of placebo infusion or angiotensin II infusion (1.5 ng/kg/min).

	Infusion:		
	Placebo	Angiotensin II	
Before	9.9 +/- 1.9	10.3 +/- 1.7	NS
After	9.8 +/- 1.5	32.1 +/- 5.0	$p<0.0025$

Paired t-testing.

Summary

This study sought evidence for an RAS/SNS interaction in man by examining how a subpressor dose of AII (1.5 ng/kg/min) influences the haemodynamic and plasma NA responses to physiological stimulation of the sympathetic nervous system. The physiological stimuli investigated were a cold pressor test, the response to standing from lying, bicycle exercise and forearm isometric exercise.

The presence of the AII infusion had no effect on the systolic blood pressure, diastolic blood pressure, heart rate or plasma NA responses to stimulation of the sympathetic nervous system. This study has therefore found no evidence to support the enhancement of NA release by this low dose of AII in man.

[Discussion: chapter 13.8]

CHAPTER NINE

CHAPTER NINE

The effect of angiotensin II on the haemodynamic and plasma noradrenaline responses to tyramine infusion in man

Introduction

The data presented in chapter eight has shown that a subpressor infusion of AII does not alter the haemodynamic or plasma NA response to a cold pressor test, standing from lying, bicycle exercise or forearm isometric exercise. In case the kind of SNS stimulation is a crucial factor, this study seeks evidence for an AII/NA interaction by examining whether exogenous AII is able to augment the presynaptic release of endogenous NA in response to infused tyramine (TYR) in normal man.

Methods

Six normotensive volunteers (4 male and 2 female) were studied. Their mean age was 24 years (range 18-33) and mean weight 70 kg (range 54-87.5 kg). They were studied on four separate occasions at least five days apart. The subjects attended the clinical laboratory at 09.30 h after a light breakfast or at 13.30 h after a light luncheon. Each individual was investigated at the same time of day on all four study days. They were positioned supine and intravenous cannulae were placed in veins in both forearms. After 20 minutes of supine rest an infusion of either AII (2ng/kg/min) or placebo (5% dextrose) was commenced and infused at a constant rate in a volume of 0.5 ml/min throughout the rest of the study. After a further 20 min supine rest an incremental infusion of placebo (5% dextrose) or TYR was commenced at 1.25 mcg/kg/min and increased at 10 min intervals to 2.50, 3.75, 5.00, 7.50 and 10.0 mcg/kg/min. The infusions

were given in randomised single blind fashion. Blood samples were taken and divided into two aliquots for later analysis of plasma AII and plasma NA.

Results were analysed by repeated measures analysis of variance (MANOVA, SPSS/PC+) to examine the effect of time, treatments and for an interaction between treatments. A p-value of <0.05 was considered significant. Results are given as mean +/- s.e.m. in the figure and tables.

Results

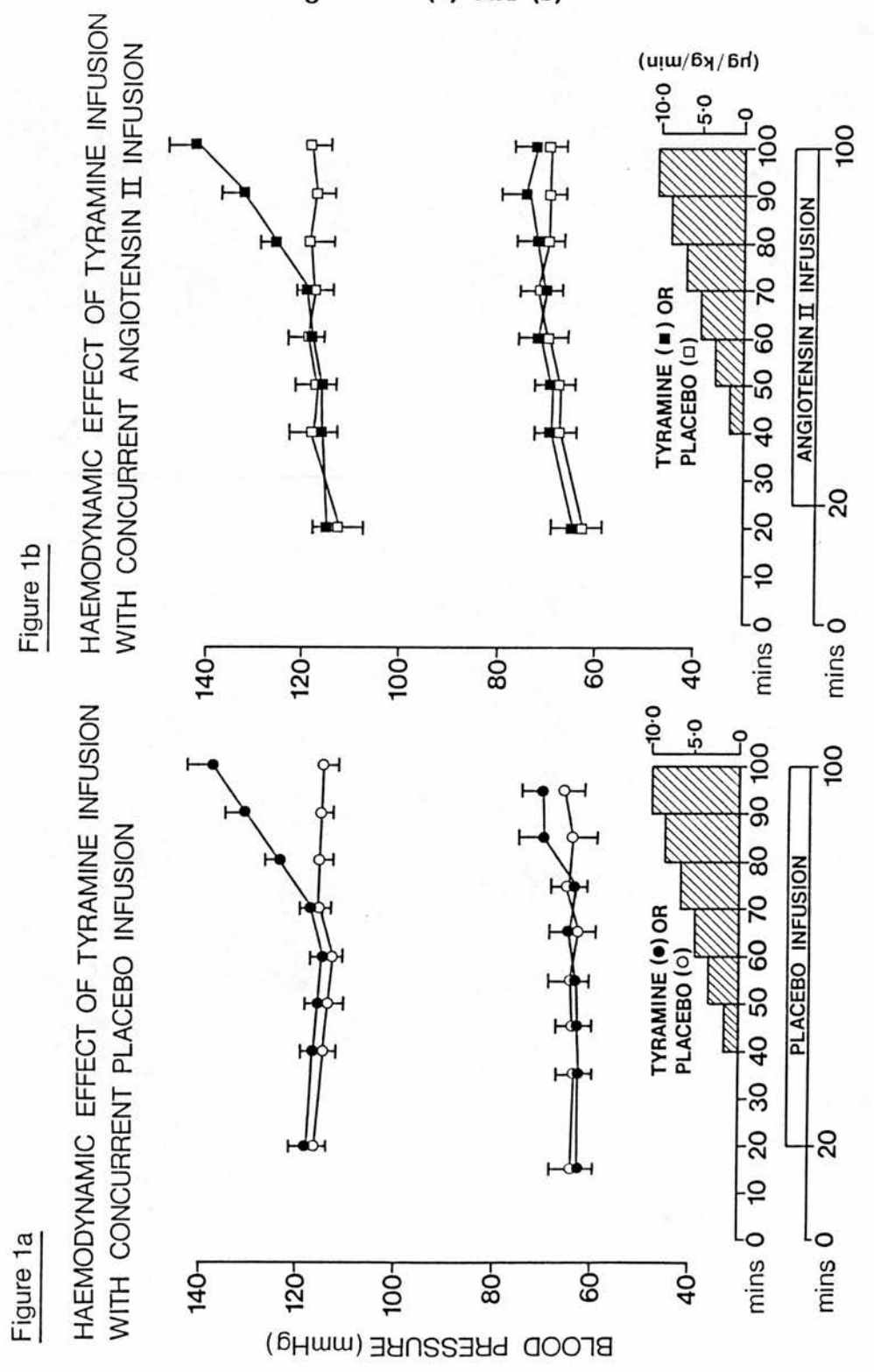
Table 9.1 shows systolic and diastolic pressure and heart rate before and after the start of an AII or placebo infusion. Resting systolic BP, diastolic BP and heart rate were not significantly different on all four study days. There were no significant changes in any of these observations in response to AII infusion.

Figure 9.1(a) shows the effect of TYR infusion on systolic and diastolic BP with concomitant placebo infusion, and figure 9.1(b) shows the effect of TYR infusion with concomitant AII infusion. AII alone produced no significant change in systolic or diastolic BP. TYR alone caused a large dose-dependent increase in systolic BP (118+/-3 to 138+/-5, $p<0.02$ MANOVA) and a smaller but significant increase in diastolic BP at the two highest doses of TYR ($p<0.03$). When infused together, AII/TYR produced a similar dose-dependent increase in systolic BP (115+/-2 to 143+/-6, $p<0.02$) and a small rise in diastolic BP (65+/-4 to 75+/-5, $p<0.03$). There was no significant interaction between AII and TYR in either the systolic BP or the diastolic BP data.

Table 9.2 shows the heart rate data for the four study days. There was no effect of AII alone, TYR alone nor their combination seen in the heart rate data (MANOVA). Table 9.3(a) shows the plasma AII response and

table 9.3(b) the plasma NA response to infusion. Plasma AII levels were no different at the beginning of the four study days and did not change on the dual placebo day. Infusion of TYR alone also had no effect on plasma AII levels. Infusion of AII caused a significant rise in plasma AII ($p<0.01$) and this rise was not altered by concomitant TYR infusion. Plasma NA levels were not significantly different at the start of the four study days. Neither dual placebo infusion nor AII infusion alone caused significant change in plasma NA levels. TYR alone and the combination of AII/TYR produced a rise in plasma NA only at the highest dose ($p<0.01$). Whilst the plasma NA levels at the highest dose of TYR showed considerable interindividual variability there was no significant difference depending on whether AII was also present or not, and the plasma NA and haemodynamic measurements are clearly the same in both TYR treated groups at the penultimate dose.

Figure 9.1 (a) and (b)



Legend to figure 9.1 (a) and (b)

The haemodynamic effect of tyramine infusion (a) with concurrent placebo infusion and (b) with concurrent angiotensin II infusion. Angiotensin II was infused at a constant rate of 2 ng/kg/min. Systolic blood pressure is shown in the top half of each panel. Diastolic blood pressure is shown in the bottom part of each panel. Bars represent mean +/- s.e.m.

Table 9.1

Systolic and diastolic pressures and heart rate before and 20 min after angiotensin II (2ng/kg/min) or placebo infusion.

	SBP(mmHg)		DBP(mmHg)		HR(beats/min)	
	before	after	before	after	before	after
Placebo (PL)	117+/-2	115+/-3	64+/-4	63+/-4	59+/-5	59+/-5
Placebo (TYR)	118+/-3	117+/-2	63+/-4	63+/-3	60+/-5	60+/-5
AII (PL)	113+/-5	118+/-4	63+/-4	68+/-3	61+/-7	56+/-6
AII (TYR)	115+/-2	117+/-3	65+/-4	69+/-3	57+/-7	58+/-6

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate. AII = angiotensin II infusion. Letters in parentheses indicate the study days in which subjects subsequently received placebo (PL) or tyramine (TYR) infusion as described in the methods. Results are given as mean +/- s.e.m.

Table 9.2

CHANGES IN HEART RATE (beats per min)

			dose of tyramine (mcg/kg/min)					
	Resting	Control	1.25	2.50	3.75	5.00	7.50	10.0
PL/PL	59+/-5	59+/-5	58+/-4	58+/-4	59+/-4	57+/-4	58+/-4	59+/-5
PL/TYR	60+/-5	60+/-5	57+/-5	56+/-4	58+/-3	57+/-3	58+/-5	56+/-4
AII/PL	61+/-7	56+/-6	57+/-4	58+/-4	55+/-5	58+/-5	56+/-5	55+/-5
AII/TYR	57+/-7	58+/-6	55+/-6	55+/-7	52+/-6	55+/-6	55+/-7	57+/-5

PL = placebo infusion (5% dextrose), AII = angiotensin II infusion (2ng/kg/min) TYR = tyramine infusion (1.25 - 10.0 mcg/kg/min as indicated). Resting = baseline measurements taken after 20 min supine rest. Control = measurements taken after a 20 mins infusion of either AII or PL as indicated. This was followed by an incremental infusion of TYR in the doses indicated. Results are given as mean +/- s.e.m.

Table 9.3(a)

PLASMA ANGIOTENSIN II (pg/ml)

			Dose of tyramine (mcg/kg/min)		
	Resting	Control	2.50	5.00	10.0
Placebo/Placebo	21+/-7	23+/-7	-	-	19+/-4
Placebo/AII	19+/-3	67+/-4*	-	-	61+/-6*
Tyramine/placebo	23+/-6	19+/-3	-	-	18+/-3
Tyramine/AII	23+/-8	63+/-7*	-	-	66+/-6*

Table 9.3(b)

PLASMA NORADRENALINE (pg/ml)

			Dose of tyramine (mcg/kg/min)		
	Resting	Control	2.50	5.00	10.0
Placebo/Placebo	400+/-46	444+/-77	379+/-43	343+/-46	394+/-53
Placebo/AII	416+/-74	433+/-82	513+/-63	386+/-53	376+/-41
Tyramine/placebo	590+/-55	451+/-45	600+/-69	623+/-73	983+/-227*
Tyramine/AII	530+/-62	509+/-60	502+/-67	636+/-60	707+/-73*

Placebo = 5% dextrose, AII = angiotensin II infusion (2ng/kg/min) and Tyramine = tyramine infusion (1.25 - 10.0 mcg/kg/min as indicated). Results are given as mean +/- s.e.m. * $p<0.01$

Summary

In this study evidence was sought for an AII/NA interaction in man by examining how an AII infusion influences the haemodynamic and plasma NA responses to endogenously released NA. TYR was infused to cause the endogenous release of neuronal NA. Six normal volunteers were studied on four separate occasions. On each occasion they received two concomitant infusions which were either placebo/placebo, placebo/TYR, AII/placebo or AII/ TYR. AII infusion was given at a constant rate of 2ng/kg/min whereas the TYR infusion consisted of 10 min increments at 1.25, 2.5, 3.75, 5, 7.5 and 10 mcg/kg/min.

TYR infusion caused a dose dependent increase in systolic blood pressure with increases in diastolic blood pressure and plasma NA only at the highest doses. These changes were not affected by concomitant AII infusion. We have therefore found no evidence to support the enhancement of haemodynamic or plasma NA responses to tyramine infusion by low dose infusion of AII in man.

[Discussion: chapter 13.9]

CHAPTER TEN

CHAPTER TEN

The effect of angiotensin II on the forearm blood flow response to noradrenaline

Introduction

This study and those reported in the following chapters use a second methodology to investigate the RAS/SNS interaction further. The methodology is that of intra-arterial infusion of hormone or placebo into the brachial artery and simultaneous measurement of forearm blood flow (FBF) by strain gauge plethysmography (see methods and figure 2.1).

In this study evidence was sought for a possible postsynaptic RAS/SNS interaction. NA was infused into the brachial artery with concomitant AII or placebo infusion and the effects compared.

Methods

Eight subjects (7 male, 1 female) were studied on one occasion each. Two infusions were given simultaneously into the brachial artery via a Y-connector which delayed mixing until the solutions entered the arterial cannula.

One infusion (infusion A, Table 10.1) started with saline for 20 min, followed by two separate periods of NA infusion, each with three sequential incremental doses (12.5, 25 and 50 ng/min), each dose being given for 10 min. The two periods of incremental NA infusion were separated by a washout period of 50 min of saline infusion.

The other infusion (infusion B, Table 10.1), given simultaneously, started with saline for 10 mins followed by AII (320 fmol/min) or placebo (saline) for 40 min. This was then replaced by saline infusion for a washout

period of 40 min, followed by a further period of AII or saline placebo for 40 min. Hence AII was given for 10 min before and then throughout either the first or second period of incremental NA infusion. Whether AII was given during the first or the second NA infusion period was governed by a balanced block design. FBF was measured for the last 5 min of each 10 min throughout the study.

Analysis of results.

Results within the text are presented as mean +/- sem for each measurement. The data were transformed to the ratios of blood flow in the infused and non-infused forearm as described in chapter two (Methods). These data were subjected to analysis of variance for repeated measures (MANOVA, SPSS/PC+), to look for an effect of NA by time and an effect of AII versus placebo. The data are illustrated in the figure as "cannulated" (C) to "non-cannulated" (NC) ratios, were C represents the recorded blood flow in the cannulated arm and NC represents the recorded blood flow in the non-cannulated arm (see chapter two for details).

Results

The effect of local infusion of AII (320 fmol/min) on the response to local NA infusion is shown in Table 10.1 and figure 10.1. There was no significant difference between the absolute resting blood flow in the infused forearm before placebo or AII infusion. Blood flow in the infused arm was 3.4+/-0.8 ml/min/100ml before placebo infusion, and 3.0+/-0.4 ml/min/100ml before AII infusion. Nor was there a significant change in blood flow associated with infusion of AII or placebo alone. Forearm blood flow went from 3.4 +/- 0.8 to 3.4 +/- 0.6 ml/min/100ml during placebo infusion alone, and from 3.0 +/- 0.4 to 2.8 +/- 0.4 ml/min/100ml during AII

infusion alone.

NA caused a significant dose-dependent reduction in blood flow in the infused forearm ($p<0.001$ MANOVA, fig 13.1), whether given during placebo or AII infusion. There was, however, no difference between the responses to NA in these circumstances ($P=0.86$). There were also no significant changes in blood flow in the non-infused forearm during these experiments.

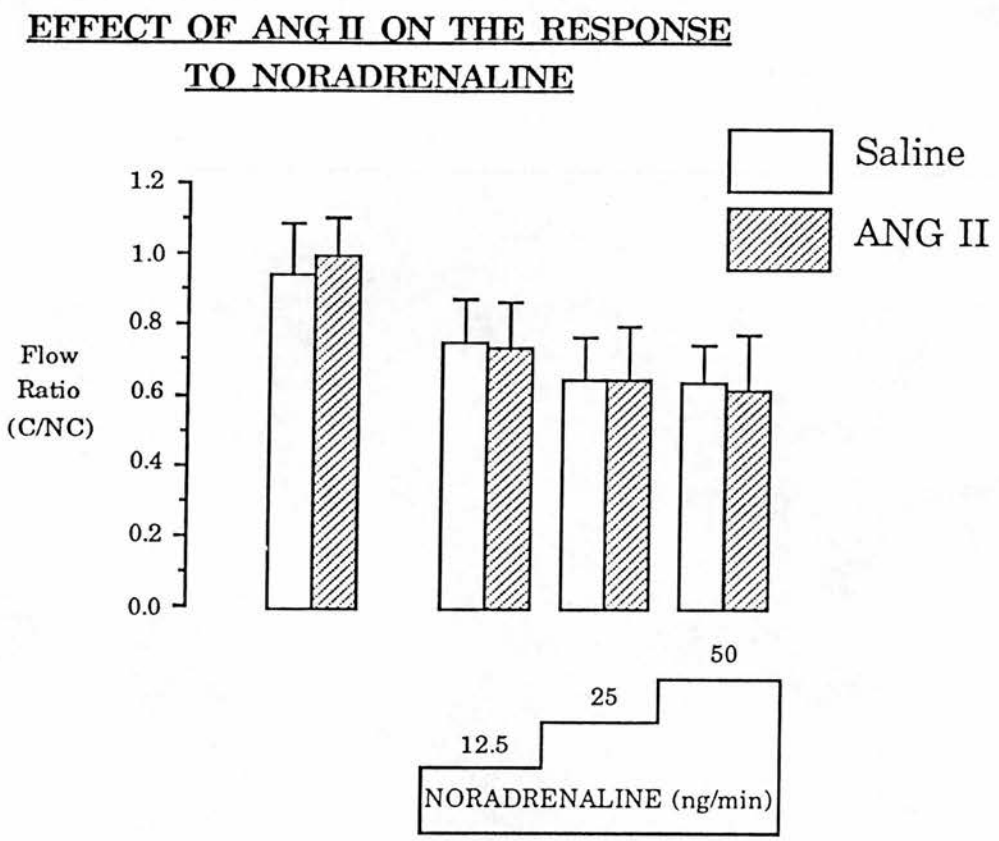
Table 10.1

The effect of angiotensin II (AII; 320 fmol/min) on resting blood flow and the response of forearm blood flow to incremental infusion of noradrenaline (NA).

Mean +/- s.e.m. For details of infusions A and B see Methods

Infusion		Blood flow (ml/min/100ml)	
		Infused arm	Non-infused arm
A	B		
Saline	Saline	3.4+/-0.8	3.7+/-0.8
Saline	Saline	3.4+/-0.6	3.6+/-0.7
NA (12.5 ng/min)	Saline	2.1+/-0.4	3.1+/-0.6
NA (25 ng/min)	Saline	2.1+/-0.5	3.3+/-0.7
NA (50 ng/min)	Saline	2.2+/-0.4	3.5+/-0.8
Saline	Saline	3.0+/-0.4	3.1+/-0.5
Saline	AII	2.8+/-0.4	3.4+/-0.5
NA (12.5 ng/min)	AII	2.1+/-0.4	3.2+/-0.6
NA (25 ng/min)	AII	1.8+/-0.4	3.4+/-0.6
NA (50 ng/min)	AII	1.8+/-0.3	3.6+/-0.6

Figure 10.1


EFFECT OF ANG II ON THE RESPONSE TO NORADRENALINE
Saline
ANG II
Flow Ratio (C/NC)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
50
25
12.5
NORADRENALINE (ng/min)

Legend to figure 10.1

Ratio of flows in the infused arm / non-infused arm during incremental infusion of noradrenaline (12.5-50 ng/min) with concomitant placebo (saline; open bars) or with concomitant angiotensin II infusion (320 fmol/min; hatched bars) in 8 supine subjects (mean +/- sem)

Summary

The effect of AII on the response of forearm blood flow (FBF) to exogenous NA (12.5-50 ng/min) was studied in eight subjects. AII had no effect on the reduction in FBF in response to incremental NA infusion.

These data argue against a postsynaptic AII/NA interaction.

[Discussion: chapter 13.11]

CHAPTER ELEVEN

CHAPTER ELEVEN

The effect of local angiotensin II on the forearm blood flow response to lower body negative pressure.

Introduction

In this study evidence was sought for an AII/SNS interaction by intra-arterial infusion of AII or placebo (saline) and SNS stimulation by lower body negative pressure (LBNP). Forearm blood flow was measured in both forearms and compared to look for an effect of AII or placebo on the reduction in forearm blood flow caused by LBNP.

Methods

Six male subjects were studied on one occasion each. Prior to brachial artery cannulation blood pressure and heart rate were recorded every 2 min for 18 min with LBNP being applied for 3 min, between 11 and 14 min. This was followed by brachial artery cannulation and sequential infusion of saline, AII (320 fmol/min), and then further saline. Each infusion was given for 15 min. FBF was recorded for the last 9 min of each infusion period, with LBNP (15 mmHg; see chapter two: Methods) being applied for the middle 3 min of FBF measurement.

Analysis of results.

Results within the text are presented as mean +/- sem for each measurement. The effect of LBNP on blood pressure and heart rate was subjected to analysis of variance for repeated measures (SPSS/PC+) using the two measures during, and the two measures immediately preceding and following LBNP. Where a significant F ratio was obtained, Student's t-test

was performed using the Bonferroni correction. The effect of AII on responses to LBNP were analysed using Wilcoxon's signed rank test, with the percentage changes in blood flow in the infused and non-infused forearm calculated by the formula given below:

$$\frac{F_{(t)} - F_{(0)} \times 100\ \%}{F(0)}$$

where $F_{(0)}$ and $F_{(t)}$ represent measured forearm blood flows (F) before (0) and during (t) intervention.

Results

The effect of LBNP on systemic BP and heart rate are shown in figure 11.1 and figure 11.2. LBNP caused an initial 5 mmHg fall in systolic BP ($p<0.01$) which returned to baseline within a further 2 min of LBNP. Neither diastolic BP nor heart rate were affected by this degree of LBNP, and blood pressure and heart rate after LBNP was similar to that before its institution.

The changes in forearm blood flow in response to LBNP are shown in table 11.1 and figure 11.3. Absolute resting blood flows during saline infusion were similar in the infused and non-infused arms at 5.8+/-1.1 vs. 6.9+/-1.5 ml/min/100ml tissue respectively, and blood flow was reduced to a similar degree in both arms by LBNP (21 +/- 6% in each forearm). At the dose of AII given (320 fmol/min) there was no alteration in forearm blood flow, with flows in the infused and non-infused arms of 5.9+/-1.6 and 6.7+/-1.4 ml/min/100ml respectively. However of major importance is the finding that, during AII infusion at this dose, there was a marked difference in response to LBNP, with blood flow reduced by 40 +/- 7% in the AII infused arm but only by 21 +/- 4% in the non-infused arm ($p<0.05$).

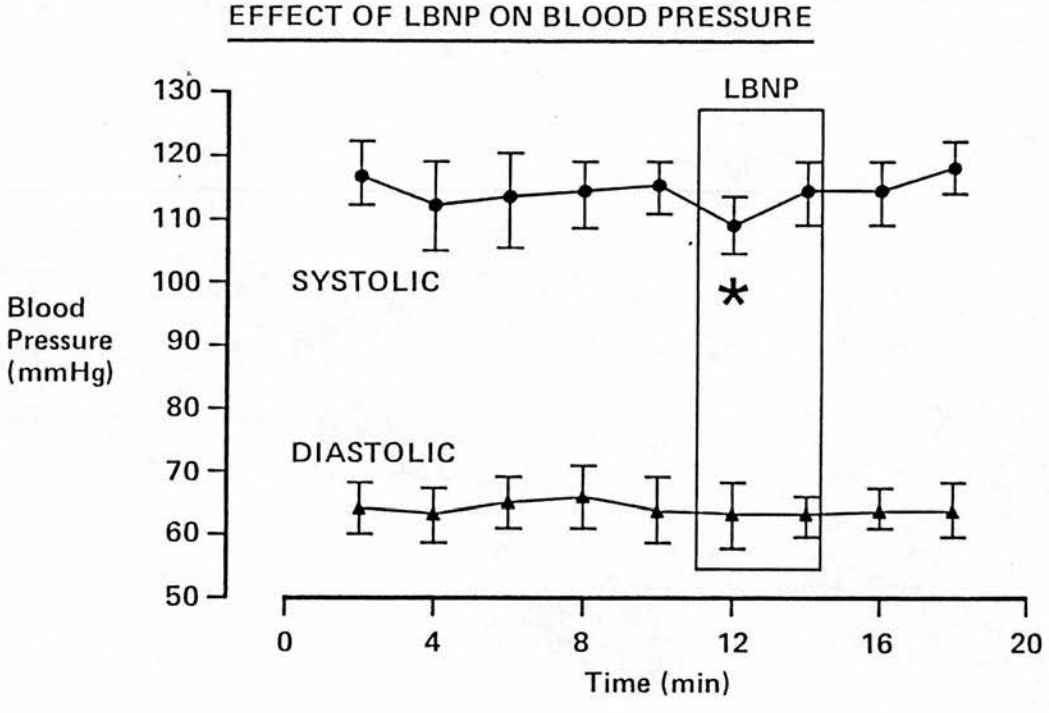
Table 11.1

The effect of angiotensin II (AII) on resting blood flow and the response of forearm blood flow to lower body negative pressure (LBNP).

Mean +/- s.e.m.

		Blood flow (ml/min/100ml)	
		Infused arm	Non-Infused arm
Saline	Resting	5.8+/-1.1	6.9+/-1.5
	LBNP	4.8+/-1.3	5.5+/-1.4
AII (320 fmol/min)	Resting	5.9+/-1.6	6.7+/-1.4
	LBNP	3.6+/-1.0	5.2+/-1.1
Saline	Resting	5.4+/-0.9	5.6+/-0.6
	LBNP	4.5+/-0.8	4.5+/-0.4

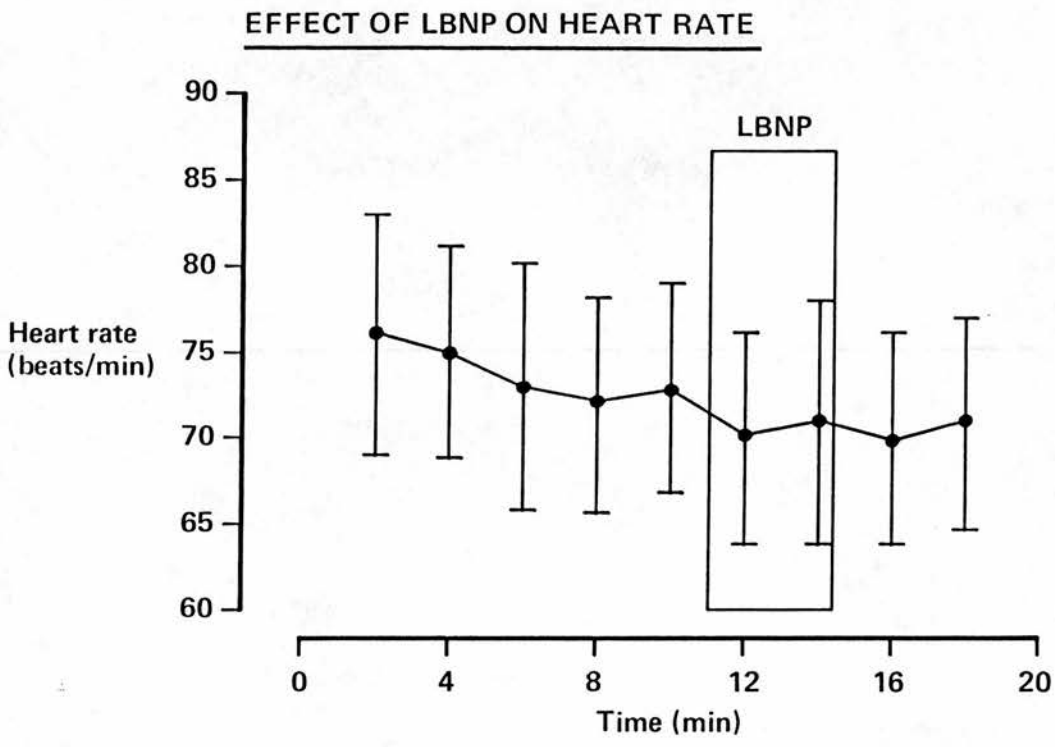
Figure 11.1


EFFECT OF LBNP ON BLOOD PRESSURE
LBNP
SYSTOLIC
DIASTOLIC
*
Blood Pressure (mmHg)
130
120
110
100
90
80
70
60
50
0
4
8
12
16
20
Time (min)

Legend to figure 11.1

Effect of 3 min application of lower body negative pressure (LBNP) on systolic and diastolic blood pressure (mmHg) in 6 supine subjects (mean +/- sem). * P<0.01

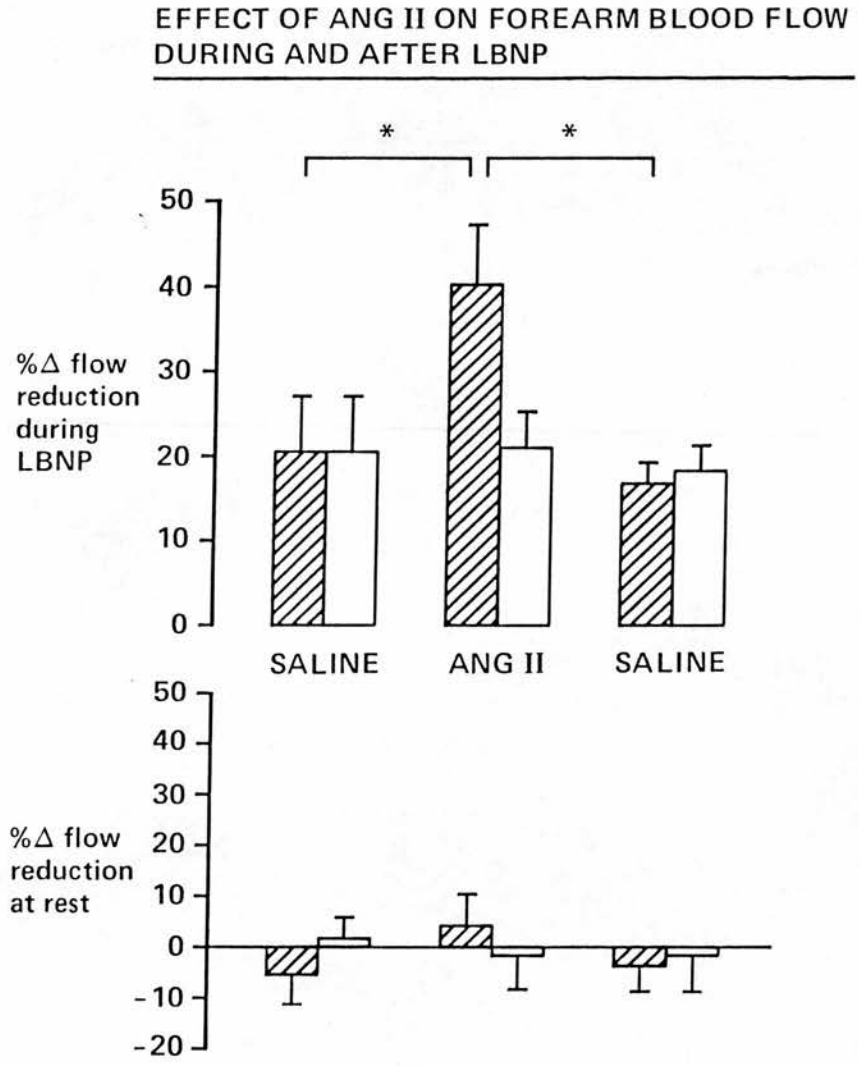
Figure 11.2


EFFECT OF LBNP ON HEART RATE
LBNP
Heart rate
(beats/min)
90
85
80
75
70
65
60
0
4
8
12
16
20
Time (min)

Legend to figure 11.2

Effect of 3 min application of lower body negative pressure (LBNP) on heart rate (beats/min) in 6 supine subjects (mean +/- sem).

Figure 11.3


EFFECT OF ANG II ON FOREARM BLOOD FLOW
DURING AND AFTER LBNP
*
*
%Δ flow reduction during LBNP
50
40
30
20
10
0
SALINE
ANG II
SALINE
%Δ flow reduction at rest
50
40
30
20
10
0
-10
-20

Legend to figure 11.3

Percentage changes (from control) in forearm blood flow with LBNP (mean +/- sem) in the infused (hatched bars) and the non-infused arm (open bars) of 6 subjects during sequential infusion of saline (control period), angiotensin II (ANG-II 320 fmol/min; upper section of figure) and further saline (Resting; lower section of figure). * $P<0.05$

Summary

The effect of brachial arterial infusion of AII on the response to sympathetic vasoconstriction produced by LBNP (15 mmHg) was studied in 6 subjects.

AII itself caused no change in resting forearm blood flow. LBNP produced no change in heart rate or diastolic BP, but caused an early 5 mmHg fall in systolic BP ($p<0.01$) which rapidly returned to baseline. Before AII infusion LBNP caused a 21+/-6% fall in forearm blood flow in the infused arm and a 21+/-6% fall in the non-infused arm. In contrast during AII infusion LBNP caused a 40+/-7% fall in forearm blood flow in the infused arm and a 21+/-4% fall in the non-infused arm ($p<0.05$).

This study provides evidence that a significant haemodynamic AII/ SNS interaction occurs in the forearm resistance vessels in man.

[Discussion: chapter 13.11]

CHAPTER TWELVE

CHAPTER TWELVE

Studies employing pharmacological interruption of the renin-angiotensin system.

Four experiments were undertaken to investigate the value of pharmacological interruption of the RAS as a tool for studying the interaction between the RAS and SNS. The forearm circulation of normal volunteers was studied and LBNP was employed as the stimulus to activate the SNS in all studies. The first approach (a) was the local intra-arterial infusion of enalaprilat (the active metabolite of the prodrug enalapril, an ACE inhibitor). The second approach (b) was to pretreat all subjects with enalapril, and subsequently infuse AII intra-arterially in an incremental fashion. The third approach (c) was to infuse saralasin (an AII antagonist) intra-arterially in salt replete subjects. The fourth approach (d) was to infuse saralasin intra-arterially in sodium deplete subjects. The results of these four studies are given in this chapter.

(a) The effect of local angiotensin converting enzyme inhibition on the forearm blood flow response to lower body negative pressure

Introduction

In chapter eleven AII was shown to augment the LBNP induced sympathetic vasoconstriction in the forearm resistance vessels. In this study evidence was sought for an effect of endogenous AII generation in the forearm vasculature on sympathetic vasoconstriction. The forearm blood flow responses to blockade of local forearm ACE activity by infusion of

enalaprilat were examined at rest and during sympathetic stimulation with LBNP.

Methods

Six male subjects were studied on one occasion each. Subjects were sequentially infused via the brachial artery with saline for 15 min, enalaprilat (5 mcg/min: low dose) for 19 min, and enalaprilat (50 mcg/min: high dose) for 19 min. [enalaprilat is the active form of the prodrug enalapril: Merck Sharp and Dohme Ltd, UK]. Enalaprilat was prepared for infusion diluted in saline immediately prior to infusion. FBF was measured for the last 9 min of each infusion period, with LBNP (15 mmHg) being applied for the middle 3 min of recording.

Analysis of results.

Results are presented as mean +/- sem for each measurement. The data were transformed to the ratios of blood flow in the infused and non-infused forearm as described in chapter two. These data were subjected to analysis of variance for repeated measures (MANOVA, SPSS/PC+), to look for an effect of enalaprilat and an effect of LBNP.

Results

The results of resting forearm blood flow are given in table 12(a).1. Resting blood flow in the infused arm was not altered by infusion of either low dose or high dose of enalaprilat (Saline, 4.7+/-1.0; low dose, 4.7+/-1.2; high dose, 5.5+/-1.1 ml/min/100ml). The small rise noted at the high dose was matched by a similar rise in the non-infused arm and the C/NC ratio was unchanged (figure 12(a).1).

LBNP caused reductions in blood flow in both infused and non-infused

forearms of the same magnitude and these were unaltered by infusion of enalaprilat. These results are given in table 12(a).1 and are illustrated by unchanged C/NC ratios in figure 12(a).1

Table 12(a).1

Effect of local ACE inhibition on the forearm blood flow response to lower body negative pressure

Forearm blood flow in infused arm and non-infused arm at rest and with lower body negative pressure (LBNP) in ml/min/100ml tissue during infusion of saline and enalaprilat (5 and 50 mcg/min)

	Infused forearm		Non-infused forearm	
Infusion	Resting	LBNP	Resting	LBNP
Saline	4.7+/-1.0	2.8+/-0.8	4.5+/-1.3	2.8+/-0.9
Low dose	4.7+/-1.2	2.9+/-0.9	4.2+/-1.4	2.7+/-1.0
High dose	5.5+/-1.1	3.7+/-0.9	5.1+/-1.2	3.8+/-1.1

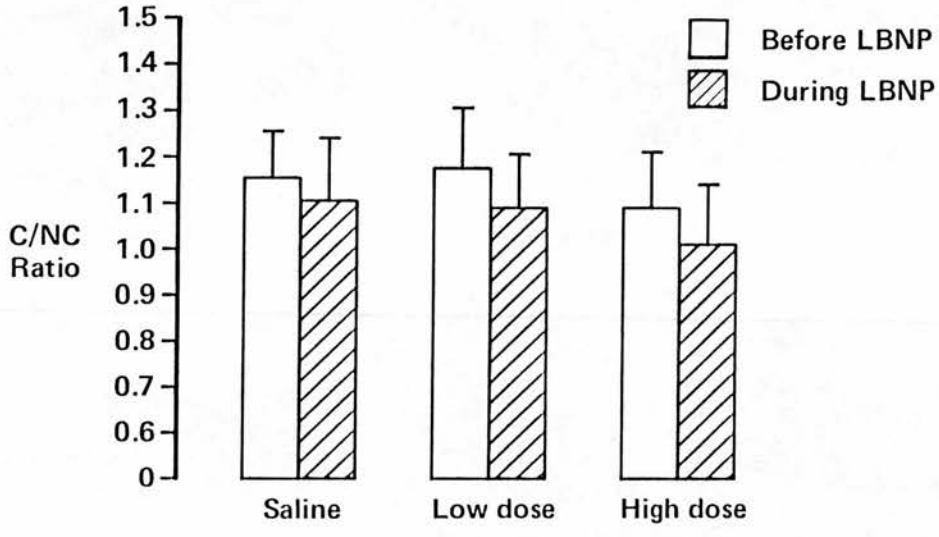
Low dose infusion = enalaprilat 5 mcg/min.

High dose infusion = enalaprilat 50 mcg/min.

Results are presented as mean +/- sem for each measurement.

Figure 12(a).1

EFFECT OF LOCAL ACE INHIBITION ON THE FOREARM BLOOD FLOW RESPONSE TO LBNP



Legend to figure 12(a).1

Changes in forearm blood flow shown as flow ratios (C/NC ratios where C represents flow in the infused forearm and NC represents flow in the non-infused arm) caused by LBNP during sequential infusion of saline, low dose enalaprilat (5 mcg/min) and high dose enalaprilat (50 mcg/min).

Summary

Evidence was sought for an effect of endogenous AII generation in the forearm vasculature on sympathetic vasoconstriction by examining the forearm blood flow responses to blockade of local forearm ACE activity by infusion of enalaprilat at rest and during sympathetic stimulation with LBNP.

Neither resting forearm blood flow, nor responses to sympathetic stimulation with LBNP were altered by local ACE inhibition with enalaprilat infusion.

These results suggest that local generation of AII may play a lesser role in the maintainance of vascular tone and sympathetic function than circulating AII.

[Discussion: chapter 13.12]

(b) The effect of systemic angiotensin converting enzyme inhibition and reinfusion of angiotensin II on the forearm blood flow response to lower body negative pressure

Introduction

In chapter eleven AII was shown to augment the LBNP induced sympathetic vasoconstriction in the forearm resistance vessels. In this study evidence was sought for an effect of circulating AII by examining the forearm blood flow responses at rest and in response to LBNP during systemic ACE inhibition with oral enalapril and also studying the effect of re-infusion of an incremental infusion of AII.

Methods

Six male subjects were studied on one occasion each. Subjects were pretreated with enalapril 20 mg twice daily for the two days preceding the study, and received a final dose of enalapril 20 mg on the morning of the study, five hours before the experiment began. Before brachial artery cannulation blood pressure and heart rate were recorded every 2 min for 12 min with LBNP being applied for 3 min, between 5 and 8 min. This was followed by sequential infusion via the brachial artery of saline, AII (160 fmol/min), AII (320 fmol/min), AII (640 fmol/min) and AII (1280 fmol/min), each given for 15 min. Forearm blood flow was recorded for the last 9 min of each infusion, with LBNP being applied for the middle 3 min of forearm blood flow measurement.

Analysis of results.

Results are presented as mean +/- sem for each measurement. The effect of LBNP on blood pressure and heart rate was subjected to analysis

of variance for repeated measures (SPSS/PC+) using the two measures during, and the two measures immediately preceding and following LBNP. These were also compared with the same data from the group studied in chapter eleven, who had not received Enalapril. The forearm blood flow data were transformed to the ratios of blood flow in the infused and non-infused forearm as described in chapter two. These data were subjected to analysis of variance, to look for an effect of AII versus placebo and an effect of LBNP. Where ANOVA revealed significant differences subsequent paired t-testing was undertaken.

Results

The effect of LBNP on systolic and diastolic BP and heart rate are shown in table 12(b).1. In subjects treated with enalapril systolic BP and diastolic BP and heart rate were significantly lower than in untreated subjects ($p<0.001$ and $p<0.005$ by ANOVA, see table 12(b).1). There was no significant change in any haemodynamic parameter during LBNP in the enalapril treated group and a small non-sustained fall in systolic blood pressure only in the untreated group as noted earlier (chapter 11 and figure 11.1).

The results of resting forearm blood flow and blood flow during LBNP are given in table 12(b).2. AII produced a dose dependent reduction in the infused arm compared with the non-infused arm ($P<0.001$, ANOVA) illustrated in figure 12(b).1 as reductions in consecutive flow ratios. Significance of reduction of blood flow in the infused forearm at individual doses of AII compared with control (saline) is given in table 12(b).2.

LBNP produced further reductions in forearm blood flow which were more marked in the infused forearm (illustrated by reductions in C/NC flow ratios, figure 12(b).1). Overall the responses to LBNP were not

significantly altered by AII infusion. However a significant dose related difference was revealed ($p<0.05$, ANOVA). Subsequent t-testing showed that at a dose of AII 320 fmol/min the response to LBNP was significantly enhanced, with blood flow in the infused arm being reduced by 20+/-7% during saline infusion period and by 33+/-6% during the AII infusion period, compared with blood flow in the control arm which was reduced 16+/-8% during saline infusion period and by 11+/-8% during the AII infusion period ($p<0.05$). This result is indicated in figure 12(b).1.

Table 12(b).1

Haemodynamic effects of lower body negative pressure in untreated subjects and subjects pretreated with enalapril

			LBNP	
		Baseline	1 min	3 min
Untreated	SBP	114+/-5	109+/-4*	114+/-5
	DBP	65+/-4	64+/-5	63+/-5
	HR	73+/-6	70+/-6	71+/-7
Enalapril	SBP	103+/-4***	101+/-3	107+/-6
	DBP	59+/-2***	59+/-2	56+/-3
	HR	65+/-5**	68+/-6	71+/-5

SBP and DBP = systolic and diastolic blood pressure (mmHg).

HR = heart rate (beats/min). LBNP = lower body negative pressure.

Enalapril dosage as described in the methods.

Significance: * $p<0.02$ by paired t-testing with Bonferroni correction after ANOVA; ** $p<0.005$ and *** $p<0.001$ (ANOVA)

Table 12(b).2

Effect of systemic ACE inhibition and re-infusion of angiotensin II on the forearm blood flow response to lower body negative pressure

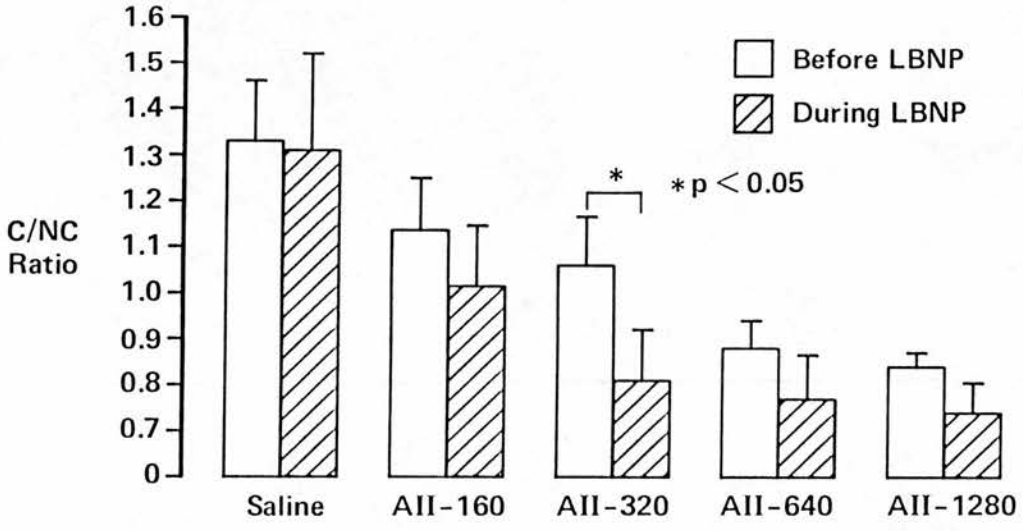
Forearm blood flow in infused arm and non-infused arm at rest and with lower body negative pressure (LBNP) in ml/min/100ml tissue during infusion of saline and angiotensin II (160-1280 fmol/min)

	Infused forearm		Non-infused forearm	
Infusion	Resting	LBNP	Resting	LBNP
Saline	4.1+/-0.7	3.2+/-0.6	3.0+/-0.3	2.4+/-0.2
AII (160)	3.2+/-0.6**	2.5+/-0.5	2.8+/-0.3	2.4+/-0.3
AII (320)	3.3+/-0.6**	2.2+/-0.5	3.1+/-0.4	2.7+/-0.4
AII (640)	2.6+/-0.3*	2.5+/-0.4	2.9+/-0.2	2.8+/-0.2
AII (1280)	2.5+/-0.2*	2.0+/-0.4	3.0+/-0.2	2.7+/-0.3

Numbers in parentheses indicate dose of AII in fmol/min.
Results are presented as mean +/- sem for each measurement.
Significance: * $p<0.05$ and ** $p<0.01$ by paired t-testing with control (Saline).

Figure 12(b).1

EFFECT OF SYSTEMIC ACE INHIBITION AND RE-INFUSION OF ANGIOTENSIN II OF THE FOREARM BLOOD FLOW RESPONSE TO LBNP



Legend to figure 12(b).1

Changes in forearm blood flow shown as flow ratios (C/NC ratios where C represents flow in the infused forearm and NC represents flow in the non-infused arm) caused by LBNP during sequential infusion of saline and angiotensin II (160-1280 fmol/min) in subjects pretreated with enalapril.

Significance * $p<0.05$

Summary

In this study evidence was sought for an effect of circulating AII by examining the forearm blood flow responses at rest and in response to LBNP during systemic ACE inhibition with oral enalapril and also studying the effect of re-infusion of an incremental infusion of AII.

Resting blood pressure and heart rate were significantly reduced by treatment with enalapril. Infusion of AII caused a significant reduction in flow in the infused arm ($p<0.001$, ANOVA).

In addition AII (at a dose of 320 fmol/min) significantly enhanced the response to LBNP ($p<0.05$). This finding provides further evidence for AII enhancing sympathetic vasoconstriction in the human forearm and suggests that circulating AII may play a part in peripheral sympathetic neurotransmission.

[Discussion: chapter 13.12]

(c) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium replete subjects

Introduction

This study sought to investigate whether circulating AII plays a role in peripheral sympathetic neurotransmission by examining the effect of blockade of AII by saralasin infusion into the brachial artery at rest and during sympathetic stimulation with LBNP. Subjects were on free diet and were considered sodium replete.

Methods

Eight male subjects were studied on one occasion each. The brachial artery was cannulated followed by sequential infusion of saline, saralasin (10 ng/min), saralasin (100 ng/min) and saralasin (1000 ng/min). Each infusion was given for 15 min. FBF was recorded for the last 9 min of each infusion period, with LBNP being applied for the middle 3 min of FBF measurement.

Analysis of results

Results are presented as mean +/- sem for each measurement. The data were transformed to the ratios of blood flow in the infused and non-infused forearm as described in chapter two. These data were subjected to analysis of variance for repeated measures (MANOVA, SPSS/PC+) to look for an effect of saralasin and an effect of LBNP. Paired t-testing was performed where a significant effect was revealed by ANOVA.

Results

The results of resting forearm blood flow and blood flow during LBNP

are given in table 12(c).1. There were no significant differences in resting blood flow between the infused and non-infused arms.

Saralasin had a small but highly significant dose dependent effect reducing blood flow in the infused arm ($p<0.001$, ANOVA) reflected in falling C/NC flow ratios as illustrated in figure 12(c).1. Significance by paired t-testing for individual doses (compared with control, saline) is given in figure 12(c).1.

The responses to sympathetic stimulation with LBNP were not affected by saralasin ($p=0.6$, ANOVA).

Table 12(c).1

Effect of saralasin on the forearm blood flow response to lower body negative pressure in sodium replete subjects

Forearm blood flow in infused arm and non-infused arm at rest and with lower body negative pressure (LBNP) in ml/min/100ml tissue during infusion of saline and saralasin (10-1000 ng/min)

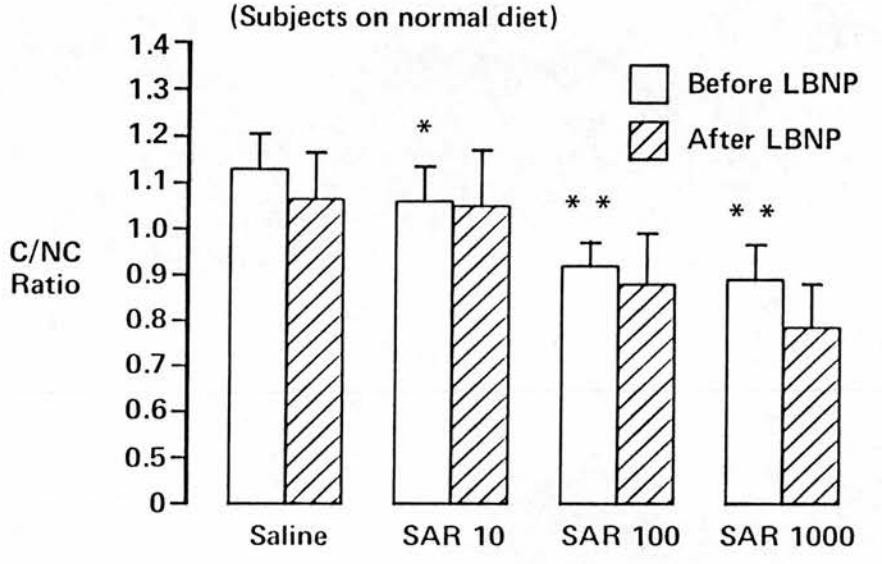
	Infused forearm		Non-infused forearm	
Infusion	Resting	LBNP	Resting	LBNP
Saline	5.0+/-1.0	3.3+/-0.7	4.3+/-0.8	3.0+/-0.6
SAR (10)	4.9+/-1.1	3.3+/-0.8	4.6+/-1.0	3.1+/-0.7
SAR (100)	4.4+/-1.1	3.0+/-0.8	4.8+/-1.2	3.3+/-0.9
SAR (1000)	4.0+/-0.8	2.5+/-0.5	4.6+/-1.0	3.2+/-0.7

SAR = saralasin infusion. Numbers in parentheses indicate dose of saralasin infused in ng/min.

Results are presented as mean +/- sem for each measurement

Figure 12(c).1

EFFECT OF SARALASIN ON THE FOREARM BLOOD FLOW RESPONSE TO LBNP



Legend to figure 12(c).1

Effect of saralasin (10-1000 ng/min) on the forearm blood flow response to LBNP in sodium replete subjects. Results are given as mean +/- sem of C/NC flow ratios (see chapter two for details of analysis). Plain bars represent flow ratios at rest and hatched bars represent flow ratios 3 min after the application of LBNP. Significance: * $p<0.02$, ** $p<0.01$

Summary

This study sought to investigate whether circulating AII plays a role in peripheral sympathetic neurotransmission by examining the effect of blockade of AII by saralasin infusion into the brachial artery at rest and during sympathetic stimulation with LBNP. Subjects were on free diet and were considered sodium replete.

Saralasin was shown to have a dose dependent effect on reducing forearm blood flow, but had no effect on the responses to LBNP.

These findings may reflect an agonist effect of saralasin at AII receptors.

[Discussion: chapter 13.13]

(d) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium deplete subjects

Introduction

This study sought to investigate whether circulating AII plays a role in peripheral sympathetic neurotransmission by examining the effect of blockade of AII by saralasin infusion into the brachial artery at rest and during sympathetic stimulation with LBNP. Subjects were salt deplete on this occasion.

Methods

Eight male subjects were studied on one occasion each. Prior to investigation subjects were rendered sodium deplete by giving a single oral dose of frusemide 40mg taken on the first morning, followed by three days of a diet providing 12 to 15 mmol sodium, and 40 to 60 mmol potassium daily. Studies were performed on the morning of the fourth day after an overnight fast from 22.00 h. This method usually produces an external sodium deficit of approximately 180 mmol (Webb et al, 1985). The brachial artery was cannulated followed by sequential infusion of saline, saralasin (10 ng/min), saralasin (100 ng/min) and saralasin (1000 ng/min). Each infusion was given for 15 min. FBF was recorded for the last 9 min of each infusion period, with LBNP being applied for the middle 3 min of FBF measurement.

Analysis of results

Results are presented as mean +/- sem for each measurement. The data were transformed to the ratios of blood flow in the infused and non-infused forearm as described in chapter two. These data were subjected to

analysis of variance for repeated measures (MANOVA, SPSS/PC+) to look for an effect of saralasin and an effect of LBNP. Paired t-testing was performed where a significant effect was revealed by ANOVA.

Results

The results of resting forearm blood flow and blood flow during LBNP are given in table 12(d).1. There were no significant differences in resting blood flow between the infused and non-infused arms.

Saralasin did not effect resting blood flow on this occasion ($p=0.6$, ANOVA). However saralasin caused significant enhancement of the response to sympathetic stimulation with LBNP ($p<0.02$, ANOVA). These results are reflected in the fall in C/NC flow ratios illustrated in figure 12(d).1.

Table 12(d).1

Effect of saralasin on the forearm blood flow response to lower body negative pressure in sodium deplete subjects

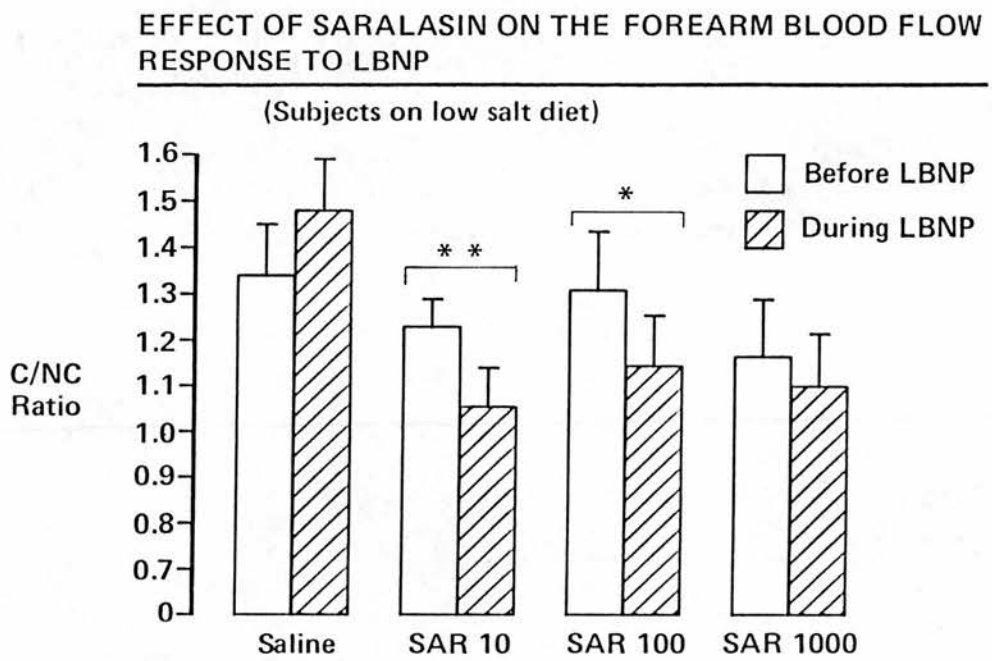
Forearm blood flow in infused arm and non-infused arm at rest and with lower body negative pressure (LBNP) in ml/min/100ml tissue during infusion of saline and saralasin (10-1000 ng/min)

	Infused forearm		Non-infused forearm	
Infusion	Resting	LBNP	Resting	LBNP
Saline	4.1+/-0.4	2.9+/-0.3	3.0+/-0.3	2.0+/-0.2
SAR (10)	4.0+/-0.5	2.7+/-0.5	3.3+/-0.4	2.6+/-0.3
SAR (100)	4.0+/-0.5	2.8+/-0.5	3.1+/-0.4	2.4+/-0.3
SAR (1000)	4.0+/-0.6	2.7+/-0.5	3.4+/-0.5	2.4+/-0.2

SAR = saralasin infusion. Numbers in parentheses indicate dose of saralasin infused in ng/min.

Results are presented as mean +/- sem for each measurement

Figure 12(d).1


EFFECT OF SARALASIN ON THE FOREARM BLOOD FLOW RESPONSE TO LBNP
(Subjects on low salt diet)
C/NC Ratio
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
* *
*
Before LBNP
During LBNP
Saline
SAR 10
SAR 100
SAR 1000

Legend to figure 12(d).1

Effect of saralasin (10-1000 ng/min) on the forearm blood flow response to LBNP in sodium replete subjects. Results are given as mean +/- sem of C/NC flow ratios (see chapter two for details of analysis). Plain bars represent flow ratios at rest and hatched bars represent flow ratios 3 min after the application of LBNP. Significance: * $p<0.05$, ** $p<0.02$

Summary

This study sought to investigate whether circulating AII plays a role in peripheral sympathetic neurotransmission by examining the effect of blockade of AII by saralasin infusion into the brachial artery at rest and during sympathetic stimulation with LBNP. Subjects were salt deplete.

In contrast to the previous study (chapter 12(c)) saralasin had no effect on resting forearm blood flow, but caused significant enhancement of the responses to LBNP.

These findings are also likely to represent an agonist effect of saralasin at AII receptors.

[Discussion: chapter 13.13]

SECTION THREE: DISCUSSION

OVERVIEW OF INVESTIGATIONS

Chapter number:

3. The effect of intravenous infusion of angiotensin II and noradrenaline alone and in combination on blood pressure, heart rate and plasma noradrenaline.
[Systemic: Raised AII: NA infusion: Postsynaptic]

4. The effect of angiotensin II on renal sodium excretion.
[Systemic: Raised AII: None: Direct AII action]

5. The effect of noradrenaline on renal sodium excretion.
[Systemic: None: NA infusion: Direct NA action]

6. The effect of angiotensin II and noradrenaline alone and in combination on renal sodium excretion.
[Systemic: Raised AII: NA infusion: Postsynaptic]

7. The effect of angiotensin II on the stroke volume response to beta agonism.
[Systemic: Raised AII: Beta-agonist infusion: Postsynaptic]

8. The effect of angiotensin II on endogenous noradrenaline release.
[Systemic: Raised AII: Physiological stimuli of SNS: Presynaptic]

9. The effect of angiotensin II on the haemodynamic and plasma noradrenaline responses to tyramine infusion.
[Systemic: Raised AII: NA release by tyramine infusion: Presynaptic]

10. The effect of local angiotensin II on the forearm blood flow response to noradrenaline.
[Local: Raised AII: NA infusion: Postsynaptic]

11. The effect of local angiotensin II on the forearm blood flow response to lower body negative pressure.
[Local: Raised AII: Physiological stimulus (LBNP): Presynaptic]

12. Studies employing pharmacological interruption of the renin-angiotensin system.

(a) The effect of local angiotensin converting enzyme inhibition on the forearm blood flow response to lower body negative pressure.
[Local: Low AII: Physiological stimulus (LBNP): Role of local ACE activity]

(b) The effect of systemic angiotensin converting enzyme inhibition and reinfusion of angiotensin II on the forearm blood flow response to lower body negative pressure
[Local: Low AII: Physiological stimulus (LBNP): Role of circulating ACE]

(c) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium replete subjects.
[Local: Low AII: Physiological stimulus (LBNP): Presynaptic]

(d) The effect of saralasin on the forearm blood flow responses to lower body negative pressure in sodium deplete subjects.
[Local: Raised AII: Physiological stimulus (LBNP): Presynaptic]

CHAPTER THIRTEEN

Discussion

13.1 The effect of intravenous infusion of angiotensin II and noradrenaline alone and in combination on blood pressure, heart rate and plasma noradrenaline (chapter three).

In designing this study, there were two opposing considerations with regard to the AII infusion rate. The first requirement was to infuse enough AII so that any AII/NA interaction would be detectable by non-invasive haemodynamic parameters. The second requirement was to use as low a dose of AII as possible so that the baroreflex would not be activated as this would further complicate interpretation of the results. As a compromise we chose 2 ng/kg/min (Nicholls et al, 1981). This dose fulfilled most of these aims in that it was a high enough dose to demonstrate a systolic AII/NA interaction but was also low enough to cause no significant change in systolic pressure or in baroreflex activity, as judged by reflex HR changes. Unfortunately, it did increase diastolic pressure. This does not however, detract from the main findings of this study since the synergistic AII/NA interaction was limited to systolic pressure, mean pressure and reflex bradycardia while AII alone had no effect on any of these parameters. It is worth noting from fig. 3.1, that at several time points when a subpressor systolic dose of NA was infused in the coincidental presence of a subpressor systolic dose of AII, then together a pressor effect was evident. In addition, plasma NA and AII were within the physiological range at this stage where the AII/NA interaction was most prominent. Furthermore, the fact that the reflex changes in HR followed the same synergistic pattern, suggests that the results were not a chance

phenomenon. On examining individual data in this study, it is evident that the augmentation of the systolic effect of NA is variable from one individual to another. However, no individual characteristics seemed to contribute to this variability, including baseline haemodynamics, plasma levels of NA or AII or urinary sodium excretion. With regard to diastolic pressure, AII alone did produce a significant increase but when AII and NA were given together, there was only an additive effect. This suggests that there was indeed no AII/NA interaction whatever with diastolic pressure.

It is not possible from this study to identify the precise location of this AII/NA interaction or to fully explain why this interaction was limited to systolic pressure. In considering possible interpretations, however, it is worth remembering that blood pressure is a combination of stroke volume, and peripheral resistance. Although there is much overlap, systolic pressure tends to preferentially reflect stroke volume, left ventricular ejection velocity and aortic compliance while diastolic pressure tends to preferentially reflect tone in the peripheral resistance vessels. In vitro, AII produces a consistent, concentration dependent, direct inotropic effect on myocardial ventricular contraction (Koch-Weser, 1964; Koch-Weser, 1965). Yet in vivo, this positive inotropic effect of AII is normally masked by peripheral vasoconstriction, so that in a human volunteer, infused AII tends to increase diastolic pressure more than systolic pressure and cardiac output is usually little altered by AII (Yu et al, 1961). Interestingly however, there are two other experimental situations apart from this study in which a link between AII and systolic pressure is unmasked so that AII becomes more closely associated with the systolic pressure than with diastolic pressure. Firstly, during the infusion of sodium nitroprusside in the presence of captopril, the withdrawl of AII augments the fall in systolic pressure but not that of diastolic pressure (Becker et al, 1986). Secondly,

in essential hypertension, when ACE inhibitors have been directly compared with beta-blockers, one repeated and surprising observation is that, while both groups of drugs reduced diastolic pressure equally, the fall in systolic pressure is normally greater with ACE inhibitors (Enalapril in hypertension study group, 1986; Lichter et al, 1986; Webster et al, 1986).

The mechanism for this link between AII and systolic inotropic activity may be twofold. Firstly, Stokland et al (1986) have shown in dogs that during isoprenaline infusion AII relocates central venous blood from the liver and spleen to the heart, hence increasing filling pressure and therefore cardiac output. Secondly, Peach et al (1969) showed that in vitro, AII is a potent inhibitor of NA uptake in ventricular muscle. Such a mechanism could explain why AII increases cardiac contractility in response to adrenergic nerve stimulation (Ackerly et al, 1976). It could also be relevant to this study where the systolic inotropic effect of the infused NA might have been augmented locally within the myocardium by AII, preventing NA re-uptake. If this uptake inhibition were to have occurred only within myocardial tissue, then it is most unlikely that our plasma NA levels would have been altered by AII, since myocardial tissue contributes only a small amount to overall NA clearance. Our plasma NA and AII levels really only exclude a global pharmacokinetic interaction. Overall, therefore, our results are consistent with either a localised pharmacokinetic AII/NA interaction as described above or a true pharmacodynamic AII/NA interaction which would have occurred either within the central veins, the myocardium or even the aorta.

Four other possible interpretations of our data are worthy of consideration. Firstly, it is possible that a subpressor infusion of AII could cause peripheral venous constriction so increasing venous return and hence myocardial contractility. A generalised increase in peripheral venous tone is

however, unlikely since AII has a much lesser effect on peripheral venous tone than NA so that the low dose of AII used in this study would be unlikely to to cause any such changes (McQueen and Morrison 1961; Collier et al, 1972). In addition, there is rapid tachyphlaxis to the peripheral venous effect of AII so that any initial effect would be liable to have waned by the later stages of our infusions. Secondly, although the HR changes are generally considered to be secondary to pressor responses, it is conceivable that the opposite is true in some cases (Mace et al, 1985). If so, the augmented bradycardia due to AII/NA might be compensated for by an increase in stroke volume leading to an augmentation of systolic pressure, as was observed. This seems possible but unlikely because it requires that the baroreflex works in the opposite direction to that which is generally accepted. Thirdly, it is often although not universally true that any vasodilator drug will non-specifically reduce the pressor response to NA and AII (Millar et al, 1983; Murphy et al, 1984). It is conceivable that the opposite is also true of pressor substances so that AII could render the peripheral vascular tree more responsive to other pressor agents such as NA. There is no corresponding human data yet on the effect of vasopressin on the pressor response to NA but there is in vitro animal data to suggest that vasopressin potentiates NA induced vasoconstriction and that NA potentiates vasoconstriction due to vasopressin (Bartelstone and Naysmith, 1965; Sueta et al, 1983). This attractive explanation does not however, fully explain the data because the changes in peripheral vascular tone are best detected by the parameter of diastolic pressure and yet in this study, a synergistic AII/NA interaction occurred with systolic pressure only and not diastolic pressure. Fourthly, it is conceivable that AII releases vasodilatory prostaglandins in small, but not in large arteries and that these prostaglandins conceal an AII/NA interaction on diastolic pressure.

Again this is possible but unlikely since AII infusions produce no overall systemic effect on prostacyclin release in humans (Barrow et al, 1986).

In this study therefore evidence is presented for a synergistic AII/NA interaction with regard to systolic but not to diastolic pressure. As we have seen one explanation of this may be an increase in stroke volume. One way in which stroke volume may be affected is by an increase in intravascular volume. The possible effect of an AII/SNS interaction on intravascular volume was explored by considering the possible intrarenal interaction to retain sodium and water. This was investigated first by defining the effects of AII and NA on renal sodium excretion and then investigating the effects of the combination of AII and NA infused concurrently.

13.2 The effect of angiotensin II on renal sodium excretion in man (chapter four)

The results of this study show that small ('physiological') increments in AII have a significant antinatriuretic action in man. The data also suggest that this effect on sodium excretion could be a direct one, independent of the actions of aldosterone. Though aldosterone levels did rise after infusion of AII, the peak was delayed beyond the nadir in sodium excretion, actually occurring at a time when sodium excretion was returning towards baseline. In support of this, previous evidence suggests that the action of intravenous aldosterone in man is delayed for over 40 min (this delayed onset of effect could explain the lack of immediate antinatriuresis with rising aldosterone levels during period D) [Sonnenblick et al, 1961]. In addition, potassium excretion in our study tended to fall, a directional change also arguing against an aldosterone-mediated antinatriuretic effect. Our results have also demonstrated, for the first

time in man, that AII decreases FE_{Li}. There is evidence that lithium is a specific marker for proximal tubular sodium handling in salt replete experimental animals and man, although not all authors accept the validity of this technique (Thomsen, 1984; Navar and Schafer, 1987). In our study subjects were salt-replete and FE_{Li} fell during AII infusion, suggesting that whole kidney proximal tubular reabsorption was increased (this would also argue against the major effect being due to aldosterone). We also measured FDD, a conventional clearance parameter believed to give some measure of proximal tubular outflow. This parameter relies on the concept of 'solute free water' generation, i.e. that sodium exiting the proximal tubule will be reabsorbed independently of water in the ascending limb of the loop of Henle generating 'solute free water', which in the absence of antidiuretic hormone, is excreted in the urine. In this study U_{osm} was reduced to less than 65 mosmol/kg, implying complete suppression of antidiuretic hormone.

There is some evidence that AII can increase antidiuretic hormone levels in man, although this is conflicting, and in this study there was no increase in U_{osm} during or after AII infusion. FDD fell in this study during AII infusion exhibiting a similar response to FE_{Li}. Though this method too has been criticized, on the basis that back diffusion of water can occur in the distal nephron, even in the absence of antidiuretic hormone, the fact that two separate measures of proximal tubular function showed an almost identical response to AII infusion suggests that this is a genuine effect. This is compatible with a wealth of evidence from animal experimentation where a variety of methods, including lithium clearance, have all suggested a proximal tubular effect of AII (Johnson and Malvin, 1977; Olsen et al, 1985).

A further interesting finding in this study was that distal reabsorption of sodium fell significantly during infusion of AII. Little is known about

the effects of AII on distal nephron function in either experimental animals or man and conflicting reports have been published (Olsen et al, 1985; Vander, 1963). One possible explanation for a reduction in RD_{Na} would simply be that enhanced proximal tubular sodium reabsorption reduces distal delivery of sodium to the extent that RD_{Na} falls. This view is supported by the observation that FRD_{Na} did not change significantly during AII infusion. These findings again support previous data obtained in animal studies and are a further argument against aldosterone contributing significantly to the observed reduction in sodium excretion (Olsen et al, 1985).

A recent study also supports our current findings. Brown (1988) has shown that captopril, an ACE inhibitor, increased FE_{Li} in salt-replete normal volunteers without changing inulin clearance. The effects of captopril during maximal water diuresis were also measured to assess segmental function by the free water clearance method; here too a proximal effect was demonstrated. While it is likely that the results of the study by Brown (1988) reflect removal of the effect of AII, a role for natriuretic prostaglandins cannot be excluded. In fact, Usberti et al (1986) have recently shown that the natriuretic action of captopril in normal subjects can be blocked by aspirin. The current results, however, are free from this difficulty in interpretation related to the use of captopril.

Our findings do not, however, allow any further interpretation of the mechanism by which AII might mediate the observed effects. We found no significant change in C_{Cr} in keeping with previous studies that have shown no change in GFR during infusion of low-dose AII (Johnson and Malvin, 1977; Olsen et al, 1985). We did not measure renal blood flow but the bulk of previous evidence suggests that renal blood flow falls and filtration fraction rises during AII infusion (Finnerty et al, 1961; Hollenberg et al,

1976; Johnson and Malvin, 1977; Olsen et al, 1985). The resultant changes in peritubular physical forces would be expected to enhance proximal tubular transport of sodium. Nevertheless it has been demonstrated that lower doses of AII can decrease sodium excretion in animals and man without changes in GFR or renal plasma flow (Johnson and Malvin, 1977; 0.1 and 0.5 ng/kg/min intra-renal artery infusion in dogs: Ljungman et al, 1983; 0.1 and 0.5 ng/kg/min intravenously in man), and without changing the intrarenal distribution of blood flow in animals (Johnson and Malvin, 1977). A direct effect of AII on proximal tubular sodium handling has also been suggested and the presence of specific proximal tubular AII receptors supports this possibility (Brown and Douglas, 1983; Schuster et al, 1984). Our results, however, do not enable a distinction to be made between an indirect haemodynamic mechanism of action or a direct tubular action. A further limitation of this study is that systemic infusion of AII may have different physiological effects than endogenous renal generation of AII. There is some evidence to suggest that components of the RAS (e.g. renin and angiotensin converting enzyme) are localised within the kidney, in different nephron subpopulations and within different parts of the nephron (Levens et al, 1981; Hollenberg, 1984). Physiological enhancement of endogenous AII production may, for example, produce local concentrations of AII sufficient to constrict the efferent arteriole or directly stimulate proximal tubular reabsorption of sodium. Only more sustained and intense stimulation might be necessary to cause sufficient intrarenal AII to be generated to affect nephron sites with a higher threshold for AII effect or to spill over into the systemic circulation (Levens et al, 1981). Viewed in this way systemic administration of AII might cause an abnormal pattern of response. Nevertheless it would seem from this study that the circulating levels of AII formed under certain physiological and

pathophysiological conditions could have a significant renal effect in their own right, albeit combined with the effects of local intrarenal production of AII. For example, the AII levels produced by infusion in this study (49+/-9 pg/ml) are similar to those produced by moderate salt restriction (Hollenberg et al, 1974), renovascular and accelerated hypertension (Robertson et al, 1986) and are at the lower end of the range found in compensated chronic heart failure (Fitzpatrick et al, 1985).

In summary, therefore, we have shown that physiological increments of AII are antinatriuretic in man, confirming previous observations. We have also attempted to elucidate some of the mechanisms by which AII alters renal sodium handling in man, presenting evidence that it enhances proximal tubular sodium reabsorption.

13.3 The effect of noradrenaline on renal sodium excretion in man (chapter five)

In this study we have shown that physiological increments in plasma NA have a significant antinatriuretic action in man. Previous investigations have concerned only high-dose infusion of NA (140-300 ng/kg/min) though a similar effect was observed (Werko et al, 1951; Smythe et al, 1952; McQueen and Morrison, 1961; Laragh et al, 1963). Basal NA levels in our study were at the upper part of the normal range because subjects were studied sitting and stood every 20 min to void. Consequently, the increment in NA achieved (280 pg/ml) resulted in steady state NA levels in the region of 830 pg/ml. These levels are only slightly higher than those found in upright salt-depleted subjects and are similar to those obtained during low-level exercise (Romoff et al, 1978; Richards et al, 1987; chapter eight). Further, these levels are typical of the circulating NA levels found in certain pathological salt-retaining conditions such as chronic

heart failure and hepatic cirrhosis (Bichet et al, 1982; Levine et al, 1982). From this study it is clear that circulating concentrations of NA can themselves reduce sodium excretion. This finding is particularly interesting in view of the haemodynamic effects of low-dose NA infusion. Several investigations have shown that exogenous infusion of NA in a dose of 20-30 ng/kg/min produces physiological plasma increments which begin to increase systemic blood pressure and lower the heart rate, probably by acting at extrasynaptic $alpha_2$-adrenoceptors, as well as intrasynaptic $alpha_1$-receptors (Izzo, 1983; Scriven et al, 1983; Jie et al, 1987 and discussion section 13.5). It is now clear, however, that circulating NA is not an inert neurotransmitter which has simply spilled over into the circulation. Rather, circulating NA appears to be a vasoactive hormone, which, at physiological concentrations, influences both renal function and the peripheral vasculature.

We also observed that the antinatriuretic effect of low-dose NA occurred without any change in creatinine clearance. This indicates that NA has a tubular action. Clearance of lithium was again used as a marker of proximal tubular sodium outflow in salt-replete subjects (Thomsen, 1984). In the present study lithium clearance declined in all subjects during NA infusion compared with placebo infusion without any concomitant change in the glomerular filtration rate. It appears, therefore, that NA enhances fractional proximal tubular sodium reabsorption in man, which is in keeping with animal experiments showing the presence of proximal tubular adrenergic receptors and demonstrating that synaptically released NA increases proximal tubular sodium reabsorption (DiBona, 1977; Gill, 1979). These experiments, however, only investigated neuronally released, rather than circulating, NA. It is probable that the effects of NA differ depending on whether the hormone is circulating or synaptically released,

as its access to different receptor subtypes differs in these two situations. Most authorities have suggested that post-synaptic alpha$_1$-receptors mediate the effects of catecholamines on renal tubular function. Peripherally infused NA may, however, have more access to extrasynaptic alpha$_2$-receptors (Jie et al, 1987). Alpha$_2$-receptors have recently been identified in both animal and human kidneys (Young and Kuhar, 1980; Umemura et al, 1986). Indeed, in certain species they may be the predominant alpha-adrenoceptor subtype found on the proximal tubules (Young and Kuhar, 1980; Nord et al, 1987). Furthermore, there is accumulating evidence that alpha$_2$-receptors may mediate catecholamine-induced proximal tubular fluid reabsorption in animals, as measured by micropuncture techniques or the lithium clearance technique (Baines, 1987; Nord et al, 1987). A further consideration is the possibility of presynaptic 'autoreceptor' activation by NA infusion. Presynaptic beta-receptors may augment neurotransmitter release while alpha$_2$-receptors in this location mediate negative feedback inhibition of NA release (Jie et al, 1987). With NA infusion, activation of the latter receptors is likely, though there is some evidence that this type of receptor mechanism is absent, or of only minor importance, in the kidney compared to the muscular bed (Robie, 1980; Insel et al, 1985).

A further observation in the present study was that the antinatriuretic effect of NA appeared to take time to develop in contrast to, for example, that of AII which reduces sodium excretion almost immediately (chapter four). This suggests an alternative view that the effect of circulating NA could be an indirect one, possibly due to intrarenal generation of AII. There is considerable evidence from animal experiments that the renal effects of sympathetic nerve stimulation may, at least in part, be mediated by the intrarenal RAS (Pelayo and Blantz, 1984; Johns, 1987a; Johns, 1987b). In the present study systemic AII and

aldosterone did not change significantly, though this does not exclude the possibility of an intrarenal effect. The theory that the intrarenal RAS could be important in explaining our findings will only be fully tested by studying the effects of ACE inhibition on the renal responses to low-dose NA infusion.

We did not detect any significant change in the sitting heart rate or blood pressure in this study. Our volunteers were seated and stood every 20 min to void. These changes in posture may have obscured the minor haemodynamic changes predicted by previous investigations performed in quietly resting, supine, subjects. Indeed, the dose of NA used (25 ng/kg/min) was a threshold one for detectable haemodynamic effects in man.

We did not measure renal blood flow in this study. High doses of NA consistently reduce renal blood flow in animals and man (Werko et al, 1951; Smythe et al, 1952; McQueen and Morrison, 1961; Laragh et al, 1963). However, low dose infusion of NA and low frequency renal SNS stimulation in animals can reduce sodium excretion without affecting glomerular filtration rate, renal blood flow or distribution of intrarenal blood flow (Slick et al, 1975; DiBona, 1978; DiBona, 1977; Gill 1979; Johnson and Barger, 1981). It is therefore possible that the decrease in sodium excretion measured in this study occurred independently of any change in renal blood flow; this requires further investigation. Another limitation of the present study is that we only studied the effects of increased circulating NA alone, as opposed to its combination with increased sympathetic nerve transmission. Clearly, the latter is the 'physiological' situation, and it is possible that the effects of a given level of plasma NA could differ when there is concomitant enhancement of intrasynaptic NA release.

In summary, we have shown that physiological increases in circulating NA reduce sodium excretion in man, at least partly by enhancing proximal tubular reabsorption. Circulating NA, per se, may therefore influence renal function under certain physiological conditions and pathological states associated with high plasma levels of this hormone.

13.4 The effect of angiotensin II and noradrenaline alone and in combination on renal sodium excretion in man (chapter six).

In studying the interaction between the RAS and SNS in the kidney, it is important to restate that the experimental evidence which supports such an interaction has shown that it occurs only when the RAS is activated (Zimmerman et al, 1984). In our study we increased "RAS" activity by infusion of exogenous AII and also altered "SNS" activity by infusion of NA, both at doses chosen to produce increments in hormone level and final hormone concentration of physiological and/or pathophysiological relevance in man. The baseline AII and aldosterone levels are higher than those reported for resting supine subjects and as noted in the last study basal plasma NA was also in the upper part of the normal range. These findings may be explained by posture as our subjects were studied in the seated position and stood every 20 minutes to pass urine. The increase in plasma AII (mean 19+/-5 pg/ml for all 14 infusions) and the final levels achieved by infusion (table 6.2) are similar to those that would be expected in normal subjects on a low salt diet (Oelkers et al, 1974), or in some groups of patients with heart failure (Dzau et al, 1981; Fitzpatrick et al, 1985). The increase in plasma NA (mean 316+/-51 pg/ml) and final levels achieved (table 6.2) are slightly higher than those that might be expected in upright salt depleted subjects and are similar to those seen during submaximal exercise (Robertson et al, 1979; Romoff et

al, 1979; chapter eight). These levels are also comparable to those found in patients with heart failure (Levine et al, 1982; Fitzpatrick et al, 1985).

The results of our study show that these small increments of AII, or NA, alone produce a significant reduction in sodium excretion (figure 6.1 and table 6.3). These decreases in sodium excretion due to AII and NA occurred without any change in creatinine clearance. Thus in our study the principal effect of AII and NA is most likely to have occurred at a tubular level. Our results in man therefore support earlier experimental work that suggests low doses of AII have a direct effect on the proximal tubule to increase sodium reabsorption (Harris and Young, 1977; chapter four), as do low doses of NA or low frequency renal SNS stimulation (DiBona, 1978; Bello-Reuss, 1980; chapter five). However we found no evidence to suggest that low doses of AII and NA given concomitantly in man act to further alter sodium excretion. The decrease in sodium excretion seen in our study again occurred without any change in systemic blood pressure and heart rate. This is important since changes in systemic pressure would have clearly altered the normal relationship between pressure and natriuresis. I have discussed above that low doses of AII and NA alone have been shown to cause reductions in sodium excretion without affecting glomerular filtration rate or renal haemodynamics. The corresponding information concerning infusions of both agents together is not available. It remains likely that the decreases in sodium excretion measured in this study occurred independently of any change in renal blood flow, though this requires further investigation (this limitation is discussed more fully below in section 13.6(a)). I have concentrated the present discussion on the evidence for a tubular RAS/SNS interaction from animal studies and the interpretation of the present results.

Experimental evidence for a renal tubular RAS/SNS interaction. In the

early 1970's it was recognised that low levels of renal nerve stimulation caused an antinatriuresis without change in GFR or renal blood flow (see references cited in section 13.3 and reviews DiBona, 1977 and Thames, 1984). The first studies of tubular interactions of the RAS and the SNS were undertaken at that time and were designed to investigate whether the antinatriuretic effect of low level renal nerve stimulation was mediated via the RAS. Saralasin was used as an AII antagonist and had no effect on the antinatriuretic effect of low level renal nerve stimulation (Zambraski and DiBona, 1976). This was interpreted as evidence against an AII involvement in the antinatriuretic effect of low level renal nerve stimulation. However this experiment is confounded as saralasin alone caused significant attenuation in blood pressure, renal plasma flow and $U_{Na}V$ prior to renal nerve stimulation.

The alternative approach to the interaction, whether the antinatriuretic effect of AII is mediated by or requires the activity of the renal SNS, has also been studied. Pelayo and Blantz (1984) showed that saralasin caused a reduction in absolute proximal reabsorption in both innervated (sham operated) and renal denervated rats, suggesting that the effects of AII and denervation are separate. However infusion of captopril in the renal denervated rats did not cause a reduction in absolute proximal reabsorption which is against that conclusion. Again in these experiments saralasin and captopril had significant effects on systemic and renal haemodynamics and indirect actions cannot be excluded.

Recent investigations by Johns and coworkers have studied this area further. Handa and Johns (1985) demonstrated that the action of low level renal nerve stimulation to decrease sodium excretion was removed by high dose captopril and was attenuated by a lower dose. Therefore in these experiments in the rat the action of the renal nerves to decrease sodium

excretion was dependent on AII. Similar results were obtained by indirect stimulation of the renal nerves in rats by brachial nerve stimulation (Handa and Johns, 1987). They showed that pharmacological inhibition of the RAS with saralasin and captopril attenuated the large falls in sodium and water output seen during brachial nerve stimulation. It is possible that small changes in renal haemodynamics may be involved in the attenuated antinatriuresis. However captopril did not alter renal blood flow during nerve stimulation and, whilst in the presence of saralasin there was a small fall in renal blood flow, the attenuation of antinatriuresis and antidiuresis was of the same magnitude. In addition a direct action of AII may have been removed by pharmacological blockade. There is some evidence against this being the explanation. Two studies have shown that the antinatriuresis mediated by renal nerve stimulation can be completely blocked by alpha$_2$-adrenoceptor antagonism with prazosin (Osborn et al, 1983: Hesse and Johns, 1985). However the rise in plasma renin was unaffected by prazosin. If the direct tubular action of AII was important in this experiment then its effects should still have been observed (Handa and Johns, 1987).

Further studies in rats have strengthened this argument (Johns, 1987a: Johns, 1987b). The effect of elevating the activity of the RAS (by salt depletion) and of decreasing RAS activity (by administration of deoxycortone acetate (DOCA) and saline to drink) were compared. Low level renal nerve stimulation caused decreases in sodium and water excretion in sodium deplete rats (high basal AII), but these responses were abolished by continuous captopril infusion. In rats given DOCA and saline to drink (low basal AII) low level renal nerve stimulation had no effect on excretion of water and sodium, however in a similar group of high salt rats given a continuous AII infusion low level renal nerve stimulation

decreased water and sodium excretion by a similar amount to that seen in normal, sodium replete rats. These data are therefore consistent with the view that AII can facilitate adrenergic transmission at a tubular level (Johns, 1987b).

Interpretation of the present results. The results of the present study showed no augmentation of antinatriuresis when AII and NA were given simultaneously in man. This study was designed to seek evidence for a postsynaptic AII/NA interaction in man. The conclusion from the animal evidence is that an AII/SNS interaction occurs but the exact location of such facilitation is unclear. Studies of renal nerve stimulation cannot differentiate between a presynaptic and a postsynaptic interaction. The present findings argue against a postsynaptic interaction, and taken with the animal evidence, suggests that the AII/SNS interaction demonstrated in animal studies may be presynaptic in origin.

Another possibility is that an action of AII to facilitate responses to SNS may be fully expressed in the salt replete state. Increments of AII and NA by infusion will then have only direct additive effects as seen rather than an augmented effect. This has not been tested in the present study. However in rats Johns (1987b) showed that the pattern and magnitude of the changes in water and sodium excretion in response to renal nerve stimulation were very similar in both normal, sodium replete rats and in sodium depleted rats. These findings support that a facilitatory effect may be maximal in the sodium replete state.

It is also worth noting that the infusion of exogenous AII and NA does not exactly mimic the effects of endogenous AII and NA, since the latter are both produced locally and systemically. Exogenous AII is able to act on all blood vessels with which it comes in contact. However intrarenally produced endogenous AII may be produced in different amounts

in differing parts of the kidney and not affect some parts in contact with exogenous AII (Levens et al, 1981). This is supported by the finding that angiotensin converting enzyme in the rabbit kidney is localised to specific parts, namely endothelial cells of renal arterioles, glomerular and intraglomerular capillaries and epithelial cells of the proximal tubule (Caldwell et al, 1976). Furthermore the increment in circulating NA brought about by infusion of NA is not accompanied by high intrasynaptic levels of NA as occurs with nerve stimulation.

Although aldosterone levels rose during AII infusion the immediate effect of AII on sodium excretion seen in our study is unlikely to be due to these changes (see discussion 13.2 above).

In summary our results have shown that low doses of AII and NA alone cause a significant reduction in urinary flow and sodium excretion in man with no change in systemic blood pressure or creatinine clearance. The concomitant infusion of AII and NA caused a simple additive but not synergistic reduction in sodium excretion. We have therefore found no evidence to support a postsynaptic interaction of low doses of AII and NA on renal sodium excretion in man.

13.5 Evidence that noradrenaline acts as a circulating hormone.

In the discussion above (13.3) I introduced the concept that NA may act as a circulating hormone as well as a neurotransmitter (Izzo, 1983; Scriven et al, 1983). More recently Chang and coworkers (1988) have reassesed the threshold for response to NA infusion in man using low, "physiological" doses (0-54 ng/kg/min). In resting, supine subjects they showed that the threshold venous plasma NA concentration for measurable effects on arterial BP was lower than earlier studies had suggested. Furthermore these concentrations were well within the physiological range.

Review of the literature shows the following threshold or lowest dose used with detectable change in blood pressure (diastolic, systolic or mean):

Fitzgerald et al, 1979	30 ng/kg/min
Grimm et al, 1980	42 ng/kg/min
Hjemdahl et al, 1983	90 ng/kg/min
Izzo, 1983	20 ng/kg/min
Scriven et al, 1983	20 ng/kg/min
Gordon et al, 1987	50 ng/kg/min
Blankestijn et al, 1988	30 ng/kg/min
Chang et al, 1988	18 ng/kg/min

The variation may by explained in part by different experimental procedure. Chang and coworkers recorded BP intra-arterially compared with non-invasive measurements in all other studies. Also Chang and coworkers excluded hand blood flow by cuff occlusion at the wrist for simultaneous measurement of forearm blood flow by strain-gauge plethysmography. Hjemdahl and coworkers studied their subjects in the semi-supine position and found that a higher rise in plasma NA and a higher NA infusion dose are needed before a detectable haemodynamic change occurs. This is important with regard to our study in seated subjects (Chapter 5, 13.3, receiving 25 ng/kg/min), though a rise in BP would be expected to reverse the renal effects we have demonstrated.

These studies support that NA may act as a circulating hormone as well as a local neurotransmitter and is relevant to the design and discussion of other studies in this thesis.

13.6 Methodological considerations concerning the studies of urinary sodium excretion (chapters 4-6).

(a) Measurement of creatinine clearance as a measure of glomerular filtration. Effect of angiotensin II and noradrenaline on renal blood flow.

(b) Lithium clearance as a marker of proximal tubular sodium handling.

13.6(a) Measurement of creatinine clearance as a measure of glomerular filtration. Effect of angiotensin II and noradrenaline on renal blood flow.

One limitation of these studies is that creatinine clearance has been used as a measure of glomerular filtration. A second limitation of these studies is that renal blood flow was not measured. As GFR did not change in any of these studies this has been taken to support that the observed effects on sodium excretion are due to an influence on tubular sodium handling. The experimental evidence supporting this has been cited above. Inulin clearance and excretion of para-aminohippurate (PAH) have been most widely used in studies to measure glomerular filtration and effective renal plasma flow respectively in man. I now review the evidence in man in more detail.

Effect of angiotensin II on glomerular filtration and renal plasma flow. The renal effects of subpressor doses of AII have been the subject of few studies. Pressor doses are recognised to cause a decrease in renal plasma flow and GFR (Finnerty et al, 1961; Vagnucci et al, 1964). Two groups have used lower doses of AII with minor pressor effects. Ajzen and coworkers (1968) using a dose of 2 mcg/min (approximately 2.9 ng/kg/min) show a fall in renal plasma flow with no change in GFR (inulin clearance). Aurell (1969) using doses between 1.1 and 1.5 ng/kg/min show a fall in renal plasma flow (PAH method) and a fall in GFR (inulin clearance).

Subpressor doses of AII have been employed by three groups of workers. Hollenberg and coworkers (1974) have shown that a low dose of AII (1 ng/kg/min) reduces renal blood flow assessed by ^{133}Xenon washout studies in both sodium replete (intake 200 mmol/24 hr) and sodium deplete subjects (intake 10 mmol/24 hr). Ljungman and coworkers (1983) studied doses of AII 0.1 and 0.5 ng/kg/min and show no significant change in renal plasma flow or GFR. In contrast Eadington and coworkers (1991) show that a dose of 0.5 ng/kg/min causes a small change in renal plasma flow and no change in GFR, whereas a dose of 1.0 ng/kg/min caused small changes in both GFR and renal plasma flow.

It therefore seems likely that the "threshold" dose of AII for effect on glomerular filtration and renal plasma flow lies between 0.5 and 1.0 ng/kg/min. The dose of AII used in these studies (1 ng/kg/min) is at upper end of this range.

Effect of noradrenaline on glomerular filtration and renal plasma flow. The effect of low doses of NA on glomerular filtration and renal plasma flow in man has also been the subject of few studies.

McQueen and Morrison (1961) showed that a dose of 140 ng/kg/min had no significant effect on GFR (inulin clearance) but significantly reduced renal plasma flow. Similarly Baldwin et al (1963) studied the effect of a range of doses of NA (38-440 ng/kg/min) and showed no effect on GFR (inulin clearance) and significant reduction in renal plasma flow (PAH method). The lowest dose used (38 ng/kg/min compared with 25 ng/kg/min used in our study) caused a small reduction in renal plasma flow.

Using a different technique (^{133}Xenon washout) Hollenberg and coworkers (1972) studied the effect of intrarenal arterial infusion of NA in healthy man. The calculated threshold increment in inflow NA

concentration for effect on renal blood flow was 100-300 pg/ml. The increment seen in venous plasma NA in our studies was 280 pg/ml (chapter five) and 316 pg/ml (chapter six).

The dose of NA used in the present studies (25 ng/kg/min) therefore has no effect on GFR and may be considered a threshold dose for effect with regard to renal plasma flow.

As noted earlier (13.4) in the discussion, the renal vascular effects of combined infusion of AII and NA in these low doses has not been subjected to systematic investigation. Renal plasma flow was not measured and GFR was estimated by creatinine clearance in this study. The absence of change in the latter argues against a major renovascular interaction. Whilst such an interaction has not been excluded I have concentrated the discussion on the possibility of an interaction at the tubular level.

13.6(b) Lithium clearance as a marker of proximal tubular sodium handling.

[This has been reviewed in detail by Koomans, Boer and Dorhout Mees, 1989. The essential arguments are presented here. I have referred to the original manuscripts of references cited and more recent findings have also been included. For more detailed referencing the reader is referred to the above review.]

The lithium clearance technique has recently been recommended as a method to quantitate sodium and water delivery from the proximal tubule by Thomsen and colleagues (1984). Conventional indirect methods do not allow quantitative analysis and the technique has been widely embraced. However Navar and Schafer (1987) point out that much of the evidence supporting the techniques use is circumstantial and do not support its

unconditional use.

Sodium is freely filtered at the glomerulus and approximately 75% is reabsorbed by the proximal tubule. Most of the remaining sodium is reabsorbed distal to this and only 1% of the filtered load is excreted in the urine. For lithium clearance to act as a marker of proximal tubule reabsorption three main criteria must be fulfilled. (1) Lithium should exert no influence on renal function. (2) Reabsorption of lithium should occur in proportion to sodium and water along the entire proximal tubule (3) No reabsorption of lithium should occur beyond the proximal tubule.

Lithium and renal function. It is recognised that high plasma lithium levels acutely alter renal function, causing increased sodium and potassium excretion, hydrogen ion retention and decreased water reabsorption (Singer 1981). The normal therapeutic range of plasma lithium is 0.6-1.2 mmol/l at 12 hours post dosage. However the doses usually given to man in lithium clearance studies (250-750 mg Lithium carbonate) give plasma levels of 0.2-0.3 mmol/l at 12 hours. It is generally assumed that renal effects of lithium are absent at concentrations below 0.4 mmol/l but this has not been studied systematically. In man recent evidence is conflicting. Jeffrey and coworkers (1988) showed that a dose of 750mg lithium carbonate caused a natriuresis ten hours later at a plasma level of 0.29 mmol/l and also that the natriuretic response to the dopamine prodrug, gludopa, was attenuated by this dose of lithium. Since the published review further information has become available. Schoors and Dupont (1990) investigated the effect of lithium on the response to intravenous dopamine using the same dose of lithium and achieving similar plasma lithium concentrations (0.26 mmol/l). The natriuretic response to dopamine was not altered, though in this study lithium per se also did not cause a natriuresis. Luik (1990) found that a dose of 600mg Lithium carbonate achieving a plasma

lithium concentration of 0.22 mmol/l was natriuretic. Peart and coworkers (1988) have shown that a dose of 500mg lithium carbonate increased sodium excretion in the 24 h post dosage and decreased sodium excretion in the following 24h. Strazzullo and coworkers (1988) show that a dose of 300mg lithium carbonate has no effect on renal function, though no plasma lithium concentration was given. In contrast Girbes and coworkers (1990) show that a dose of 300mg lithium carbonate achieving a plasma lithium concentration of 0.12 mmol/l was natriuretic. It is possible that flameless atomic absorption photometry may allow lithium clearance studies to follow normal trace lithium handling and avoid the need for oral loading with lithium altogether (Durr et al, 1990). This problem is therefore not yet resolved.

Reabsorption of lithium in the proximal tubule, loop of Henle and distal and collecting tubule. Evidence has been obtained principally from micropuncture studies in animals, studies during maximal water diuresis and pharmacological studies with diuretics.

Direct measurement of lithium delivery is possible by micropuncture in animals. This is however limited to superficial nephrons in anaesthetised animals. In addition only part of the nephron is accessible, up to the late proximal tubule and subsequently the distal convoluted tubule. The pars recta of the proximal tubule and the loop of Henle cannot therefore be studied independently. Using this technique Hayslett and Kashgarian (1979) show that 57% of the filtered lithium is reabsorbed before the late proximal convoluted tubule and a further 18% is reabsorbed before the early distal convoluted tubule. Thomsen (1984) argues that given the known anatomy and function of the pars recta it may be assumed that absorption occurs in the pars recta rather than the loop of Henle. Animal studies support this interpretation except in one circumstance. In sodium deplete

rats and dogs FE_{Li} was found to fall disproportionately with the reduction in sodium delivery to the proximal tubule (Kirchner, 1987). Lithium reabsorption in these circumstances is assumed to have occurred in the distal and collecting tubule as the disproportionate fall in FE_{Li} could be prevented by amiloride which acts selectively at this part of the nephron. However no sharp fall in FE_{Li} has been shown in man during salt restriction (Koomans et al, 1989). In addition two groups have shown that amiloride does not alter lithium clearance in sodium deplete man (Boer et al, 1988; Bruun et al, 1989). Leyssac and coworkers (1990) have recently confirmed the findings of Hayslett and Kashgarian by micropuncture studies, but also show that during osmotic diuresis with mannitol lithium reabsorption follows more closely the net water reabsorption rather than net sodium reabsorption. Thus during osmotic diuresis lithium clearance may slightly overestimate the water outflow from the proximal tubules.

Indirect evidence supporting the technique has been obtained by studying FE_{Li} during manoeuvres supposed to alter proximal sodium reabsorption. In general FE_{Li} is found to alter as well. FE_{Li} is increased by saline infusion, volume expansion by mineralocorticoid, hypotonic expansion with antidiuretic hormone and decreased by volume depletion due to thiazides, acute elevation of plasma oncotic pressure and upright posture.

Pharmacological studies are complex. Acetazolamide, a carbonic anhydrase inhibitor, has a major action on the proximal tubule and causes a marked rise in FE_{Li}. However carbonic anhydrase is not confined to the proximal tubule. Thiazides with carbonic anhydrase inhibitor activity also cause a rise in FE_{Li}. The "loop" diuretic frusemide causes a large increase in FE_{Li} but lesser increases in some other proximal markers. This may be due to an effect of frusemide both on the proximal tubule and to

abolishment of pre-existing lithium transport in the loop of Henle. A recent study by Colussi and coworkers (1989) using acetazolamide and frusemide alone and in combination in salt replete normal volunteers, shows that the frusemide increase in FE_{Li} was not an action on carbonic anhydrase. This further supports a distal effect of frusemide on lithium transport, though an effect of frusemide on the proximal tubule independent of carbonic anhydrase inhibition has been demonstrated. Amiloride which acts at late distal tubule and collecting duct produces no change in FE_{Li} in the salt replete state. However triamterene, which also acts at collecting tubules, causes a rise in FE_{Li} and FE_{Na} but no rise in fractional excretion of phosphate, a proximal marker (Wetzels et al, 1989). Pharmacological studies are therefore inconclusive.

Three other findings suggest that lithium can be reabsorbed by the loop of Henle. Firstly in humans during volume expansion with mineralocorticoid there was an exceptionally large increase in FE_{Li}, which was greater than the fall in maximal free water clearance, a second method of estimating fractional proximal sodium reabsorption (Boer et al, 1987). Studies in animals suggest that this is at least partly due to reduced reabsorption in the loop of Henle. Secondly in healthy volunteers indomethacin, a prostaglandin synthetase inhibitor, markedly reduced FE_{Li} whilst FE_{Na} was unaltered (Gaillard et al, 1987; Rabelink et al, 1989). Proximal solute (phosphate and uric acid) handling was not altered, agreeing with the absence of a proximal effect. Prostaglandin synthetase inhibitors increase medullary thick ascending limb reabsorption, diminish papillary blood flow and thus increase the corticomedullary osmotic gradient. Increased lithium reabsorption may therefore have occurred in the loop of Henle. Finally in water loaded volunteers the administration of the vasopressin analogue dDAVP reduced both FE_{Na} and FE_{Li} (Boer et al,

1988). This may be explained in the same way as for the effect of indomethacin, by direct and indirect (increasing the corticomedullary osmotic gradient) effects. Koomans and coworkers (1989; Boer et al, 1990) conclude the falls in FE_{Li} with indomethacin (9% of the filtered load) and dDAVP (2 to 3% of the filtered load) confirm that distal lithium reabsorption can be stimulated under certain conditions. The large rise in FE_{Li} during mineralocorticoid escape suggests that lithium reabsorption may also take place in the loop of Henle under normal conditions.

Summary. Estimation of proximal tubule sodium reabsorption by the lithium clearance technique method comes closer to direct measurement than any other indirect method. Lithium per se may alter tubular sodium handling. Most lithium reabsorption occurs in the proximal tubule. However there are clearly circumstances when reabsorption in the loop of Henle or distal nephron occurs. The known conditions invalidating the method are in the presence of antidiuretic hormone, NSAID therapy, salt depletion (probably not in humans) and during osmotic diuresis.

With regard to the studies presented in this thesis there are important practical points to be made. (1) The subjects were studied sodium replete (2) A placebo control study day with lithium alone administered was included. (3) The observed effect of intervention (AII infusion and NA infusion) fits with the known effects on sodium excretion when studied without lithium pretreatment. (4) The observed effect on FE_{Li} was compared with a second method for determining delivery from the proximal tubules, fractional distal delivery (for AII infusion). (5) Subjects were studied during maximal water diuresis to ensure suppression of antidiuretic hormone, which avoids an antidiuretic hormone effect on lithium reabsorption in collecting tubules. (6) Subjects were taking no regular oral medication and were forbidden to take aspirin or other NSAID.

13.7 The effect of angiotensin II on the stoke volume response to beta-agonism in man (chapter seven).

In this study we return to the possibility that there is an AII/SNS interaction occurring at the level of the myocardium or an effect on intra-thoracic blood volume raised by the findings in chapter three, discussion 13.1

In considering the results of this study it is important to emphasise several aspects of the use if ISO and AII in experimental animals and man.

The haemodynamic effects of isoprenaline infusion: There is a well recognised dose related increase in CO. This is associated with an increased systolic BP and a fall in TPR reflected by a fall in diastolic BP. The increase in CO caused by ISO can be separated into two components. Firstly there is a rise in HR in a dose related manner. Secondly there is an initial ISO induced increase in SV at low doses but this SV increase attains its maximum quickly with no further increase and even decreases as the dose of ISO in increased further. This has been previously shown in man with 6-15% increases in SV described (Krasnow et al, 1964; Stephens et al, 1979; Goldstein et al, 1986), though the rise is frequently non-significant (Krasnow et al, 1964; Stephens et al, 1979). Our results confirm these findings.

These actions of ISO contrast markedly with isolated chronotropic change. When HR is increased by pacing, in the absence of inotropic stimulation SV falls and CO is unchanged. When pacing occurs in the presence of an inotrope SV is maintained and CO increases (Ross et al, 1965). Thus an important inotropic effect of ISO is the maintenance of SV, which with rising HR leads to an increase in CO. It is also important to note that sympathetic stimulation similarly results in a rise in heart rate and an increase in myocardial inotropy, reflected by an increase in SV, and

that these two effects are independent (Ilebekk et al, 1978). Thus, provided cardiac preload and cardiac afterload are not altered, change in SV more closely reflects change in myocardial inotropy than CO, a composite of SV and heart rate.

Cardiac effects of angiotensin II: AII has been shown to have variable effects on the heart and circulation, dependent in part on the preparation used for investigation. Thus AII has been shown to have a positive inotropic effect in isolated kitten papillary muscles (Koch-Weser, 1964), rabbit atria (Trachte et al, 1981) and isolated cat whole heart (Dempsey et al, 1971), but a negative inotropic effect on the myocardium in the intact circulation of dogs (Frank et al, 1970). AII also exerts a positive inotropic effect on isolated human atrial muscle trabeculae (Moravec et al, 1990), however infusions of AII in man are associated with no change or small falls in CO (Segel et al, 1960; Yu et al, 1961). These findings in the intact circulation are attributed to an increase in TPR and cardiac afterload, and baroreflex induced reduction in CO (Yu et al, 1961). Similar findings are seen when an inotrope which also causes an increase in TPR such as NA is infused. The main effect is to cause increases in systolic and diastolic BP and TPR with a baroreflex bradycardia and little change or a fall in CO.

Interpretation of the present study: In this study our major finding is that when AII is infused with ISO, there is an augmented stroke volume response with no effect on the chronotropic response. This occurs with a dose of AII which alone causes no change or a small fall in SV. AII alone did cause some peripheral vasoconstriction as evidenced by small increases in systolic and diastolic BP and TPR. However it is important that there was clearly no change in HR confirming that the baroreflex was not activated.

Our results confirm that AII interacts with beta-sympathetic

stimulation to enhance the stroke volume response. There are three main influences on SV, the force of ventricular contraction (cardiac inotropy as discussed above), cardiac preload, the Starling mechanism and cardiac afterload. Our study is however limited in confirming the mechanism of this interaction as further measures such as central venous pressure or echocardiographic left ventricular dimensions were not available. Nevertheless our data suggest that afterload is well matched in the PL/ISO and the AII/ISO study days. Afterload is determined largely by peripheral vascular resistance, the physical characteristics of the arterial tree and the volume of blood that it contains at onset of ventricular ejection and is importantly influenced by arterial pressure. Our data show that TPR and systolic BP and diastolic BP are no different on these study days and together suggest that afterload is also similar. Thus it appears likely that the interaction occurs either by enhanced contractility or by increased preload. The latter mechanism is supported by animal data, which use more invasive techniques and show that during ISO infusion AII relocates venous blood from the splanchnic circulation to the heart hence increasing SV (Stokland et al, 1986). Although our data agree with that of Stokland et al, we were not ethically able to measure such regional blood flow changes in normal man by invasive cannulation. The hormonal data in our study showing a trend for ANP to rise during infusion of both AII and ISO support the relocation of blood in the circulation towards the mediastinum. Other investigators have shown that a rise in ANP is an indicator of right atrial filling (Richards et al, 1986). However peripheral venous ANP levels are liable to be a fairly insensitive measure of cardiac filling pressures in normal man. It is therefore not surprising that the relatively minor changes in filling pressures expected in this study were not reflected by significant changes in the peripheral venous ANP levels. Furthermore previous data in

man also support this proposed mechanism since Benjamin et al (1988) have shown that AII augments the venoconstrictive effects of sympathetic stimulation, which is also liable to increase cardiac filling pressure. With regard to this study the effect of infused ISO fits in with this theory in that it has previously been shown to increase venous return in man (Leenan and Reeves, 1987). There are however three alternative mechanisms which may contribute or explain the observed AII/ISO interaction and these are considered below.

Firstly AII may increase myocardial catecholamine release or decrease catecholamine reuptake locally within the myocardium. There is a wealth of animal data to support either of these possibilities (see Introduction). Measurement of venous plasma NA as in this study is a useful indicator of general SNS activity but is clearly not sensitive enough to reflect localised changes in catecholamine release or uptake in the heart. With regard to the possibility that AII facilitates NA release we measured venous plasma NA. Clearly no increase in plasma NA was seen with AII alone. Neither were the small increases in plasma NA caused by our low dose range of ISO infusion affected by concomitant AII infusion. It is possible that coronary sinus blood sampling may reveal local changes (Schwartz et al, 1979) but such investigation is ethically impossible in normal human volunteers. With regard to the possibility that AII inhibits myocardial NA uptake it is worth noting that ISO is not taken up by uptake-1 in the same way as NA but is rather taken up by uptake-2 (Goldstein et al, 1985). Therefore any postulated inhibitory effect of AII on NA uptake would not similarly effect ISO uptake. This makes it unlikely that the augmented stroke volume effect of ISO and AII together is due to any direct effect on NA uptake. If this did occur, the sequence of events would have to have two stages ie. ISO might release myocardial NA, the reuptake of

which is then inhibited by AII.

Secondly it is interesting that in an analogous experiment in man AII was able to increase SV and CO when given with a vasodilator (Landes and Kummer, 1959). In our study AII alone did cause some peripheral vasoconstriction but ISO induced peripheral vasodilatation was able to totally overcome this. It is therefore possible that the pharmacologically induced afterload reduction allowed AII to manifest its inherent inotropic effect.

Thirdly a further factor tending to limit the myocardial response to AII is that AII causes a decrease in coronary blood flow (Fowler and Holmes, 1964; Xiang et al, 1985). This reduction may also have been abolished in our study by ISO, as ISO infusion as well as reducing TPR also increases coronary blood flow (Krasnow et al, 1964; Gwirtz and Stone, 1982).

We also found no augmentation of the systolic BP response with the AII /ISO combination, which contrasts with our earlier experiment in which AII and NA combined caused a significant synergistic interaction in rise in SBP (chapter three). SBP depends not only on SV and the character of ventricular ejection during systole but also on aortic and large vessel compliance. ISO may have increased large vessel compliance and, as with TPR, this vasorelaxant effect may have been unaffected by concomitant AII infusion. In this way the synergistic increase in SV seen in the present study may not manifest itself as an augmented rise in SBP due to a concomitant ISO induced increase in aortic compliance.

In summary, our results suggest that small increments of AII can augment the stroke volume response to beta-agonism in man. This action is probably due to AII relocating blood into the mediastinum and hence

increasing cardiac filling pressures, but several other possibilities merit further investigation.

13.8 The effect of angiotensin II on endogenous noradrenaline release in man (chapter eight).

In this study, when we were choosing an AII infusion rate to use, we were aware of two opposing considerations once more. We wanted to infuse enough AII so that our methodology could detect any AII/NA interaction. On the other hand, we aimed to use as low a dose of AII as possible so that the baroreflex would not be activated as this would complicate interpretation of the results. As a compromise we chose 1.5 ng/Kg/min. This dose was certainly low enough to bring about no haemodynamic effects (Table 8.1). However we were unable to demonstrate any augmented increase in NA release in response to physiological stimulation of the SNS in the presence of this low dose of AII, despite a wide range of different levels of sypmathetic activation. It is possible that our chosen dose of AII was too low to reveal such an interaction. However, the rise in plasma AII during infusion was similar to that seen in chapter three in which there was a significant AII/NA interaction with regard to systolic blood pressure. Also the control values of AII obtained in our study correspond well with those obtained by others with constant sodium diets (129-150 mmol/day) in normal volunteers and the rise in plasma angiotensin II is similar to that found during low sodium intake diets (9-12 mmol/day) (Oelkers et al, 1974) as noted earlier. Indeed our AII samples for this study were all assayed in the same laboratory as Oelkers et al 1974. The effect of higher doses of AII infusion during SNS stimulation have not yet been investigated.

One further possibility for our failure to demonstrate an interaction

is that venous plasma NA may not be an adequate reflection of intrasynaptic NA release. In general the measurement of venous plasma NA provides a useful estimation of average sympathetic outflow (Goldstein et al, 1983). Thus plasma NA is increased by a variety of stimuli thought to activate the SNS (Lake et al, 1976; Robertson et al, 1979; Watson et al, 1979) and is decreased by physical disruption of sympathetic nerves (Nielsen et al, 1980) or pharmacological interference such as postganglionic blockade by debrisoquine (Flammer et al, 1979). Wallin et al (1981) have shown by direct measurement of sympathetic neural activity that plasma NA is directly related to measured sympathetic activity. In addition plasma NA has been shown to reflect activation and deactivation of the baroreflex during pharmacological induced changes in BP (Eckberg et al, 1986). On the other hand Brown et al (1982) have shown that synaptic cleft and plasma catecholamine concentrations can be manipulated independently. More recently Wallin et al (1987) have shown that the relative change of venous plasma NA during isometric handgrip is less than the change in recorded muscle sympathetic activity. Furthermore, although plasma NA is a useful indicator of global sympathetic activity, it cannot necessarily reflect changes if selective activation of parts of the sympathetic nervous system occurs. There is evidence of the non-uniformity of sympathetic efferent activity to various organs (Abboud, 1979a; Shepherd, 1982). Thus there are well recognised limitations in using venous plasma NA as an index of sympathetic activity and these limitations may contribute to our inability to detect a facilitatory effect of AII. It is however clearly not possible to measure intrasynaptic NA directly in man but the fact that there were no significant haemodynamic effects in this study does give us further confidence that AII genuinely does not have any facilitatory effect on intrasynaptic NA release.

In summary within the constraints of this study we have found no evidence to support the enhancement of endogenous NA release by the infusion of this low dose of AII.

13.9 The effect of angiotensin II on the haemodynamic and plasma noradrenaline responses to tyramine infusion in man (chapter ten).

In this study we have therefore found that a low dose of AII does not potentiate the pressor response nor plasma NA response to TYR infusion in man. This compares with our earlier finding that AII does not enhance the endogenous release of NA in response to physiological stimulation of the SNS in man (chapter eight). However our results would initially appear to contrast with those of Kaneko et al (1966) who show that AII can augment the pressor response to TYR in man. However the studies are not directly comparable. The dose of AII in the study of Kaneko et al was much higher (approximately 4-15 ng/kg/min) and was associated with a considerable rise in blood pressure during AII infusion (16 mmHg rise in mean arterial pressure). When designing this study, it was important that the dose of AII chosen should have no effect on diastolic BP in order to avoid the complicating effect of baroreflex changes. A subpressor dose of AII was therefore deliberately chosen for this reason and also so that our study would complement that of Kaneko et al (1966) rather than reproduce it.

The haemodynamic effects of infused tyramine: In man infused TYR is used as a standard method by which to release endogenous neuronal NA. To understand these and other results, it is worth considering its haemodynamic effects in a little more detail. As we have found, TYR is known to cause a marked increase in systolic BP with little change in diastolic BP (Cohn, 1965; Scriven et al, 1983). This by itself would suggest

that TYR raises BP mainly by stimulating cardiac contractility. Furthermore, as has also been described before, TYR causes a late and relatively minor change in venous plasma NA in comparison to the large increment in systolic BP (Bianchetti et al, 1982; Colombo et al, 1988). This and its haemodynamic effects can be attributed to the fact that TYR causes localised rather than generalised release of NA. Forman et al (1984) have shown that despite causing only small non-significant changes in arterial plasma NA levels, peripheral venous infusion of TYR results in a more than fourfold rise in myocardial release of NA as demonstrated by coronary sinus blood sampling in man. Localised myocardial release of NA makes little contribution to the total circulating plasma NA (Goldstein et al, 1983; Esler et al,1984) and would therefore not be reflected by changes in peripheral blood.

Further support for a mainly myocardial action of TYR in man comes from the study of Scriven et al (1984). The effect of propranolol on the response to TYR infusion was examined. Propranolol attenuated both the cardiac inotropic effects (estimated by systolic time intervals) and the rise in BP seen with TYR. They conclude that TYR exerts its pressor effect mainly by stimulation of cardiac beta-receptors. In a similar study Colombo et al (1988) using a non-selective and a selective beta-blocker have shown that the pressor effect of tyramine is mainly due to stimulation of cardiac $beta_1$-receptors. Thus it would appear that TYR mainly acts by releasing myocardial NA which then stimulates cardiac $beta_1$-adrenoceptors.

Interpretation of the current study: The current study is limited in its power to exclude an interaction by virtue of the small group studied which may introduce a Type II error (see discussion in section 13.10 below). However the clearly superimposable haemodynamic and plasma NA results argue against a major interaction. This study using TYR examines pre- and

postsynaptic mechanisms at the same time. Venous plasma NA responses after TYR infusion are a crude index of presynaptic NA release particularly if the release is at a local, myocardial level, and the power of the study can exclude only a major interaction (see discussion in section 13.10 below for power calculation). However the haemodynamic changes reflect the end result of both pre- and postsynaptic effects and the power of this study is sufficient to detect a small haemodynamic interaction. Within these limitations this study did not find evidence for an AII/NA interaction at either the pre- or postsynaptic site. This agrees with our previous inability to detect a presynaptic interaction (chapter eight), but contrasts with our earlier studies of postsynaptic effects. Firstly, we have seen that a subpressor dose of AII synergistically augmented the systolic but not the diastolic BP response to exogenous NA (chapter three). Secondly we found that a subpressor dose of AII augments the cardiac stroke volume response to an ISO infusion (chapter seven). The apparent difference between these postsynaptic results and our previous positive postsynaptic findings are difficult to explain.

The previous studies described in chapter three and chapter seven used exogenous NA and exogenous ISO so that differences between TYR on the one hand and NA and ISO on the other hand may provide an explanation. One possible explanation lies in intrasynaptic events. TYR releases neuronal NA which acts principally on intrasynaptic $alpha_1$- and $beta_1$- adrenoceptors whereas exogenous NA and ISO act principally on extrasynaptic receptors of the $alpha_2$ and beta subtypes. In addition, TYR causes high local intrasynaptic concentrations of NA, and may therefore be more efficient at activating intrasynaptic feedback mechanisms which limit sympathetic activity. Clearly there are major differences which may explain the different responses in the presence of AII. The second possible

explanation lies in the fact that TYR, NA and ISO all produce different patterns of regional haemodynamic changes. For example, TYR is mainly inotropic (Scriven et al, 1984; Colombo et al, 1988), ISO is inotropic and an arterial vasodilator (Krasnow et al, 1964) whereas NA increase venous tone, inotropic activity and arterial tone (Cohn, 1965). In view of these complex effects, it is perhaps not surprising that AII interacts with TYR, NA and ISO to produce different haemodynamic patterns. Further detailed studies of regional haemodynamic changes are required before a full explanation can be given for our current results.

In summary we have found no evidence to support the enhancement of haemodynamic or plasma NA responses to TYR infusion by AII in man. TYR exerts its pressor effect mainly by release of myocardial NA and stimulation of cardiac beta-receptors. We have found no evidence to suggest that this process is facilitated by AII in normal man.

13.10 Limitations of studies of the RAS/SNS interaction using intravenous infusions and/or ACE inhibitors in man.

The use of ACE inhibitors: ACE inhibitors are potent inhibitors of the angiotensin converting enzyme in animals and man and are a useful way to manipulate RAS activity in experimental studies in addition to their established therapeutic role. However some problems may be encountered in using ACE inhibitors to investigate an AII/NA interaction. One premise noted earlier is that the animal studies which support an interaction between the RAS and the SNS have shown that this interaction occurs only when the RAS is activated (Zimmerman et al, 1984). Despite this, previous attempts to demonstrate an RAS/SNS interaction in man have nearly always administered ACE inhibitors to normal volunteers and have hence

investigated the differential effects of normal and low levels of RAS activity.

Secondly inhibition of ACE, the same enzyme as kininase II, blocks the degradation of bradykinin, a potent vasodilator. Opinion is divided as to whether this may be of therapeutic importance with regard to the hypotensive effect of ACE inhibitors. Nevertheless some groups have shown increases in either plasma or urinary kinins (Swartz et al, 1979; Vinci et al, 1979; Mimran et al, 1980), whereas other groups have not (Mookherjee et al, 1983; Rasmussen et al, 1985). However the kallikrein-kinin system may be more important as a local hormonal system regulating regional blood flow (Carretero and Scicli, 1980) and local changes can occur without change in circulating bradykinin levels or in urinary kallikrein (Johnston et al, 1982). Furthermore alterations in bradykinin metabolism may stimulate prostaglandin production (Regoli and Barabe, 1980). Increases in PGE_2 in plasma and urine metabolites have been demonstrated during ACE inhibitor treatment (Swartz and Williams, 1982; Usberti et al, 1986). In experimental studies Ishida and coworkers (1989) have confirmed that ACE inhibitor treatment promotes very high bradykinin levels during bradykinin infusion. Furthermore in dogs pretreated with ACE inhibitors higher plasma concentrations of AII were needed to produce pressor responses equivalent to those seen in the same dogs under control conditions (Tree and Morton, 1980). The mechanism of this difference was not explored. In pithed rats Antonaccio and Kerwin (1981) show that pressor responsiveness to sympathetic nerve stimulation after ACE inhibitor treatment is not fully restored by AII infusion but was restored by AII and indomethacin, a prostaglandin synthetase inhibitor. These latter two studies and those cited above show that bradykinin and prostaglandins may be altered by ACE inhibitor therapy, though this may occur at a local rather than a

circulating level. This may have little importance clinically but is important in the design of studies reported in this thesis.

Finally the use of ACE inhibitors may be complicated by effects on autonomic reflexes. Treatment with ACE inhibitors has been shown to be associated with increased parasympathetic activity (Campbell et al, 1985) and to cause resetting of the baroreflex set point (Dusing et al, 1987) without affecting baroreflex sensitivity or sympathetic nervous reflexes (Sturani et al, 1982; Dusing et al, 1987).

These effects are important complications rendering ACE inhibitors imperfect tools with which to investigate the effects of AII on SNS activity. Thus, for the most part, in the studies presented here RAS "activity" has been increased by infusion of AII, and ACE inhibitors rarely used.

The measurement of venous plasma NA as an index of SNS activity: The general limitations of using venous plasma NA as an index of SNS activity are discussed in section 13.8. It is also important to consider the statistical power of these studies to demonstrate an interaction, considering both a change in plasma NA and the power to demonstrate a change in non-invasively recorded haemodynamics.

For illustration it is useful to consider the power in the smallest of the studies, that is the effect of AII on plasma NA and haemodynamic responses to TYR infusion. This study, in six subjects, has a power of more than 80% to detect an interaction in systolic BP of 10 mmHg and of 180 pg/ml in plasma NA. It is therefore clear that whilst small interactions with regard to BP may be demonstrated, only large changes in global SNS activity will be revealed by this approach, and more localised changes may be overlooked.

Because of these limitations a second approach was used to investigate a possible haemodynamic AII/SNS interaction. The approach chosen was that of infusion of hormone, drug and/or placebo into the brachial artery of the non-dominant arm in healthy subjects. The vascular effects were recorded by strain-gauge plethysmography. There are several features of this technique that make it uniquely attractive for examining a vascular interaction:

1. The vascular bed of the forearm is amenable to local infusion of drugs in healthy man.

2. The blood flow to the forearm is approximately 1% or less of total cardiac output. Thus by infusion of very low doses (many times lower than used systemically), systemic effects of the drug can be avoided. The forearm vascular bed may therefore be studied effectively in "isolation".

3. By avoidance of systemic effects the opposite, non-infused arm can be used as control.

4. By avoidance of changes in systemic arterial pressure the local vascular effect of the drug can be studied without activating neural or hormonal reflexes.

5. Conversely the effect of the drug on vascular responses to stimulation such as LBNP can be studied and again compared with the control arm.

The studies described in chapters 10-12 use this technique and are now discussed. The methodology and analysis of results are discussed in the Methods (chapter two).

13.11 The effect of angiotensin II on the forearm blood flow response to noradrenaline (chapter ten).

and

The effect of local angiotensin II on the forearm blood flow response to lower body negative pressure (chapter eleven).

In these studies we have shown that local intra-arterial infusion of AII, employing a dose which had no direct effect on blood flow, enhanced the response to LBNP. In marked contrast, AII did not affect responses to locally infused NA. These studies employed doses of AII (Chinn and Dusterdieck, 1972) and NA (Chang et al, 1988) insufficient to cause a systemic pressor response, and in the case of AII, 1000-fold greater doses do not affect blood flow in the opposite arm (Benjamin et al, 1989). Hence the effects of the infusions used in the present studies may be assumed to be limited to the infused forearm. LBNP has been shown to be a reliable stimulus for reflex sympathetic vasoconstriction in the upper limb (see Methods, chapter two for detailed discussion).

The dose of AII infused (320 fmol/min), was chosen on the basis of previously constructed dose-responses in the forearm (Benjamin et al, 1989), to produce little effect on resting blood flow. Assuming a resting blood flow in the forearm of 40-50 ml/min, this dose will have produced an increment in AII concentration in blood perfusing the forearm of approximately 6-8 fmol/min, i.e. within the physiological range. In our study it was not possible to confirm the increment in plasma concentration of AII, as angiotensinases within the peripheral circulation lead to marked AII clearance (Campbell, 1985) so that its measurement in venous effluent would be of little value. In addition, distal arterial blood sampling would have required a second arterial puncture and this was felt to be ethically unacceptable. It was however important that the dose of AII given was

insufficient to produce reduction in local blood flow directly (tables 10.1 and 11.1), as reduction in resistance vessel calibre may non-specifically alter responses to other vasoconstrictor stimuli (Doyle et al, 1959).

Our investigation has shown (figure 11.3) that with this small increment in concentration of local AII, producing no change in resting blood flow, there was significant augmentation of the reduction in forearm blood flow during sympathetic stimulation with LBNP, approximately doubling the response. This effect of AII confirms an interaction with the sympathetic nervous system, and being confined to the infused limb, acts at a peripheral site. From the results of our this experiment, this interaction could occur presynaptically to produce increased NA release or reduced reuptake, or act postsynaptically, either at the receptor or at a postreceptor level.

In our first experiment the same dose of AII (320 fmol/min) did not affect the reduction in blood flow produced by local NA infusion (figure 10.1). As with LBNP, NA infusion reduced local blood flow, resulting in a further small increment in local plasma concentration of plasma AII. Resting blood flow was, however, lower in subjects receiving NA, leading, if anything, to greater increments in AII concentration in these subjects. As LBNP and NA produced similar reductions in forearm blood flow, differences in AII concentration are unlikely to explain the absence of an interaction between NA and AII. The absence of a postsynaptic interaction might be explained by tachyphylaxis to either AII or NA in the forearm vessels. Although this may occur in vitro, it does not occur with either agent during prolonged intra-arterial infusion in man (Whelan, 1967; Clarke et al, 1989). Alternatively, the response to NA might be maximal with the doses employed. We did not directly assess the response to higher doses of NA. However, other workers have shown similar responses to our own with

NA at 50 ng/min (Taddei et al, 1990), as well as further dose-dependent reduction in local blood flow, at least to 200 ng/min (Taddei et al, 1990; Collier et al, 1972b), and an 80% reduction in flow is achieved with 500 ng/min. On this basis, we are confident that the response to NA is not maximal with the doses employed. Under these circumstances the failure of AII to affect the responses to NA implies that the major interaction in the forearm in man is presynaptic rather than postsynaptic.

Studies examining the interaction between AII and the SNS in man have generally involved manipulation of circulating plasma AII, either through intravenous AII infusion or systemic converting enzyme inhibition (see introduction). In these circumstances it is not possible to localise the site of any interaction. In the present experiments, with local AII infusion, interpretation of the findings is not confounded by the influences of central, renal or cardiovascular reflex effects associated with systemic administration. Based on the findings in animals and in vitro preparations, the likely mechanism for presynaptic interaction is through an increase in NA release from nerve endings, rather than by an effect on NA reuptake. A major effect on reuptake in the present studies is unlikely in view of the lack of interaction between AII and NA. Other neurotransmitters may be involved in peripheral sympathetic responses as, in man, forearm vasoconstriction to LBNP is not abolished by the alpha-blocker phenoxybenzamine (Taddei et al, 1990). There is evidence that stimulation of purinoceptors may affect peripheral sympathetic responses in man (Taddei et al, 1990) and animals (Holycross and Jackson, 1989). However, at present, the evidence is against an interaction between purines and AII-induced potentiation of sympathetic neurotransmission (Holycross and Jackson, 1989). Neuropeptide Y is also co-localised with NA in sympathetic nerves. However, neuropeptide Y does not influence responses either to

LBNP or NA in man (Clarke et al, in press). Thus, at present, there is no evidence that a co-transmitter plays an important role in the interaction between AII and peripheral sympathetic nerves.

Basal concentrations of AII in blood may also facilitate adrenergic tone within peripheral blood vessels. Support for this hypothesis comes from the experiments of Morganti and colleagues (1985a) who have shown that, in patients with essential hypertension, the systemic administration of an ACE inhibitor attenuates the increase of forearm vascular resistance produced by LBNP. This issue may be further resolved by experiments using the novel nonpeptide AII antagonists (Timmermans et al, 1991).

In summary we have shown that AII augments LBNP-induced sympathetic vasoconstriction in forearm resistance vessels in man. The absence of an effect of AII on the response to infused NA suggests that AII facilitates sympathetic neurotransmission by a presynaptic action, probably through increased NA release. The results of these studies, taken together with studies in veins (Benjamin et al, 1988), suggest that presynaptic facilitation of sympathetic neurotransmission represents an important physiological action of AII.

13.12 The effect of local angiotensin converting enzyme inhibition on the forearm blood flow response to lower body negative pressure (chapter twelve (a)).

and

The effect of systemic angiotensin converting enzyme inhibition and reinfusion of angiotensin II on the forearm blood flow response to lower body negative pressure (chapter twelve (b)).

In the first of these two studies evidence was sought for an effect of endogenous AII generation in the forearm vasculature on sympathetic

neurotransmission. Assuming a resting blood flow in the forearm of 40-50 mls/min as before, infusion of enalaprilat (5 mcg/min) into the forearm circulation should produce local concentrations of ACE inhibitor 2-3 times the peak levels found in venous blood following oral administration of enalapril 10mg (Biollaz et al, 1982). This dose of enalaprilat, when infused into the forearm, has been shown previously (Benjamin et al, 1989) to abolish the response to intra-arterial infusion of constrictor doses of angiotensin-I, and markedly enhance the response to dilator doses of bradykinin. This confirms that enalaprilat causes effective local blockade of angiotensin converting enzyme and blockade of local generation of AII. In addition this dose of enalaprilat has no effect on resting blood flow when infused alone. These results imply that local AII and bradykinin metabolism by ACE does not contribute to the maintainance of resting forearm vascular resistance. The kinetics of the response are consistent with an action on endothelially located, rather than plasma ACE (Benjamin et al, 1989). In the present experiment this dose, and a higher dose of enalaprilat had no effect on resting forearm blood flow, or sympathetic vasoconstriction with LBNP. The results suggest that, overall, local angiotensin and bradykinin metabolism does not contribute to the maintainance of forearm vascular resistance in these circumstances.

In this study therefore a major effect for locally generated AII has been excluded and in the second of these two studies, in which subjects were pretreated by oral enalapril, the effects of circulating AII were examined. The dose of enalapril used (20 mg twice daily for two days) produces maximal inhibition of converting enzyme and maximal reduction of plasma AII (Davies et al, 1984). The main difference between the approach in the two studies is therefore that with local forearm administration of enalaprilat (the active form of the prodrug enalapril)

local forearm vascular ACE activity is blocked and local effects are examined but circulating AII is unaltered. In contrast, with systemic ACE inhibition, tissue, serum and vascular ACE activity is blocked throughout the body and there is a major reduction in circulating AII.

During systemic ACE inhibition there was a small but significant reduction in systolic and diastolic BP and heart rate (Table 12(b).1) which has been shown to occur in normotensive subjects in other studies. LBNP caused no significant change in BP or heart rate and the forearm blood flow responses to LBNP during systemic ACE inhibition were similar to those seen earlier in the untreated group (chapter 11 and table 12(b).1). These findings compare with those of others who have shown that there was an enhanced response to LBNP (40 mmHg negative pressure) during systemic ACE inhibition in normal subjects (Rasmussen et al, 1986) and with the findings of Mancia et al (1988) who showed an attenuation of the response to LBNP in hypertensive subjects treated with an ACE inhibitor.

Infusion of AII during systemic ACE inhibition caused a significant dose dependent fall in resting forearm blood flow. Indeed, there was a significant reduction in flow at both the same dose of AII (320 fmol/min) and a lower dose (160 fmol/min) as that which had no effect on resting forearm blood flow in subjects who were not treated with systemic ACE inhibition (chapter eleven). Reduction in circulating AII may therefore increase sensitivity to infused AII possibly by altering receptor availability or numbers (Mendelsohn, 1985).

During AII infusion LBNP had no overall effect on reductions in forearm blood flow. However, at a dose of AII 320 fmol/min the response to LBNP was significantly enhanced ($p<0.05$). This dose was chosen to produce plasma AII levels that approximate to normal physiological levels, replacing the circulating AII withdrawn by systemic ACE inhibition. The

finding that AII enhances the response to LBNP in this study further supports that circulating AII facilitates sympathetic neurotransmission.

13.13 The effect of saralasin on the forearm blood flow response to lower body negative pressure in sodium replete subjects (chapter twelve (c)).

and

The effect of saralasin on the forearm blood flow response to lower body negative pressure in sodium deplete subjects (chapter twelve (d)).

These studies sought to investigate whether circulating AII plays a role in peripheral sympathetic neurotransmission by examining the effect of blockade of AII by saralasin infusion into the brachial artery at rest and during stimulation with LBNP in both sodium replete and sodium deplete subjects. An initial review of the effects of saralasin is necessary before discussing the present studies.

Saralasin: A variety of specific peptide competitive antagonists of AII have been described. All are analogues of AII with modifications in the 8 position with or without other position modification (Turker et al, 1974; Marshall, 1976; Oparil, 1983). The substitution at position 8 with an aliphatic side chain leads to a conformational change that impairs activation of the AII receptor. Substitution at the 1 position with amino acids resistant to aminopeptidase cleavage leads to increased duration of antagonism by blocking degradation. Sarcosine substitution at position 1 also increases binding affinity to the receptor site. The product with combined substitution of sarcosine in position 1 and alanine in position 8, saralasin, ([Sar^1-Val^5Ala^8]-AII), is the most extensively used competitive antagonist of AII.

Actions of saralasin in vitro: Early experiments in vitro demonstrated that these peptide analogues antagonised the effects of AII in a wide

variety of species and preparations (Pals et al, 1971a; Turker et al, 1971; Yamamoto et al, 1972). The antagonism is specific for AII in that the responses to a variety of pressor agents including noradrenaline, tyramine, phenylephrine and vasopressin were unaffected (Pals et al, 1971b; Yamamoto et al, 1972). However antagonism occurred only in situations where blood pressure was maintained by AII, either by infusion of AII, the acute phase of unilateral renal hypertension, dehydration or in pithed rats, but did not occur in normotensive rats, spontaneously hypertensive rats and mineralocorticoid hypertensive rats (Pals et al, 1971a; Needleman et al, 1976). In addition the analogues exhibit some intrinsic agonist properties under certain conditions (Turker et al, 1974; Needleman et al, 1976). These analogues are therefore specific competitive antagonists of AII with a variable degree of partial agonist activity.

Actions of saralasin in man: Saralasin also antagonises the actions of AII in normal man during AII infusion, salt deplete man and in patients with "high renin" hypertension (Streeton et al, 1975; Case et al, 1976; Hollenberg et al, 1976; Fagard et al, 1981). However in circumstances in which plasma AII is normal or low, saralasin infusion causes a mild pressor response and aldosterone release (Hollenberg et al, 1976; Fagard et al, 1981). In man therefore saralasin has proven to be a potent antagonist of AII, but also to exhibit some agonist activity particularly when plasma AII concentrations are low (Case et al, 1976). This latter action is a limitation in studies of specific AII blockade. It is possible that in the future specific antagonists without agonist action may be more reliable. Recently such non-peptide antagonists have been described (Wong et al, 1989).

Interpretation of the present studies: The expected findings if AII exerts a tonic role in peripheral sympathetic neurotransmission, and that this is blocked by saralasin acting as antagonist, are twofold. Firstly

resting blood flow may be increased by the removal of direct and indirect action of AII, and secondly the reduction in forearm blood flow in response to sympathetic stimulation with LBNP would be diminished compared with control. The main finding of the first study in salt replete subjects was that saralasin caused a dose dependent fall in forearm blood flow. This suggests that saralasin was acting as an agonist at these doses in salt replete subjects. The main finding of the second study was that whilst resting forearm blood flow was not affected by saralasin infusion in salt deplete subjects, there was significant enhancement of the reduction in forearm blood flow with LBNP. This also suggests that saralasin is acting as agonist in these conditions. The results of these studies are therefore inconclusive, but the second finding is consistent with AII agonism facilitating sympathetic neurotransmission.

13.14 Methodological lessons learnt during the course of these studies for future investigations.

1. **Plasma noradrenaline.** The limitations of measuring venous plasma NA as an index of sympathetic activity have been discussed earlier (section 13.8 and 13.10). I would anticipate that measurement of arterial and venous NA across a more limited vascular circuit would have a greater sensitivity to demonstrate small differences and interactions. Examples of such vascular circuits are the heart with arterial and coronary sinus sampling, renal arterial and venous sampling, arterial and venous sampling in the ipsilateral forearm and comparison of infused and contralateral non-infused venous NA samples during intra-arterial infusion in one arm. There are ethical restrictions to such studies.

2. **Haemodynamic measurements.** The studies described in this thesis have relied upon non-invasive assessments of systemic haemodynamics including blood pressure, cardiac output and forearm blood flow. However the studies suggest that the measurement of stroke volume, cardiac output and central venous pressure are preferable.

3. **Regional haemodynamic measurements.** Regional haemodynamic assessments would be a valuable extension of these studies, including venous return, renal artery flow, liver blood flow and capillary blood flow. Ethical constaints are likely to restrict such studies to ultrasound and echocardiographic techniques with inherent limitations.

4. **Pharmacological blockade of the RAS.** The limitations of using ACE inhibitors in these studies has been discussed in section 17.11. The value of saralasin as an investigatory tool was examined in two studies (chapter 13) with inconclusive results, most likely due to partial agonist activity. However the recently described specific nonpeptide AII blockers will be exciting tools for use in future studies of RAS/SNS interactions in man (Wong et al, 1990; Timmermans et al, 1991).

CHAPTER FOURTEEN

CHAPTER FOURTEEN

Conclusion

This thesis has set out to systematically investigate the interaction between the RAS and SNS in healthy man. There is a limited amount of human data investigating this interaction directly with which to compare the results of these studies. Earlier studies in the literature infused AII in doses of 1-10 ng/kg/min and showed no changes in venous plasma NA (Beretta-Piccoli et al, 1980; Mendelsohn et al, 1980; Nicholls et al, 1981). They are interpreted as providing evidence against an AII/SNS interaction. Our results confirm a lack of effect of AII on resting plasma NA. We also showed no effect of AII infusion in responses to a wide range of stimuli known to activate the SNS and cause NA release. These stimuli include the cold pressor test, forearm isometric handgrip, standing from lying, bicycle exercise and tyramine infusion. These findings have also been confirmed by recent studies of subpressor and pressor infusions of AII both at rest and during baroreceptor loading and unloading by head-down and head-up tilt respectively (Goldsmith and Hasking, 1990; Goldsmith and Hasking, 1991). In addition to haemodynamic recordings and plasma NA levels, NA spillover was measured and the effects of AII were compared with equipressor doses of phenylephrine. Whilst there was no difference in plasma NA and NA spillover with either infusion compared with control, a significant bradycardia was seen with phenylephrine, which was not seen with AII infusion. They conclude that AII may inhibit the efferent response to baroreceptor loading in humans (Goldsmith and Hasking, 1991). This finding has been seen in both animals and man (Lumbers et al, 1979; Mace et al, 1985). The evidence from this type of investigation seeking a presynaptic AII/SNS interaction using systemic intravenous infusion in man has not revealed such an interaction.

This conclusion however contrasts with our findings in the human forearm vasculature in vivo. AII caused a marked enhancement of reduction in forearm blood flow in response to sympathetic stimulation with LBNP. We have also shown in the same circulation that AII does not alter the responses to infused NA. Our findings suggest that within the "isolated" human forearm vasculature presynaptic facilitation of sympathetic neurotransmission occurs. These findings await confirmation by measurement of the arteriovenous difference in plasma NA across the forearm circulation and NA spillover studies in the forearm. The technical difficulty of performing this experiment has not yet been overcome. Compelling evidence is also found in the venous circulation of the hand in man. Benjamin et al (1988) show that doses of AII that do not cause venoconstriction alone cause dramatic enhancement of venoconstriction caused by the deep breath reflex. The same dose of AII had no effect on the venoconstriction caused by infused NA. These findings suggest that AII augments sympathetically induced venoconstriction via a presynaptic mechanism.

Also in contrast is that several studies in man now support a postsynaptic interaction. In our first experiment we demonstrated that during combined AII and NA infusion there was a significant interaction with regard to rise in systolic blood pressure but not diastolic blood pressure. In a similar and simultaneous experiment Reams and Bauer (1987) observed the same effect. They demonstrated that during a subpressor infusion of AII the dose of NA and the plasma NA level required to cause small changes in blood pressure were significantly lower than during control infusion. We also showed that AII augments the stroke volume response to ISO infusion in man, suggesting a postsynaptic AII/beta-agonist interaction. The major possibilities are that during ISO infusion AII causes a relocation

of blood from the splanchnic circulation to the heart or that AII augments myocardial contractility.

There are a number of conclusions that may be drawn. Firstly an AII/SNS interaction has been clearly and directly demonstrated in man. This interaction occurs at low doses of AII that may be of physiological and pathophysiological importance. The most compelling evidence for this interaction comes from studies of the human forearm vasculature which support a presynaptic AII/SNS interaction. The problems demonstrating a more generalised presynaptic interaction with systemic intravenous infusion are likely to be due to the gamut of cardiovascular reflexes tending to obscure changes. In addition plasma NA is a crude index of SNS activity with limited power in small studies to demonstrate an interaction. NA spillover used by others has similar limitations, though takes account of changes in NA clearance. The studies in this thesis suggest that AII increases the presynaptic release of NA but this is not of sufficient magnitude to spill over into plasma, altering plasma NA. With regard to the effect of NA after it has been released (postsynaptic effect), AII does not influence the effects of NA on peripheral resistance vessels, but at a systemic level the intravenous infusion experiments show that AII synergistically augments the rise in systolic blood pressure seen with NA and increases the effect of beta-agonism on stroke volume. The exact location and mechanism of this interaction is not confirmed, but the most likely mechanism is a redistribution of blood from the splanchnic circulation to the heart.

My final paragraphs propose a general hypothesis. I suggest that both presynaptic and postsynaptic interactions occur but that they do not necessarily occur at the same location in the circulation. Thus in the peripheral resistance vessels a presynaptic interaction occurs allowing rapid

response to changes in RAS activity and thus increased vascular response to a given amount of sympathetic outflow. In contrast within the heart a postsynaptic interaction occurs whereby the heart, in addition to responding to all relevant reflex changes, is also responsive to the circulating level of NA, acting in a role as a circulating hormone. The full five litres of cardiac output must pass through the heart which is therefore in an ideal situation in the circulation to be exposed to and to respond to circulating hormone or infused inotrope. On the other hand the peripheral vasculature has an enormous surface area at the level of arteriolar resistance vessels and large amounts of cardiac output is diverted to major organs. The peripheral vasculature is under the influence of basal sympathetic tone and a variety of cardiovascular reflexes alter sympathetic outflow. A presynaptic interaction in the peripheral vasculature would therefore exert a more responsive influence.

What evidence is there to support this hypothesis? Firstly the evidence in humans and animals clearly demonstrates that when an isolated circulation is examined a potent interaction is revealed. Our studies in the human forearm suggest that a presynaptic interaction occurs. However we have not been able to fully rule out a postsynaptic interaction. By contrast studies in isolated vascular circuits of animals such as the dog hind limb, paw and a variety of species mesenteric circulation, demonstrate beyond doubt that a presynaptic interaction occurs. An increase in NA released per nerve impulse has been demonstrated by radiolabelling studies in addition to perfusate effluent studies. It is possible that species differences occur. However our studies suggest that in the "isolated" vasculature of the human forearm a similar interaction occurs.

Under what circumstances does the human heart demonstrate response to circulating hormones? The only relevant "isolated" heart studies clearly

demonstrating such a response have been performed in patients after cardiac transplantation or heart-lung transplantation. In such patients the heart is denervated, evidenced by resting tachycardia due to an absence of vagal tone. Investigation has shown that their heart rate and cardiac output response to dynamic exercise is sluggish and is heavily dependent on circulating catecholamines as are responses to other stimuli (Stinson et al, 1972; Banner et al, 1990).

What evidence is there for a postsynaptic interaction between AII and the SNS at the myocardial or intrathoracic level? As discussed above a number of human studies support a postsynaptic interaction. However these have been performed by studying the effect of intravenous infusion of AII altering total systemic levels. The effects of AII infusion on physiological or pharmacological alteration of the SNS by stimuli such as LBNP, head-down tilt and head-up tilt, and infusion of NA or other agonist have been studied. It is not possible from these studies to separate the peripheral vasculature from its reflex effects on cardiac performance, nor a myocardial or intrathoracic effect in isolation from the peripheral vasculature. I have argued that the study with ISO and AII combined (chapter seven), by matching afterload, "removes" the peripheral resistance vessels from consideration and comes closest to achieving separation. The interaction in augmenting stroke volume may have occurred within the splanchnic circulation or venous capacitance vessels rather than the myocardium.

There are a number of exciting possibilities to be explored within this area of research and following on from this thesis. One area of rapid growth and interest is the new opportunities opened by the discovery of specific nonpeptide antagonists of AII and the demonstration of two types of AII receptor (Wong et al, 1990; Timmermans et al, 1991).

SECTION FOUR: REFERENCES

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ABSTRACTS / PRESENTATIONS TO LEARNED SOCIETIES:

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