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The effect of angiotensin II on endogenous noradrenaline release in man

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1 Considerable data from animal studies suggest that angiotensin II exerts a facilitatory effect on noradrenaline release. We sought evidence for such an effect in man by examining how a subpressor dose of angiotensin II (1.5 ng kg^{-1} min^{-1}) influences the haemodynamic and plasma noradrenaline responses to physiological stimulation of the sympathetic nervous system.

2 The physiological stimuli investigated were a cold pressor test, the response to standing from lying, bicycle exercise and forearm isometric exercise.

3 The presence of the angiontensin II infusion had no effect on the systolic blood pressure, diastolic blood pressure, heart rate or plasma noradrenaline responses to stimulation of the sympathetic nervous system.

4 We have therefore found no evidence to support the enhancement of noradrenaline release by this low dose of angiotensin II in man.

Keywords angiotensin II sympathetic nervous system noradrenaline

Introduction

The renin-angiotensin-system (RAS) and sympathetic nervous system (SNS) are involved in the regulation of blood pressure and their independent roles have been extensively investigated. Evidence from studies in animals suggests that there is a major interaction between the RAS and the SNS (Zimmerman *et al.*, 1984). In these studies either isolated organ or intact animal models have shown that exogenous angiotensin (AII) can influence sympathetic nervous transmission in several different ways. Firstly Knape (1985) has shown in pithed normotensive rats that AII has a tyramine-like effect, releasing endogenous NA from neuronal stores. Secondly, Zimmerman & Whitmore (1967) have shown that AII facilitates sympathetic neurotransmission in vascular smooth muscle by enhancement of the amount of noradrenaline released per nerve impulse. The above suggests that AII facilitates presynaptic release of NA but Hatton & Clough (1982) have also suggested that AII facilitates the effect of released NA by a postsynaptic mechanism.

In man we have previously shown that there is a postsynaptic AII/NA interaction in that AII synergistically augments the systolic blood pressure response to NA (Struthers *et al.*, 1987). 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.

Methods

Nine normotensive male volunteers were studied. Their mean age was 26 years (range 19–38 years) and mean weight 80 kg (range 67–109kg). Each subject gave written informed consent to the study which had been approved by the Medical and Dental research ethical committee of the University of Dundee.

No subject was on any regular medication and all were instructed not to take any medication for 7 days prior to each study day. They were

also instructed to abstain from alcohol and smoking for 24 h prior to each study day and to take no caffeine containing drinks from 22.00 h the day before each study day. They were studied on three occasions, the first a pre-study assessment as described below and 2 formal study days at least 1 week apart. Sodium intake was not strictly controlled but each subject was asked to maintain the same approximate sodium intake for 3 days before each study day which was assessed by 24 h urinary sodium excretion and baseline plasma AII levels. The subjects attended 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 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 watts and the workload was then increased at 1 min intervals until a heart rate of 130–140 beats min^{-1} was achieved as recorded by oscillometer (Hewlett Packard). After a period of 15 min 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 min supine rest, baseline recordings of heart rate and blood pressure and blood samples were taken.

Infusion was then commenced with either a subpressor dose of angiotensin II (1.5 ng kg^{-1} min^{-1} Ciba Geigy) or placebo (5% dextrose) in a randomised single-blind fashion. After a further 15 min supine rest, blood pressure and heart rate recordings and blood sampling were repeated. Thereafter each subject underwent a series of standard tests to stimulate the sympathetic nervous system. These tests were always administered in the same order as described below.

Cold pressor test

The subject immersed one foot to the ankle in melting ice for 2 min. Blood pressure and heart rate were recorded at 0, 1 and 2 min and a blood sample taken at 2 min. The subject then rested in the supine position for a further 15 min during which blood pressure and heart rate were measured every 5 min 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 2 min standing erect.

Bicycle exercise

The subject then sat on a bicycle ergometer and, after baseline haemodynamic recordings, performed 5 min exercise at a workload predetermined in each individual at the pre-study assessment to cause a rise in heart rate to 130–140 beats min^{-1}. The same workload was used on both study days. Blood pressure and heart rate were recorded at 2 and 5 min of exercise and 1, 2 and 3 min after exercise. Blood samples were taken during the fifth minute of exercise.

Forearm isometric exercise

Following a further rest period of 15 min, baseline haemodynamic recordings and blood samples were taken. The subject then undertook 3 min 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 1 min intervals during handgrip and blood samples taken after 3 min.

The electrocardiograph and heart rate were monitored throughout the study on an oscilloscope and blood pressure recorded semi-automatically (Dinamap-Vital signs monitor 1846, Critkon).

Blood samples for noradrenaline were kept on ice until centrifuged at 4°C. Plasma was stored at −80°C until assayed. Noradrenaline was measured by our double-isotope enzymatic technique (Brown & Jenner, 1981). Plasma AII was measured by Sep-pak C_{18} cartridge extraction of plasma followed by radioimmunoassay by the method of and in the laboratory of Morton & Webb (1985).

Angiotensin II (Hypertensin, Ciba-Geigy, Switzerland) solutions, 500 ng ml^{-1} in 5% dextrose, were prepared immediately before use and infused at a rate of 1.5 ng kg^{-1} min^{-1}.

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 1 shows the initial recordings of blood pressure, heart rate and plasma NA before and 15 min after the start of an AII (1.5 ng kg^{-1} min^{-1}) or a placebo infusion. There were no changes in any of these observations in response to AII.

The haemodynamic and plasma NA responses to the 2 min cold pressor testing, after 2 min standng from lying, at 5 min bicycle exercise and at 3 min isometric handgrip are shown in Figure 1. 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 combined data from both study days in order to compare the results of each physiological stimulus. The results of this analysis of haemodynamic and plasma NA responses are shown in Table 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

Table 1 Systolic and diastolic pressure, heart rate and plasma noradrenaline before and 15 min after angiotensin II (1.5 ng kg^{-1} min^{-1}) or placebo infusion. Results are expressed as mean ± s.e. mean (n = 9)

	SBP (mmHg)		*DPB (mmHg)*		*HR (beats min^{-1})*		*NA (ng l^{-1})*	
	Placebo	*AII*	*Placebo*	*AII*	*Placebo*	*AII*	*Placebo*	*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 ± 3	63 ± 4	510 ± 50	480 ± 20

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

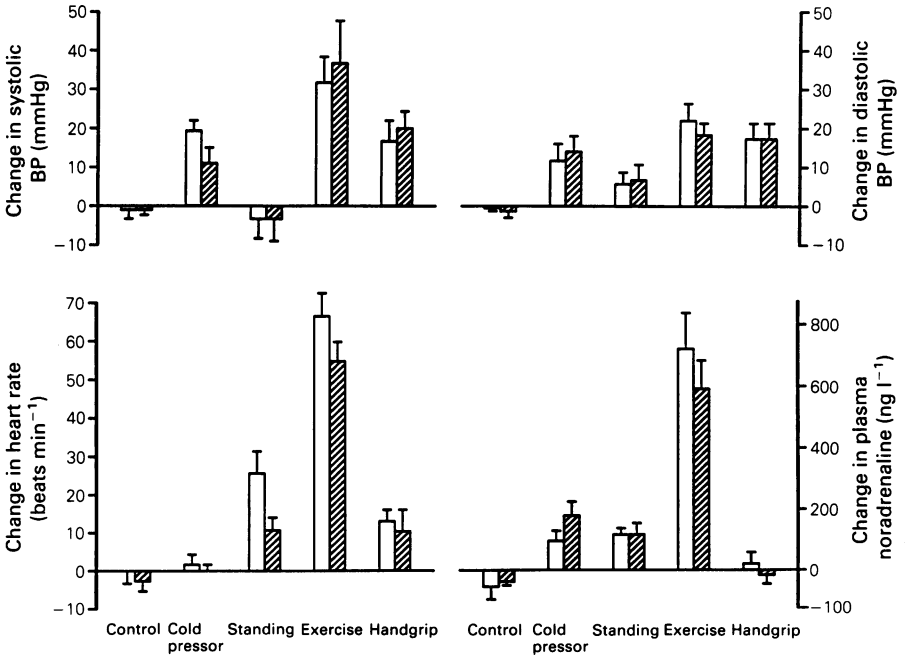

Figure 1 Haemodynamic and plasma noradrenaline responses (mean ± s.e. mean) to physiological stimuli in the presence of angiotensin II infusion (▨) or dextrose infusion (□).

Table 2 Haemodynamic and plasma noradrenaline response to physiological stimuli. (Mean data only are displayed for clarity. This table combines data from the AII pretreatment and placebo pretreatment days as they were not significantly different from each other)

	SBP (mmHg)	*DBP (mmHg)*	*HR (beats min^{-1})*	*NA (ng l^{-1})*
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 is significantly different between placebo and angiotensin II. * $P < 0.05$ and $^{+}P < 0.01$ represent significant differences from control values as obtained by the Duncan procedure.

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. mean 24 h urinary sodium excretion was 174 ± 16 (n = 8) prior to the placebo study day and 201 ± 20 (n = 7) prior to the AII infusion study day, which was not significantly different by paired t-testing. The plasma AII levels (Table 3) show that there was no difference between resting levels on the two study days and no change during placebo infusion but there was the expected 312% rise in plasma AII levels during the AII infusion.

Discussion

It is worth noting 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 angiotensin converting enzyme (ACE) inhibitors to normal volunteers and have hence investigated the differential effects of normal and low levels of RAS activity. The results of such studies have been conflicting. Treatment with ACE inhibitors has been shown to produce either no change in resting plasma NA (Niarchos *et al.*, 1982; Millar *et al.*, 1982; Ajayi *et al.*, 1985) or an increase in resting plasma NA (MacGregor *et al.*, 1981; Ibsen *et al.*, 1983). Pretreatment with ACE inhibitors had no effect on the plasma NA responses to stimulation of the SNS (Millar *et al.*, 1982; Ibsen *et al.*, 1983; Campbell *et al.*, 1985; Becker *et al.*, 1986).

There is a second problem with the use of ACE inhibitors as experimental agents in this regard. ACE inhibitors not only lower AII levels but also cause major changes in vasodilatory prostaglandins, bradykinin and possibly other peptides (Tree & Morton, 1981; Campbell *et al.*, 1985). These other effects are a major complication which make ACE inhibitors imperfect tools with which to investigate the effects of AII.

In the present study we used a different approach and sought to increase 'RAS' activity by infusion of AII itself. Other workers have infused pressor doses of AII into man and found that plasma NA does not change (Beretta-Piccoli *et al.*, 1980; Mendelsohn *et al.*, 1980; Nicholls *et al.*, 1981). This has been interpreted as evidence against AII facilitating NA release. However an alternative explanation could be the exact opposite of this. In other words, when a pressor dose of AII is infused, the baroreflex is activated and a reduction in NA release might be expected. The lack of a fall in plasma NA after AII infusion

Table 3 Plasma angiotensin II levels before and after 15 min of angiotensin II (1.5 ng kg^{-1} min^{-1}) or placebo infusion

	Placebo (pg ml^{-1})	*Angiotensin II (pg ml^{-1})*	
Before	9.9 ± 1.9	10.3 ± 1.7	$^{+}$NS
After	9.8 ± 1.5	32.1 ± 5.0	$^{+}P < 0.0025$

$^{+}$Paired t-test

could be interpreted as indirect evidence for AII facilitating NA release.

In this study, when we were choosing an AII infusion rate to use, we were aware of two opposing considerations. 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^{-1} min^{-1}. This dose was certainly low enough to bring about no haemodynamic effects (Table 1) but 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. 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 the same as that in our earlier study in which we showed a significant postsynaptic AII/NA interaction with regard to systolic blood pressure (Struthers *et al.*, 1987). 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^{-1}) in normal volunteers and the rise in plasma angiotensin II is similar to that found during low sodium intake diets (9–12 mmol day^{-1}) (Oelkers *et al.*, 1974). Indeed our AII samples 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 noradrenaline 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. 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. 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 directly intrasynaptic NA in man. The fact that there were no significant haemodynamic effects in this study does give us some further confidence that this dose of AII does indeed have no facilitatory effect on intrasynaptic NA release in man.

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.

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References

Ajayi, A. A., Campbell, B. C., Howie, C. A. & Reid, J. L. (1985). Acute and chronic effects of the converting enzyme inhibitors enalapril and lisinopril on reflex control of heart rate in normotensive man. *J. Hypertension*, **3**, 47–53.

Becker, R. H. A., Struthers, A. D. & Brown, M. J. (1986). Effect of captopril on changes in plasma noradrenaline induced by sodium nitroprusside. *Br. J. clin. Pharmac.*, **22**, 409–413.

Beretta-Piccoli, C., Weidmann, P., Keusch, G., Gluck, Z., Grimm, M., Meier, A. & Minder, I. (1980). Responsiveness of circulating catecholamines, renin and aldosterone to angiotensin II. *Mineral Electrolyte Metab.* **4**, 137–148.

Brown, M. J. & Jenner, D. A. (1981). Novel double-isotope technique for enzymatic assay of catecholamines, permitting high precision, sensitivity and plasma sample capacity. *Clin. Sci.*, **61**, 591–598.

Brown, M. J., Lhoste, F. J. M., Zamboulis, C., Ind, P. W., Jenner, D. A. & Dollery, C. T. (1982). Estimation of sympathetic activity in essential hypertension. *Clin. Pharmac. Ther.*, **31**, 16–22.

Campbell, B. C., Sturani, A. & Reid, J. L. (1985). Evidence of parasympathetic activity of the angiotensin converting enzyme inhibitor, captopril, in normotensive man. *Clin. Sci.*, **68**, 49–56.

Flammer, J., Weidmann P., Gluck, Z., Ziegler, W. H. & Reubi, F. C. (1979). Cardiovascular and endocrine profile of adrenergic neurone blockade in normal and hypertensive man. *Am. J. Med.*, **66**, 34–42.

Goldstein, D. S., McCarty, R., Polinsky, R. J. &

Kopin, I. J. (1983). Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension*, **5**, 552–559.

Hatton, R. & Clough, D. P. (1982). Captopril interferes with neurogenic vasoconstriction in the pithed rat by angiotensin dependent mechanisms. *J. cardiovasc. Pharmac.*, **4**, 116–123.

Ibsen, H., Egan, B., Osterziel, K., Vander, A. & Julius, S. (1983). Reflex-hemodynamic adjustments and baroreflex sensitivity during converting enzyme inhibition with MK-421 in normal humans. *Hypertension*, **5** (Suppl. I), I–184–I–191.

Knape, J. T. A. (1985). Cardiovascular actions of angiotensin II in the pithed normotensive rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **329**, R248.

Lake, C. R., Ziegler, M. G. & Kopin I. J. (1976). Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. *Life Sci.*, **18**, 1315–1326.

MacGregor, G. A., Markandu, N. D., Roulston, J. E. & Jones, J. C. (1981). Maintenance of blood pressure by the renin-angiotensin system in man. *Nature*, **291**, 329–331.

Mendelsohn, F. A. O., Doyle, A. E. & Gray, G. W. (1980). Lack of response of sympathetic nervous system to angiotensin infusion. *Lancet*, **i**, 492–493.

Millar, J. A., Derkx, F. H. M., McLean, K. & Reid, J. L. (1982). Pharmacodynamics of converting enzyme inhibition: the cardiovascular, endocrine and autonomic effects of MK421 (enalapril) and MK521. *Br. J. clin. Pharmac.*, **14**, 347–355.

Morton, J. J. & Webb, D. J. (1985). Measurement of plasma angiotensin II. *Clin. Sci.*, **68**, 483–484.

Niarchos, A. P., Pickering, T. G., Morganti, A. & Laragh, J. H. (1982). Plasma catecholamines and cardiovascular responses during converting enzyme inhibition in normotensive and hypertensive man. *Clin. exp. Hypertension*, A4 (4 & 5), 761–789.

Nicholls, M. G., Espiner, E. A., Miles, K. D., Zweifler, A. J. & Julius, S. (1981). Evidence against an interaction of angiotensin II with the sympathetic nervous system in man. *Clin. Endocrinol.*, **15**, 423–430.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrunner, K. & Bent, D. H. (1975). *SPSS: statistical package for the social sciences*. 2nd ed. New York: McGraw-Hill.

Nielsen, S. L., Christensen, N .J., Olsen, N. & Lassen, N. A. (1980). Raynaud's phenomenon: peripheral catecholamine concentration and effect of sympathectomy. *Acta Chir. Scand.*, **502**, 57–62.

Oelkers, W., Brown, J. J., Fraser, R., Lever, A. F., Morton, J. J. & Robertson, J. I. S. (1974). Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. *Circ.Res.*, **34**, 69–77.

Robertson, D., Johnson, G. A., Robertson, R. M., Nies, A. S., Shand, D. G., & Oates, J. A. (1979). Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation*, **59**, 637–643.

Struthers, A. D., Burrin, J. M. & Brown, M. J. (1986). Exercise-induced increases in plasma catecholamines and growth hormone are augmented by selective α_2-adrenoceptor blockade in man. *Neuroendocrinology*, **44**, 22–28.

Struthers, A. D., Pai, S., Seidelin, P. H., Coutie, W. J. R. & Morton, J. J. (1987). Evidence for a postsynaptic interaction between noradrenaline and angiotensin II with regard to systolic but not diastolic blood pressure. *J. Hypertension*, (in press).

Tree, M. & Morton, J. J. (1980). Evidence that the acute hypotensive effect of captopril in dogs is not wholly explained by a reduction of plasma angiotensin II and its direct vasoconstrictor effect. *Clin. Sci.*, **59**, 451–456.

Wallin, B. G., Sundlof, G., Eriksson, B-M., Dominiak, P., Grobecker, H. & Linblad, L. E. (1981). Plasma noradrenaline correlates to sympathetic muscle nerve activity in normotensive man. *Acta Physiol. Scand.*, **111**, 69–73.

Wallin, B. G., Morlin, C. & Hjemdahl, P. (1987). Muscle sympathetic activity and venous plasma noradrenaline concentrations during static exercise in normotensive and hypertensive subjects. *Acta Physiol. Scand.*, **129**, 489–497.

Watson, R. D. S., Hamilton, C. A., Jones, D. H., Reid, J. L., Stallard, T. J. & Littler, W. A. (1980). Sequential changes in plasma noradrenaline during bicycle exercise. *Clin. Sci.*, **58**, 37–43.

Zimmerman, B. G. & Whitmore, L. (1967). Effect of angiotensin and phenoxybenzamine on release of norepinephrine in vessels during sympathetic nerve stimulation. *Int. J. Neuropharmac.*, **6**, 27–38.

Zimmerman, B. G., Sybertz, E. J. & Wong, P. C. (1984). Interaction between sympathetic and renin-angiotensin system. *J. Hypertension*, **2**, 581–587.

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