



Direct positive chronotropic effects of angiotensin II and angiotensin III in pithed rats and in rat isolated atria

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1 The direct positive chronotropic effects of angiotensin II (AII) and its degradation products angiotensin III (AIII) and angiotensin IV (AIV) were established in pithed rats and in rat spontaneously beating right atria.

2 In pithed rats, AII, AIII and AIV caused dose-dependent tachycardia with similar maximal responses (110 beats min⁻¹). The β -adrenoceptor antagonist propranolol (3.37×10^{-6} mol kg⁻¹) but not the α_1 -adrenoceptor antagonist prazosin (2.38×10^{-7} mol kg⁻¹) significantly reduced these effects ($P < 0.05$; $n = 7-8$), but 20–25% of the responses could not be blocked by propranolol.

3 In isolated atria, AII, AIII and AIV caused concentration-dependent increases in beating rate with similar maximal responses to AII and AIII (34.3 ± 0.4 and 34.7 ± 0.4 beats min⁻¹; $n = 9-10$), and a lower maximal response to AIV (26.8 ± 0.6 beats min⁻¹; $P < 0.05$; $n = 8$). AIII was about 9 times less potent than AII, whereas AIV proved approximately 3800 times less potent than AII. Neither propranolol (1 μ M) nor prazosin (1 μ M) could influence the effects of the angiotensin peptides.

4 In isolated atria, the selective AT₁-receptor antagonist, losartan (10, 100 and 300 nM) caused parallel rightward shifts of the concentration-response curves for AII and AIII, whereas the selective AT₂-receptor antagonist PD123177 (1 μ M) did not influence the effects of AII and AIII. The aminopeptidase-A and -M inhibitor amastatin (10 μ M), significantly steepened the slope of the AIII curves and increased the potency of AIII about 6 fold. Amastatin did not influence the responses to AII.

5 Our results indicate that both *in vivo* and *in vitro*, exogenous AII and AIII induced a direct dose-dependent chronotropic effect, which is independent of the adrenergic system. This chronotropic effect is mediated by AT₁-subtype receptors.

Keywords: Angiotensin II; angiotensin III; angiotensin IV; angiotensin (1–7); chronotropic effect; losartan; PD123177; amastatin

Introduction

Recent biochemical, molecular biological and functional studies have suggested the existence of a local renin-angiotensin system in the heart (Lindpaintner & Garten, 1991). Angiotensin I converting enzyme (ACE) is known to be present in various non-pulmonary tissues (e.g. kidney, brain and heart) (Caldwell *et al.*, 1976). Angiotensin II (AII) can also be formed in the human heart via the chymase pathway, in addition to its generation in the lung as a result of ACE activity (Urata *et al.*, 1993). These findings indicate that locally generated AII may play a role in the modulation of cardiac contractile force and frequency.

Previous investigations in our department (Knappe & van Zwieten, 1988; Zhang *et al.*, 1993) have shown that the tachycardic response induced by AII in the pithed rat is generated by a direct positive chronotropic effect as well as by an indirect action of AII, which is known to stimulate the sympathetic ganglia, thus leading to the release of catecholamines. It has also been found (Allen *et al.*, 1988) that AII increases the spontaneous contractile frequency in cultured heart myocytes from neonatal rats. The rat heart contains a high concentration of AII-binding sites in the presynaptic neurones and in the conducting system, as substantiated by recent radioligand binding studies (Sechi *et al.*, 1992; Saavedra *et al.*, 1993).

The octapeptide AII may be primarily degraded at its N-terminal position by aminopeptidase-A (Lindpaintner & Garten, 1991), resulting in the formation of the heptapeptide angiotensin III (AIII). This can be further degraded at its N-terminal position by aminopeptidase-B or -M (Lindpaintner & Garten, 1991; Abhold & Harding, 1988), to produce the hex-

apeptide angiotensin IV (AIV). It has been demonstrated that AII, AIII and AIV have similar efficacies in inducing vasoconstrictor effects in rat aorta (Li *et al.*, 1995) and in the rat pulmonary vascular bed (Nossaman *et al.*, 1995). Several studies have been devoted to the vascular effects of AII, AIII and AIV, but much less attention has been paid to the cardiac effects of these peptides. We therefore performed a comparative investigation of the influence of AII, AIII and AIV on heart rate, both in pithed rats and in rat isolated atria. We also studied the effects of the AT₁-subtype selective antagonist losartan (Timmermans *et al.*, 1991) and the AT₂-subtype selective antagonist PD123177 (Chiu *et al.*, 1989) on the responses induced by AII, AIII and AIV on heart rate, in order to characterize the receptor subtypes that are involved.

It has been shown that angiotensin (1–7), a heptapeptide formed directly from angiotensin I in the brain and other tissues (Chappell *et al.*, 1989), can mimic some effects of AII (Lees *et al.*, 1993). Therefore, we also investigated the chronotropic effect of angiotensin (1–7) in isolated atria.

Several studies have indicated that endogenous degradative proteases may interfere with the quantitative evaluation of the effects of angiotensin peptides (Robertson *et al.*, 1992; Fujimoto *et al.*, 1992). In the present study, the chronotropic effects of AII and AIII in isolated atria were therefore compared in the absence and presence of an aminopeptidase-A and -M inhibitor, amastatin.

Methods

Animals

Male Wistar rats (IFFA CREDO, Les Oncins, France) weighing 250–350 g were used in all experiments.

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Pithed rat preparation

Rats were anaesthetized with ether. After the trachea had been cannulated, the animals were pithed by a blunt needle introduced into the vertebral canal via the orbit and artificially ventilated with room air (20 ml kg⁻¹ beat⁻¹, 40 beats min⁻¹) by a Braun Melsungen pump. The body temperature of the animals was maintained at 37°C by a heating pad. Both vagal nerves were sectioned in the cervical region. The left jugular vein and carotid artery were cannulated for drug administration and blood pressure (BP) monitoring, respectively. The cannulae were maintained patent with heparinized saline (500 u ml⁻¹). Heart rate (HR) was derived from the BP signal by a rate meter (MacLab/8 A.D. Instruments Ltd, London) and both parameters were recorded and continuously displayed with a MacLab/8-computer system (A.D. Instruments).

After instrumented animals had been allowed to stabilize for 15 min, saline, prazosin (2.38 × 10⁻⁷ mol kg⁻¹, i.v.) or propranolol (3.37 × 10⁻⁶ mol kg⁻¹, i.v.) was injected. The cardiovascular responses caused by intravenous injection of AII, AIII or AIV were measured 20 min after one of the two antagonists or combination of the two antagonists had been administered. Changes in HR were measured and expressed by means of dose-response curves.

All drugs were administered intravenously in a volume of 0.5 ml kg⁻¹ body weight followed by a flush with 0.07 ml of saline. Only a single complete cumulative dose-response curve was determined per individual animal.

Isolated right atria

Rats were killed by stunning and exsanguination, and the heart was quickly removed and immediately placed in a Krebs solution (at room temperature) of the following composition (mm): NaCl 119.0, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2 and glucose 11.1, gassed with 95% O₂ + 5% CO₂. The entire right atrium was carefully and rapidly cut perpendicularly to the axis of the heart. Each atrium was suspended in a 10 ml organ bath with Krebs solution at 37°C (pH = 7.4) and connected by a silk thread to a force transducer (UFL Co. California, U.S.A.). Isometric force was recorded by a MacLab/8-computer system (A.D. Instruments) and the frequency (defined as the mean basal rate of spontaneously beating) of the right atria was derived and recorded from the contractile force signal via a rate meter (A.D. Instruments). The resting tension of each atrium was adjusted to 0.5 g and the tissues were equilibrated for 60 min before starting the experiments. At 30 min intervals the medium was exchanged with fresh Krebs solution.

After equilibration, cumulative concentration-response curves to AII, AIII, AIV or angiotensin (1-7) were constructed. The concentration of the peptides was increased only after the previous addition of the peptide had caused the maximal response. Only a single complete cumulative concentration-response curve was obtained in each atrium.

In separate experiments, cumulative concentration-response curves to AII, AIII or AIV were constructed after 30 min of

incubation with one of the following antagonists: propranolol (1 μM), prazosin (1 μM), amastatin (10 μM), PD123177 (1 μM), losartan (10, 100 and 300 nM).

Drugs used

AII, AIII, AIV and angiotensin (1-7) (Bachem, Hannover, Germany); losartan, PD123177 (1-[(4-amino-3-methylphenyl) methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazole[4,5-C]pyridine-6-carboxylic acid) (Thomae GmbH; Biberach/Riss, Germany); prazosin hydrochloride (Pfizer, Groton, U.S.A.); (-)-propranolol hydrochloride (Imperial Chemical Industries, Macclesfield, UK); amastatin (ICN Biomedicals B.V., Zoetermeer, the Netherlands). Prazosin was dissolved in 5% glucose solution and PD123177 was taken up in a small volume of 1 M NaOH, and subsequently diluted with saline.

Statistical analysis

All data are presented as means ± s.e.mean for *n* observations. The pD₂ value [-log effective concentration which produces 50% of the maximal effect (EC₅₀)] and the maximal effect (E_{max}) were calculated from the dose- or concentration-response curves by the sigmoid curve-fitting programme GraphPAD (San Diego, CA, U.S.A.). According to the basic criteria of Schild analysis (Arunlakshana & Schild, 1959), when angiotensin curves obtained in the presence of the antagonists were parallel with the control curves and no significant depression of the maximal effect occurred, angiotensin concentration-ratios (DR) were calculated. Accordingly, the concentration of the agonist in the presence of antagonist, which produced the same response as that caused by the EC₅₀ in the absence of antagonist, was divided by the EC₅₀. The results were visualized by means of a Schild plot, as log(DR - 1) versus log antagonist concentration (log[B]), and a regression of log(DR - 1) on log[B] was calculated. Only when the regression was linear and the slope was not significantly different from unity, the pK_B value of the antagonist was calculated. Differences between values were analysed for significance by one way analysis of variance (ANOVA) or Student's *t* test. *P* values less than 0.05 were considered as significant.

Results

Chronotropic effects of AII, AIII and AIV i.v. in pithed rats

In pithed rat preparations the initial HR after stabilization amounted to 283 ± 4 beats min⁻¹ (*n* = 47). Intravenous injections of AII (3 × 10⁻¹⁰–3 × 10⁻⁸ mol kg⁻¹), AIII (10⁻⁹–10⁻⁷ mol kg⁻¹) and AIV (3 × 10⁻⁸–3 × 10⁻⁶ mol kg⁻¹) caused dose-dependent positive chronotropic effects with comparable maximal responses (Table 1; Figure 1a). Pre-treatment with the β-adrenoceptor antagonist propranolol

Table 1 E_{max} (maximal response), pD₂ and slope values of dose-response curves for the chronotropic effects and pressor effects of AII, AIII and AIV in pithed rats

	Agonist	E _{max}	pD ₂	Slope
HR (beat min ⁻¹)	AII	112.7 ± 2.6	8.93 ± 0.08	1.74 ± 0.13
	AIII	111.5 ± 4.0	8.42 ± 0.16*	1.56 ± 0.21
	AIV	112.2 ± 1.5	7.16 ± 0.15*	1.45 ± 0.08
DBP (mmHg)	AII	100.2 ± 2.8	9.78 ± 0.05	0.85 ± 0.09
	AIII	98.5 ± 1.8	9.11 ± 0.02*	0.84 ± 0.03
	AIV	97.9 ± 2.0	7.56 ± 0.04*	0.75 ± 0.06

Data are shown as mean ± s.e.mean (*n* = 7–11).

*Significantly different from the data obtained with AII (*P* < 0.05).

(3.37×10^{-6} mol kg⁻¹) reduced the maximal responses to AII, AIII and AIV by $74.5 \pm 2.7\%$, $71.4 \pm 4.2\%$ and $81.2 \pm 3.2\%$, respectively. However, the α_1 -adrenoceptor antagonist prazosin (2.38×10^{-7} mol kg⁻¹) caused no change of the dose-response curves for AII, AIII and AIV (data not shown). Higher doses (1.01×10^{-5} and 1.69×10^{-5} mol kg⁻¹) of propranolol alone or in combination with prazosin (2.38×10^{-7} mol kg⁻¹) failed to cause further changes of the AII, AIII and AIV curves (data not shown).

The basal diastolic blood pressure (DBP) after stabilization amounted to 44.3 ± 1.3 mmHg ($n = 36$). Intravenous injections of AII (3×10^{-12} – 3×10^{-8} mol kg⁻¹), AIII (10^{-11} – 10^{-7} mol kg⁻¹) and AIV (3×10^{-10} – 3×10^{-6} mol kg⁻¹) caused dose-dependent increases in DBP with similar maximal responses (Table 1; Figure 1b).

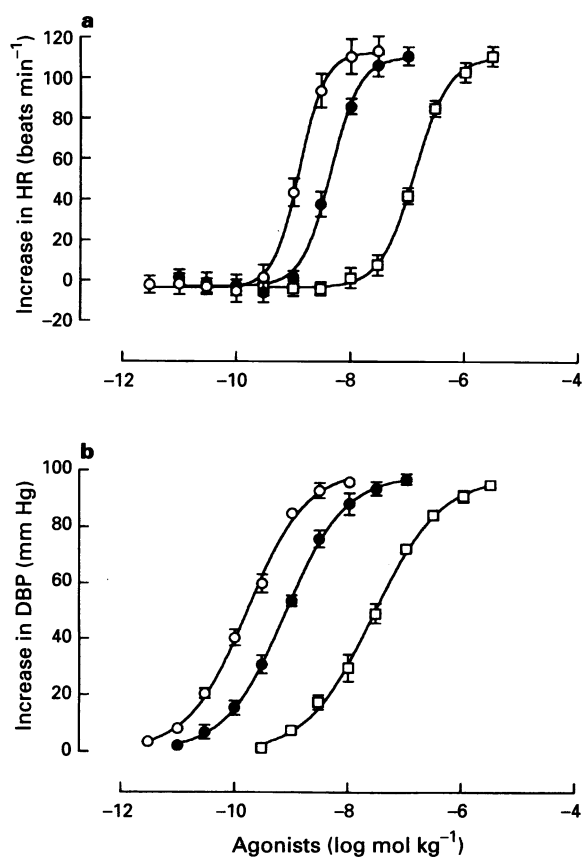


Figure 1 Dose-response curves for the chronotropic effects (a) and pressor effects (b) of angiotensin II (○), angiotensin III (●) and angiotensin IV (□) in pithed rat preparations. Data are shown as mean \pm s.e.mean ($n = 7-9$).

Chronotropic effects of AII, AIII and AIV in rat right atria preparations

In rat isolated right atria preparations, the basal contractile frequency after equilibration amounted to 267 ± 3 beats min⁻¹ ($n = 42$). Cumulative additions of AII (0.1–100 nM), AIII (0.3 nM–1 μ M) and AIV (0.3–100 μ M) elicited concentration-dependent increases in frequency (Figure 2). The maximal responses induced by AII and AIII were similar, but the AIV-induced maximal increase in frequency was significantly less than that of AII ($P < 0.05$). AIII was 9 times less active than AII, whilst AIV was approximately 3800 times less potent than AII (Table 2).

The basal contractile amplitude of the isolated right atria amounted to 0.58 ± 0.07 g ($n = 38$). Cumulative additions of AII, AIII and AIV slightly but significantly reduced the amplitude by maximally $10.2 \pm 0.3\%$, $11.4 \pm 0.6\%$ and $9.3 \pm 0.3\%$, respectively ($P < 0.05$; $n = 7-8$), which occurred simultaneously with the changes in frequency.

Cumulative additions (1–100 μ M) of angiotensin (1–7) neither influenced the frequency nor the amplitude of these preparations (data not shown).

Effects of propranolol and prazosin on the chronotropic effects of AII, AIII and AIV in isolated right atria

Propranolol (1 μ M) significantly decreased the basal frequency of beating of the isolated right atria by $8.4 \pm 1.3\%$ ($P < 0.05$; $n = 18$), whilst prazosin (1 μ M) did not influence this parameter. Both antagonists caused no significant changes of the concentration-response curves for AII, AIII and AIV in rat right atria preparations (Table 3).

In separate experiments, (0.1–30 μ M) tyramine-induced

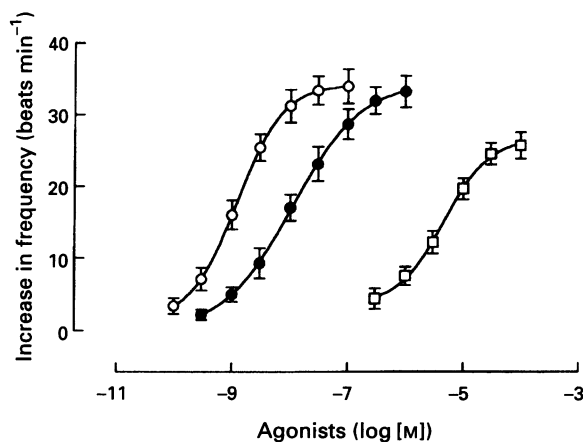


Figure 2 Concentration-response curves for the chronotropic effects of angiotensin II (○), angiotensin III (●) and angiotensin IV (□) in rat isolated right atria. Data are shown as mean \pm s.e.mean ($n = 9-11$).

Table 2 E_{max} (maximal response), pD_2 and slope values of concentration-response curves for the chronotropic effects of AII, AIII and AIV in rat isolated right atria preparations (in the absence and presence of 10 μ M amastatin)

Agonist		E_{max} (beats min ⁻¹)	pD_2	Slope
AII	-Amastatin	34.3 ± 0.4	8.91 ± 0.01	1.11 ± 0.02
	+ Amastatin	35.4 ± 0.4	8.81 ± 0.04	1.17 ± 0.04
AIII	-Amastatin	34.7 ± 0.4	$7.96 \pm 0.03^*$	$0.73 \pm 0.04^*$
	+ Amastatin	34.0 ± 0.2	$8.75 \pm 0.01^{\ddagger}$	$1.19 \pm 0.04^{\ddagger}$
AIV	-Amastatin	$26.8 \pm 0.6^*$	$5.33 \pm 0.05^*$	1.10 ± 0.08

Data are shown as mean \pm s.e.mean ($n = 7-11$).

*Significantly different from 'AII-Amastatin', group ($P < 0.05$). \ddagger Significantly different from '- Amastatin group' ($P < 0.05$).

positive chronotropic effects could be shifted to the right and virtually abolished by $1 \mu\text{M}$ propranolol (Table 3), indicating that the sympathetic nerve endings were intact and that the concentration of propranolol was sufficiently high to block β -adrenoceptors.

Effects of AII receptor antagonists on the chronotropic effects of AII and AIII in rat right atria preparations

The basal contractile amplitude and frequency of beating of the atria remained unchanged in the presence of selective angiotensin-receptor antagonists, losartan and PD123177. The selective AT_1 -receptor antagonist losartan (10, 100 or 300 nM) concentration-dependently shifted the AII and AIII curves to the right, without depressing the maximal response (Figure 3). The pK_B values of losartan for the AII and AIII curves amounted to 8.75 ± 0.07 and 8.66 ± 0.10 , respectively. The selective AT_2 -receptor antagonist PD123177 ($1 \mu\text{M}$) caused no significant change of the concentration-response curves for AII and AIII (Figure 3).

Influence of the aminopeptidase-A and -M inhibitor amastatin on AII and AIII induced chronotropic effects in rat right atria preparations

The basal contractile amplitude and frequency of beating of the preparations remained unchanged by the aminopeptidase-A and -M inhibitor, amastatin ($10 \mu\text{M}$). Neither the potency nor the maximal response to AII was affected by $10 \mu\text{M}$ amastatin (Table 2; Figure 4a). However, amastatin ($10 \mu\text{M}$) significantly shifted the concentration-response curve of AIII to the left and steepened the slope without changing the maximal response ($P < 0.05$; Table 2; Figure 4b). Accordingly, amastatin ($10 \mu\text{M}$) increased the potency of AIII approximately 6 fold (Table 2).

Discussion

Several studies have shown that angiotensin peptides may influence HR via their central inhibitory effects on cardiac vagal afferents (Thompson, 1970; Ferrario *et al.*, 1972; Lee *et al.*, 1980). It has also been shown that in pithed rats and guinea-pigs endogenous AII may depress cardiac vagal transmission (Rechtman & Majewski, 1993). In the present study, we investigated the chronotropic effects of angiotensin peptides in pithed vagotomized rats, thus ruling out the potential central effects and vagal inhibitory actions of these peptides. Increased endogenous angiotensin II level in pithed rats may contribute to some extent to the basal heart rate (Knape & van Zwieten, 1988). The same study also showed that in pithed rats with a low level of circulating endogenous angiotensin II due to pretreatment with captopril, angiotensin II-induced tachy-

cardia was comparable to that observed in saline-treated rats, indicating that receptor desensitization was not involved in this experimental model. We observed that in pithed rat preparations, both AIII and AIV elicited dose-dependent increases in DBP and HR, with a comparable maximal effect to AII, indicating a similar efficacy of these three peptides. Similar observations concerning their vasoconstrictor effects have been obtained in several other species and tissues (Cheng *et al.*, 1994; Li *et al.*, 1995; Nossaman *et al.*, 1995).

Previous studies (Knape & van Zwieten, 1988) have demonstrated that the tachycardia induced by AII in pithed rats is generated by two different actions of AII: a predominantly

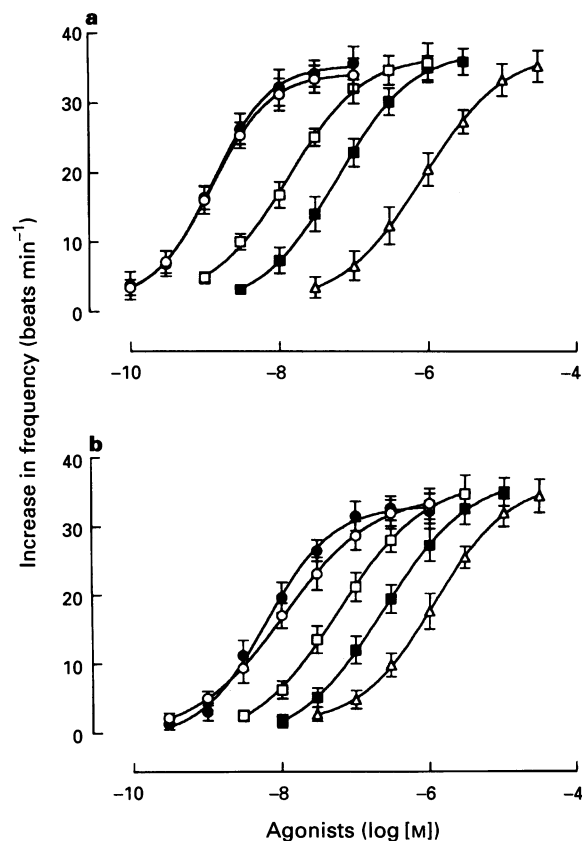


Figure 3 Concentration-response curves for the chronotropic effects of angiotensin II (a) and angiotensin III (b) in rat right atria preparations, in the absence (○) or presence of PD123177 (●) and losartan (□, 10 nM; ■, 100 nM and △, 300 nM). Data are shown as mean \pm s.e.mean ($n = 6-8$).

Table 3 E_{max} (maximal response), pD_2 and slope values of concentration-response curves for the chronotropic effects of AII, AIII and tyramine in rat isolated right atria preparations (in the absence and presence of $1 \mu\text{M}$ prazosin or $1 \mu\text{M}$ propranolol)

Agonist		E_{max} (beats min^{-1})	pD_2	Slope
AII	Saline	34.3 ± 0.4	8.91 ± 0.01	1.11 ± 0.02
	Prazosin	33.8 ± 0.6	8.88 ± 0.06	1.04 ± 0.09
	Propranolol	35.1 ± 0.7	8.90 ± 0.04	1.23 ± 0.06
AIII	Saline	34.7 ± 0.4	7.96 ± 0.03	0.73 ± 0.04
	Prazosin	32.8 ± 0.7	8.01 ± 0.05	0.84 ± 0.09
	Propranolol	32.7 ± 0.4	7.91 ± 0.02	0.78 ± 0.03
Tyramine	Saline	139.4 ± 3.5	5.99 ± 0.05	1.58 ± 0.08
	Prazosin	136.2 ± 2.2	6.01 ± 0.05	1.67 ± 0.07
	Propranolol	$19.6 \pm 1.0^*$	$5.36 \pm 0.07^*$	$1.13 \pm 0.11^*$

Data are shown as mean \pm s.e.mean ($n = 6-11$).

*Significantly different from 'Saline' group ($P < 0.05$).

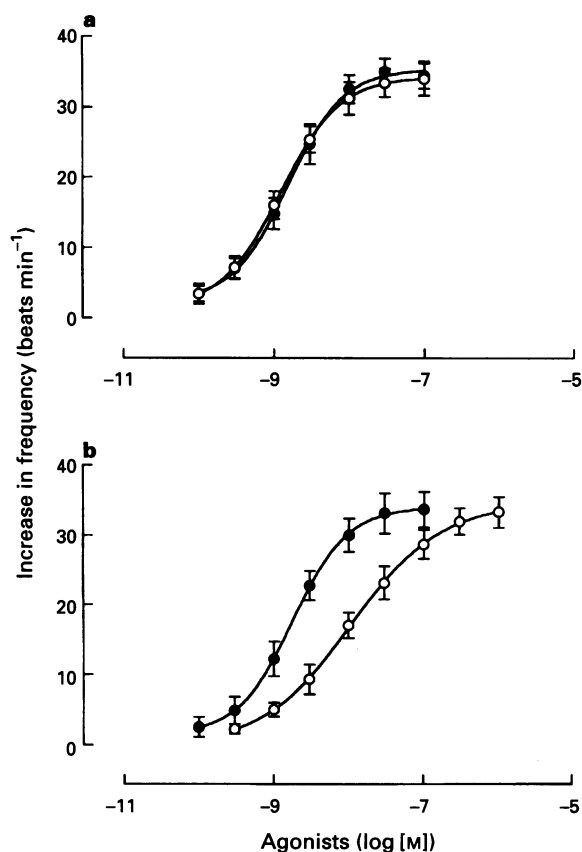


Figure 4 Concentration-response curves for the chronotropic effects of angiotensin II (a) and angiotensin III (b) in rat right atria preparations, in the absence (○) and presence (●) of amastatin (10 μM). Data are shown as mean ± s.e.mean (n = 7).

indirect effect of AII causing the release of noradrenaline which activates cardiac β -adrenoceptors, as well as a small direct positive chronotropic effect on the heart. Similar results were observed for AIII and AIV in the present study. Pre-treatment with the non-selective β -adrenoceptor antagonist propranolol effectively decreased the tachycardic responses to the angiotensin peptides. However, complete suppression of the effects on frequency could not be obtained by using higher doses of propranolol or a combination with the α_1 -adrenoceptor antagonist prazosin, thus suggesting the occurrence of a small direct chronotropic effect, independent of the sympathetic system. This suggestion was further confirmed by the *in vitro* experiments.

We are the first to observe that in spontaneously beating rat atria, AIII and AIV concentration-dependently increases the frequency of beating. AIII induced a similar maximal response to that of AII, whereas a weaker maximal response to AIV was seen. Several authors (Starke, 1971; Ziogas *et al.*, 1985; Xiang *et al.*, 1985) have shown that in rabbit and guinea-pig isolated hearts, AII stimulates angiotensin-receptors located on sympathetic nerve terminals, and hence facilitates sympathetic neurotransmission, resulting in a rise in HR. This effect appears to occur only with respect to the release of noradrenaline induced by nerve impulses, but not for spontaneous or tyramine-induced noradrenaline release. Majewski (1989) found a tonic activation of facilitatory prejunctional AII receptors at the sympathetic nerve endings in pithed rats. However, this effect was predominantly triggered by the release of renin from the kidney, but not by locally generated AII. In our study, propranolol and prazosin did not influence the positive chronotropic responses to angiotensin peptides in isolated right atria, thus indicating that these effects were independent of catecholamine release and β -adrenoceptors.

We observed that the difference between the potency of AII

and AIV in isolated atrial preparations was more pronounced than that in pithed rats. *In vitro*, the maximal response induced by AIV was significantly weaker than those to AII and AIII, although a similar maximal response of these three peptides was observed *in vivo*. These observations suggest a stronger activation of AIV on noradrenaline release in intact animals, which may mask the differences between the direct chronotropic effects of AIV and its precursors.

Both in pithed rat preparations and in isolated atria, the differences between the potency of AII and AIII were less than 10 fold, whereas AIV proved much less potent than its precursors. These data, together with the observation that angiotensin (1-7) did not influence the frequency of isolated right atria, suggest that the amino acids in the positions 3 to 8 are essential for the expression of agonist activity, and that the amino acid arginine in position 2 of the angiotensin peptides is essential for the high affinity binding to the angiotensin-receptors in the rat heart.

The newly developed nonpeptide angiotensin antagonists have led to the identification of at least two different angiotensin-receptor subpopulations: AT₁- and AT₂-receptor subtypes (Wong *et al.*, 1990). These two receptor subtypes have been demonstrated to exist in the rat heart, and they are distributed in similar proportions over the myocardium (Chang & Lotti, 1991; Sechi *et al.*, 1992). The present functional experiments strongly suggest that the angiotensin receptors mediating the chronotropic effects of AII and AIII in the rat atria belong to the AT₁-subtype, since the AT₁-receptor selective antagonist losartan competitively antagonized the tachycardic responses to AII and AIII, whereas the AT₂-receptor selective antagonist PD123177 caused no significant change in the tachycardic responses to these two peptides. Accordingly, AT₂-receptors did not play a role in these responses. These results are supported by the recent finding (Saavedra *et al.*, 1993) that AII receptors in the conduction system of the rat heart belong to the AT₁-subtype; as concluded from quantitative autoradiographic studies. Our results are also in agreement with a recent study by Lambert (1995), who found that the chronotropic effect induced by a direct injection of AII into the sinus node artery of dogs was mediated via AT₁-receptors.

Several studies have indicated that endogenous degradative proteases may interfere with the quantitative evaluation of the effects of angiotensin peptides (Ahmad & Ward, 1990; Robertson *et al.*, 1992; Fujimoto *et al.*, 1992). In isolated atria, the slope of the AIII concentration-response curves proved more shallow than that of the AII curves, although no significant difference between those of AIV and AII was found. This difference may be explained by the rapid enzymatic degradation of AIII (Robertson *et al.*, 1992). This presumption is supported by the observations in the present study that amastatin, an aminopeptidase-A and -M inhibitor, significantly increased the slope of the AIII curves as well as the potency of AIII. The absence of an effect of amastatin on AII responses may be attributed to its much stronger inhibitory effects on aminopeptidase-M activity than on aminopeptidase-A activity (Ahmad & Ward, 1990). The present data are consistent with those of Robertson *et al.* (1992) and Ahmad & Ward (1990), who found that the contractile responses to AIII, but not AII, were potentiated by the presence of amastatin. Since the possible effects of endogenous degradative proteases and effective protease inhibition on the concentration-response curves for AIV so far remain unknown, it remains uncertain whether the potency of AIV may be underestimated in the present study.

It has been demonstrated that AII can cause positive inotropic effects in rats (Zhang *et al.*, 1993) and other species (Lindpaintner & Garten, 1991). However, in isolated right atria preparations, we observed that AII, AIII and AIV slightly decreased the amplitude of beating simultaneously with the increases in frequency. These effects were not influenced by angiotensin receptor antagonists. Therefore these effects appear to be restricted to the rise in frequency, which may influence the shortening velocity and the relaxation ve-

locity. Similar observations have been obtained in cultured heart myocytes (Allen *et al.*, 1988) of the rat, but the underlying mechanisms remain known.

In isolated atria losartan and PD123177 did not cause significant changes of the basal parameters, suggesting that endogenous angiotensin peptides do not contribute to the basal beating rate in this animal model. Dzau (1987) and Falkenhahn *et al.* (1994) have discussed the role of abnormal angiotensinogen gene expression or regulation of tissue renin-angiotensin systems in cardiovascular disorders. Further studies are needed to investigate the physiological or pathological

roles of angiotensin peptides-induced chronotropic effects. However, our results, in agreement with those of others, suggest that angiotensin peptides may contribute to the control of heart rate and the genesis of arrhythmias.

In conclusion, the present study has demonstrated that both in pithed rats and in rat isolated right atria, AII, AIII and AIV cause direct positive chronotropic effects, which are independent of the adrenergic system. The tachycardic responses to AII and AIII in isolated atria are mediated by angiotensin AT₁-subtype receptors but not the AT₂-subtype.

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