Br. J. Pharmacol. (1993), **110**, 159–162

Regional haemodynamic effects of angiotensin II (3-8) in conscious rats

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1 It has been reported that angiotensin II (AII) (3-8) causes endothelium-dependent renal cortical vasodilatation, in anaesthetized rats, through interaction with a novel receptor that shows no affinity for the AT_1-receptor antagonist, losartan. Therefore in order to get a fuller profile of the regional haemodynamic effects of AII (3-8) in conscious rats we assessed its renal, mesenteric and hindquarters vascular effects, and compared them to the responses elicited by AII and AIII.

2 AII and AIII (1.25, 12.5 and 125 pmol kg^{-1}) caused dose-dependent pressor and renal and mesenteric vasoconstrictor effects. At doses up to 125 pmol kg^{-1}, AII (3-8) was without any cardiovascular effects, but with doses of 1.25 and 12.5 nmol kg^{-1} there were dose-dependent increases in mean arterial blood pressure and reductions in renal and mesenteric flows and vascular conductances. The responses to AII (3-8) (12.5 nmol kg^{-1}) were abolished by losartan (20 μmol kg^{-1}).

3 Since it has been found that pretreatment with L-arginine can reveal a vasodilator effect of AII (3-8) on rabbit pial arterioles, we assessed responses to AII (3-8) (12.5 nmol kg^{-1}) before and 5 min after onset of a primed infusion of L-arginine (1.4 mmol kg^{-1} bolus, 1.4 mmol kg^{-1} h^{-1} infusion). Responses to AII (3-8) were unchanged by L-arginine.

4 The results are consistent with AII (3-8) being a less effective agonist than AII (or AIII) at the AT_1-receptor, but provide no evidence for AII (3-8) interacting with a novel receptor that shows no affinity for losartan.

Keywords: Angiotensin II; angiotensin II (3-8); losartan; L-arginine; haemodynamics

Introduction

Recently, Swanson *et al.* (1992) described a specific binding site for angiotensin II (AII) (3-8), distinct from the AT_1- or the AT_2- receptor. Swanson *et al.* (1992) found that AII (3-8) injected into the renal artery in anaesthetized rats caused an increase in superficial renal blood flow, as assessed by a laser Doppler flowmeter. Under the same conditions, Swanson *et al.* (1992) found that AII caused a reduction in renal cortical blood flow consistent with the potent renal vasoconstrictor effect of this peptide seen in conscious rats (Gardiner *et al.*, 1988). In support of their findings, Swanson *et al.* (1992) cited the work of Haberl *et al.* (1991), showing that AII (3-8) could cause cerebral vasodilatation. However, in the latter study, AII (3-8) only caused vasodilatation after L-arginine pretreatment, whereas AII and AIII both caused dilatation when applied topically to pial arterioles. Since the vasodilator effects of AII and AIII were inhibited by amastatin (which blocks enzymatic degradation of AII and AIII), Haberl *et al.* (1991) suggested that release of the AII degradation products, AII (3-8) and L-arginine, 'specifically produce endothelium-dependent dilation of cerebral resistance vessels.'

Against this background, we compared the effects of AII (3-8), AII and AIII on renal, mesenteric and hindquarters haemodynamics in conscious rats. In addition, we assessed the effects of pretreatment with L-arginine, or the AT_1-receptor-selective antagonist, losartan (Timmermans *et al.*, 1991), on responses to AII (3-8).

Methods

Male Long Evans rats (350–450 g), bred in our Animal Unit, were used in this study. Under sodium methohexitone anaesthesia (Brietal, Lilly; 60 mg kg^{-1}, i.p. supplemented as required), pulsed Doppler flow probes (Haywood *et al.*, 1991) were sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor hindquarters flow). Animals were given an i.m. injection of ampicillin (7 mg kg^{-1}) and were kept in individual home cages to recover for 7–14 days with free access to food (Biosure GLP grade 41B) and water. After that time, the animals were anaesthetized again (sodium methohexitone, 40 mg kg^{-1}, i.p.), and those with acceptable signals (signal: noise $>$20:1) from all 3 probes had a catheter implanted in the distal abdominal aorta (via the ventral caudal artery), and 3 separate catheters implanted in the right jugular vein. The catheters ran subcutaneously to emerge at the back of the neck, with the probe wires. These wires were soldered into a microconnector (Microtech Inc, Boothwyn, U.S.A.) that was clamped in a harness fitted to the rat. The harness was connected to a flexible spring through which the catheters were threaded for protection. The spring and all connections to the rat were supported by a counter-balanced lever system that allowed the animal free movement in its home cage to which it was returned, with free access to food and water, until experiments began at least 1 day after catheter implantation. The following experiments were performed:-

Responses to AII and AII (3-8)

The same animals ($n = 7$) were given increasing bolus doses of AII (1.25, 12.5 and 125 pmol kg^{-1}) or AII (3-8) (0.125, 1.25 and 12.5 nmol kg^{-1}) in random order, in the morning or afternoon of the same experimental day. Injections were separated by at least 20 min to allow variables to return to baseline. In pilot experiments, we found that AII (3-8) had no consistent effects over the range of doses used for AII, so we used the highest dose employed for AII (i.e., 125 pmol kg^{-1}) as the lowest dose of the range for AII (3-8).

Effects of L-arginine on responses to AII (3-8)

Since there is some evidence that L-arginine promotes the vasodilator effects of AII (3-8) applied topically to rabbit pial

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arterioles (Haberl et al., 1991), we assessed regional haemodynamic responses to AII (3-8) before, and 5 min after, the onset of a primed infusion of L-arginine (1.4 mmol kg^{-1} bolus, 1.4 mmol kg^{-1} h^{-1} infusion) in 3 of the animals studied above; this experiment was carried out on a separate day to that above.

Responses to AIII

In order to determine if responses to AII (3-8) resembled those to AIII more than those to AII, another three of the animals used in the first experiment also received increasing bolus doses (1.25, 12.5 and 125 pmol kg^{-1}) of AIII; this experiment was carried out on the same day as the first experiment, but at least 1 h before or after administration of AII or AII (3-8).

Responses to AII (3-8) before and after administration of the AT_1-receptor antagonist, losartan

Swanson et al. (1992) reported that the site which bound AII (3-8) showed no affinity for losartan, but they did not assess the effects of losartan on functional responses to AII (3-8). Therefore, in a separate group of rats ($n = 5$) we measured regional haemodynamic responses to AII (3-8) (12.5 nmol kg^{-1}) before, and 5 min after i.v. bolus injection of losartan (20 μmol kg^{-1}). Elsewhere we have shown this dose of losartan completely abolishes the AT_1-receptor-mediated haemodynamic effects of AII for several hours (Batin et al., 1991a,b).

Data analysis

Throughout an experiment, continuous recordings were made of instantaneous heart rate, mean and phasic arterial blood pressure, and mean and phasic Doppler shift signals with a modified (Gardiner et al., 1990) VF-1 pulsed Doppler flowmeter (Crystal Biotech, Hopkinton, U.S.A.). At time points representative of the profile of response to any particular intervention, variables were averaged over 20 s epochs. Changes relative to baseline were assessed by Friedman's test (Theodorsson-Norheim, 1987), applied to the areas under or over curves. Because the duration of responses to AII and AII (3-8) differed (see Results), areas were calculated over the 5 min period following AII and over the 2 min period after AII (3-8) administration. A P value <0.05 was taken as significant.

Drugs

AII (H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH), AIII (H_2N-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) and AII (3-8) (H_2N-Val-Tyr-Ile-His-Pro-Phe-OH), were obtained from Bachem (U.K.). Peptides were dissolved in sterile isotonic saline (154 mmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma). L-Arginine hydrochloride (Sigma) and losartan potassium (gift from Dr R. Smith, DuPont U.S.A.) were dissolved in isotonic saline. All injections were given in a volume of 100 μl and flushed in with 100 μl of saline. Administration of saline alone in these volumes had no consistent cardiovascular effects.

Results

AII caused dose-dependent pressor effects and reductions in renal and mesenteric flows and vascular conductances (Figure 1, Table 1). Only the highest dose of AII caused significant bradycardia and hindquarters vasoconstriction (Figure 1, Table 1).

AII (3-8) also caused dose-dependent pressor effects, but these were of shorter duration than those seen with AII (Figure 1, Table 1). Moreover, although with the doses chosen the peak pressor effects of AII and AII (3-8) were similar, the doses of the latter were 100 fold higher than those of AII (Figure 1, Table 1).

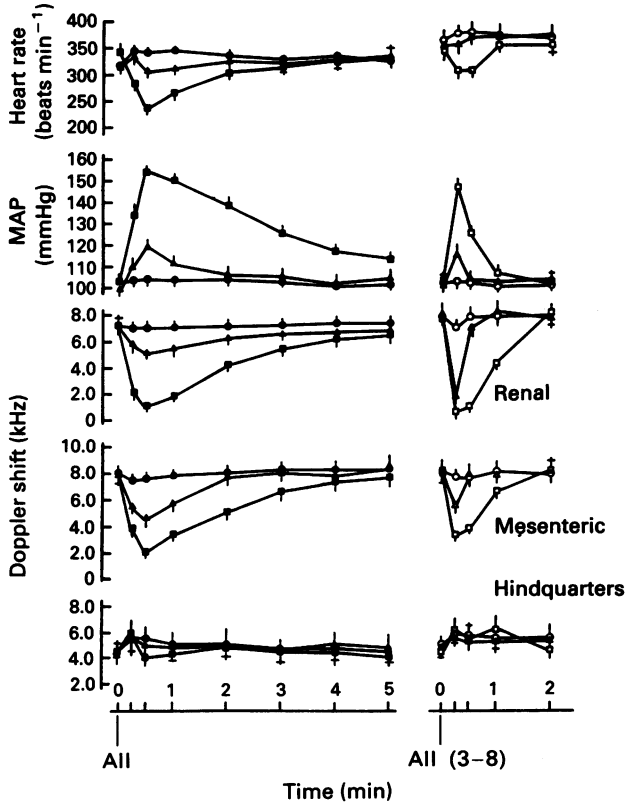

Figure 1 Cardiovascular responses to angiotensin II (● 1.25; ▲ 12.5; ■ 125 pmol kg^{-1}; left-hand panels) or angiotensin II (3-8) (○ 0.125; △ 1.25; □ 12.5 nmol kg^{-1}; right-hand panels) in the same conscious Long Evans rats ($n = 7$). Values are mean with s.e.mean. Statistics for the integrated changes are shown in Table 1.

The pressor effects of AII (3-8) were accompanied by dose-dependent reductions in renal and mesenteric flows and vascular conductances, but the dose-effect relation was particularly steep in the renal vascular bed (Figure 1, Table 1). As with AII, the changes in heart rate and hindquarters haemodynamics in response to AII (3-8) were variable (Figure 1, Table 1).

Pretreatment with L-arginine ($n = 3$) had no consistent effects on responses to AII (3-8) (12.5 nmol kg^{-1}) (Table 2). However, losartan abolished the effects of AII (3-8) (Figure 2).

Cardiovascular responses to AIII were very similar to those seen with AII, and over the same dose-range (Figure 3).

Discussion

The present work has provided no evidence for AII (3-8) acting as a vasodilator agent in the renal (Swanson et al., 1992) or in the mesenteric vascular bed. Although AII (3-8) was capable of causing an increase in hindquarters flow, this effect was not different from that seen with AII, and, as with the latter, was probably, in part, due to release of adrenal medullary adrenaline, causing activation of β_2-adrenoceptors in the hindquarters vascular bed (Gardiner et al., 1988). However, the effects of AII (3-8) (and of AII) on hindquarters haemodynamics and heart rate were less reproducible or prominent than its pressor and renal and mesenteric vasoconstrictor actions, which were abolished by the AT_1-receptor antagonist, losartan.

These results do not agree with those of Swanson et al. (1992), who considered that AII (3-8) interacted with 'an

Table 1 Integrated (areas under or over curves) cardiovascular responses to incremental bolus doses of angiotensin II (AII) or AII (3-8) in the same conscious, Long Evans rats ($n = 7$)

	AII (pmol kg^{-1})			*AII (3-8)* (nmol kg^{-1})		
	1.25	12.5	125	0.125	1.25	12.5
Δ Heart rate (beats)	66 ± 19*	68 ± 29	− 253 ± 47*	23 ± 7*	27 ± 13	− 43 ± 17*
Δ Mean arterial blood pressure (mmHg min)	3 ± 1	38 ± 5*	146 ± 15*	1 ± 1	8 ± 1*	23 ± 3*
Δ Renal flow (kHz min)	− 1.0 ± 0.2	− 5.4 ± 0.8*	− 15.7 ± 2.0*	− 0.6 ± 0.1	− 3.1 ± 0.3*	− 8.8 ± 0.6*
Δ Mesenteric flow (kHz min)	− 1.2 ± 0.5	− 5.2 ± 0.8*	− 12.9 ± 1.6*	− 0.5 ± 2	− 1.6 ± 0.2*	− 4.4 ± 0.5*
Δ Hindquarters flow (kHz min)	2.2 ± 1.3	3.1 ± 1.3	2.5 ± 1.1	1.2 ± 0.4	1.3 ± 0.3*	2.9 ± 0.7*
Δ Renal conductance ([kHz mmHg^{-1} min] 10^3)	− 6 ± 2	− 74 ± 10*	− 189 ± 19*	− 5 ± 1	− 32 ± 4*	− 88 ± 6*
Δ Mesenteric conductance ([kHz mmHg^{-1} min] 10^3)	− 11 ± 4	− 69 ± 9*	− 172 ± 19*	− 4 ± 2	− 20 ± 4*	− 50 ± 6*
Δ Hindquarters conductance ([kHz mmHg^{-1} min] 10^3)	22 ± 12	47 ± 21	− 109 ± 26*	22 ± 6*	11 ± 4	16 ± 6*

Values are mean ± s.e.mean.
*$P < 0.05$ versus baseline.

Table 2 Integrated (areas under or over curves) cardiovascular responses to angiotensin II (3-8) (AII (3-8) (12.5 nmol kg^{-1}) in the same conscious, Long Evans rats ($n = 3$) in the absence (control) or presence of L-arginine (1.4 mmol kg^{-1} bolus, 1.4 mmol kg^{-1} h^{-1} infusion)

	Control	*+ L-Arginine*
Δ Heart rate (beats)	− 69 ± 27	− 55 ± 16
Δ Mean arterial blood pressure (mmHg min)	23 ± 4	37 ± 12
Δ Renal flow (kHz min)	− 9.3 ± 1.2	− 7.6 ± 0.5
Δ Mesenteric flow (kHz min)	− 4.5 ± 1.1	− 6.2 ± 1.4
Δ Hindquarters flow (kHz min)	− 1.7 ± 0.5	1.1 ± 0.2
Δ Renal conductance ([kHz mmHg^{-1} min] 10^3)	− 95 ± 10	− 79 ± 7
Δ Mesenteric conductance ([kHz mmHg^{-1} min] 10^3)	− 55 ± 14	− 76 ± 21
Δ Hindquarters conductance ([kHz mmHg^{-1} min] 10^3)	21 ± 13	4 ± 2

Values are mean ± s.e.mean

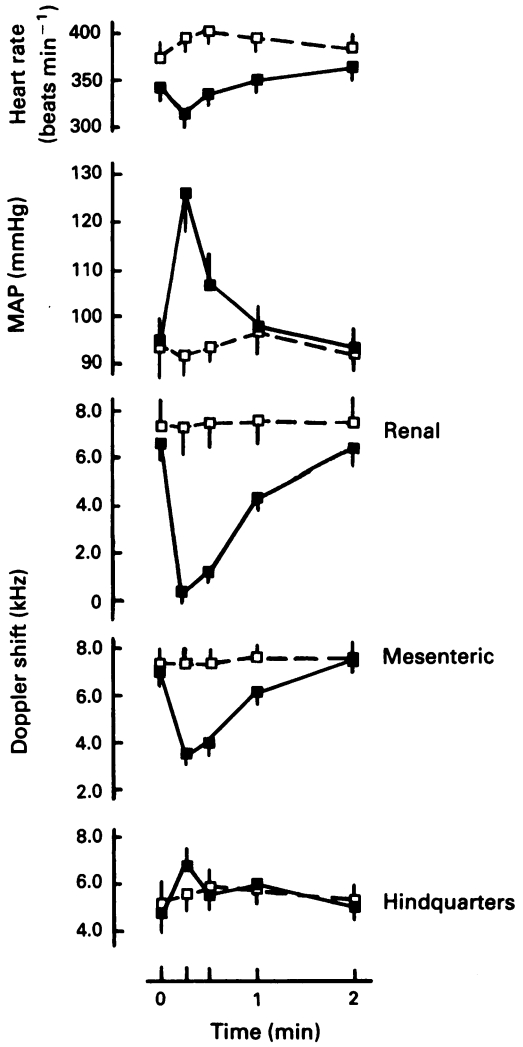

Figure 2 Cardiovascular responses to angiotensin II (3-8) (12.5 nmol kg^{-1}) in the same conscious Long Evans rats ($n = 5$) in the absence (■) or presence (□) of losartan (20 μmol kg^{-1}). Values are mean ± s.e.mean.

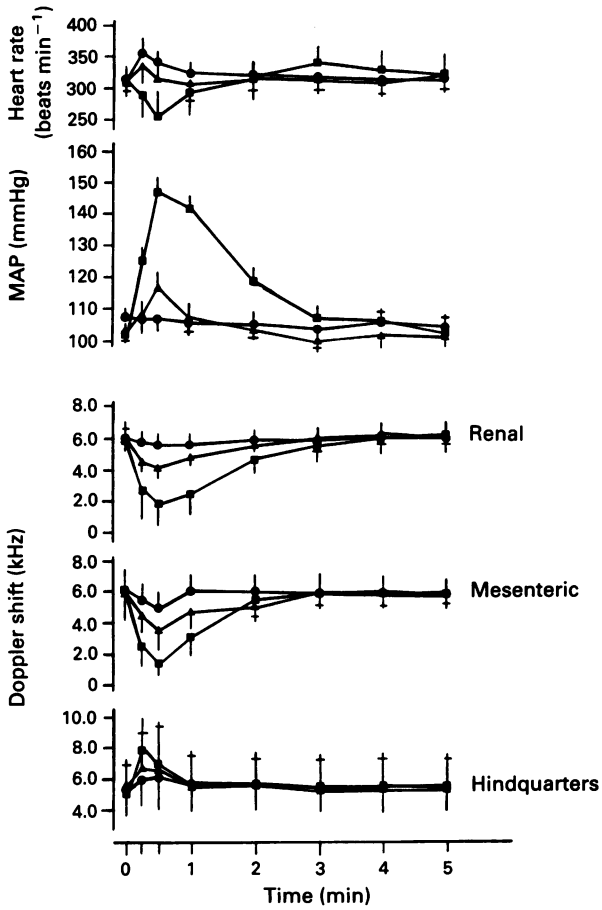

Figure 3 Cardiovascular responses to angiotensin III (● 1.25; ▲ 12.5; ■ 125 pmol kg^{-1}) in the same conscious Long Evans rats ($n = 3$). Values are mean ± s.e.mean.

entirely new angiotensin receptor with unique specificity, distribution and functional characteristics'. Swanson *et al.* (1992) speculated that the interaction of AII (3-8) with its receptor on endothelial cells stimulated the synthesis and release of endothelium-derived relaxing factor (EDRF). In support of their findings, Swanson *et al.* (1992) cited the work of Haberl *et al.* (1991) which, they claimed, showed that AII (3-8) acted as a vasodilator in rat cerebral arteries via an EDRF-mediated mechanism. In fact, Haberl *et al.* (1991) studied anaesthetized rabbits, and showed that AII (3-8) was without effect on pial arterioles, whereas AII and AIII caused vasodilatation. However, when AII (3-8) (which differs from AIII only in the absence of the amino-terminal L-arginine residue) was applied after L-arginine, vasodilatation was seen. As mentioned in the Introduction, Haberl *et al.* (1991) considered that their results indicated availability of L-arginine and AII (3-8) was necessary for endothelium-dependent cerebral vasodilatation. However, there is no clear evidence that L-arginine can enhance the release of nitric oxide (NO) contingent upon receptor-mediated activation of constitutive NO synthase, and in the present work, L-arginine had no effect on responses to AII (3-8). Moreover, AIII had effects very like AII, causing marked renal and mesenteric vasoconstrictions. A similar pattern of response was seen with AII (3-8) although it was about 100 fold less potent than AII, and its duration of action was considerably shorter. Thus, our results are consistent with AII (3-8) being a less effective agonist at the receptor with which AII interacts.

Considering the findings of Swanson *et al.* (1992), showing that AII (3-8) caused marked renal cortical vasodilatation, it is particularly notable that, in our model, the effects of AII (3-8) were characterized by dramatic renal vasoconstriction. One difference between our study and that of Swanson *et al.* (1992) is that we gave i.v. bolus injections of AII (3-8) to conscious animals, whereas they infused the peptide into the renal artery of anaesthetized rats. Thus, it is possible that our assessment of total renal flow masked a specific vasodilator effect of AII (3-8) in the renal cortex. It is also feasible that, given i.v., AII (3-8) activated mechanisms that concealed its direct vasodilator effects, Indeed, it could be suggested that the short duration of the pressor and vasoconstrictor action of AII (3-8), compared to AII, was due to concurrent activation of vasodilator mechanisms, possibly also in vascular beds not studied by us. However, these arguments are difficult to reconcile with our finding that losartan blocked all the effects of AII (3-8), unless the former was not acting as a selective antagonist of AT_1-receptors, but was also inhibiting the action of AII (3-8) on a distinct AII-receptor subtype. In that case, one is left with the problem of aligning this proposal with the finding of Swanson *et al.* (1992) that AII (3-8) interacted with a receptor that showed no affinity for losartan. Whatever the explanation of the disparities between our findings and those of Swanson *et al.* (1992), the present work provides no evidence for AII (3-8) exerting acute effects, other than through activation of AT_1-receptors.

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(Received February 25, 1993
Revised March 26, 1993
Accepted April 28, 1993)