British Journal of Pharmacology (1999) 127, 1256–1262
http://www.stockton-press.co.uk/bjp

Prejunctional angiotensin receptors involved in the facilitation of noradrenaline release in mouse tissues

*,[1]S.L. Cox, [1]A.U. Trendelenburg & [1]K. Starke

[1]Pharmakologisches Institut, Freiburg University, Hermann-Herder-Strasse 5, D-79104 Freiburg, Germany

1 The effect of angiotensin II, angiotensin III, angiotensin IV and angiotensin-(1–7) on the electrically induced release of noradrenaline was studied in preparations of mouse atria, spleen, hippocampus, occipito-parietal cortex and hypothalamus preincubated with [^{3}H]-noradrenaline. The prejunctional angiotensin receptor type was investigated using the non-selective receptor antagonist saralasin (AT_1/AT_2) and the AT_1 and AT_2 selective receptor antagonists losartan and PD 123319, respectively.

2 In atrial and splenic preparations, angiotensin II (0.01 nM–0.1 μM) and angiotensin III (0.01 and 0.1 nM–1 μM) increased the stimulation-induced overflow of tritium in a concentration-dependent manner. Angiotensin IV, only at high concentrations (1 and 10 μM), enhanced tritium overflow in the atria, while angiotensin-(1–7) (0.1 nM–10 μM) was without effect in both preparations.

3 In preparations of hippocampus, occipito-parietal cortex and hypothalamus, none of the angiotensin peptides altered the evoked overflow of tritium.

4 In atrial and splenic preparations, saralasin (0.1 μM) and losartan (0.1 and 1 μM), but not PD 123319 (0.1 μM), shifted the concentration-response curves of angiotensin II and angiotensin III to the right.

5 In conclusion, in mouse atria and spleen, angiotensin II and angiotensin III facilitate the action potential induced release of noradrenaline *via* a prejunctional AT_1 receptor. Only high concentrations of angiotensin IV are effective in the atria and angiotensin-(1–7) is without effect in both preparations. In mouse brain areas, angiotensin II, angiotensin III, angiotensin IV and angiotensin-(1–7) do not modulate the release of noradrenaline.

Keywords: Angiotensin; angiotensin receptors; noradrenaline release; mouse atria; mouse spleen; mouse hippocampus; mouse occipito-parietal cortex; mouse hypothalamus

Abbreviations: Ang, angiotensin; con, control; los, losartan; PD, PD 123319; PD 123319, S(+)-1-[(4-dimethylamino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c] pyridine-6-carboxylic acid di-trifluoroacetate; PSS, physiological salt solution; sar, saralasin

Introduction

Prejunctional facilitation of noradrenaline release from postganglionic sympathetic axons is a classical effect of angiotensin II (see Zimmermann *et al*., 1972; Peach, 1977; Starke, 1977). The majority of studies carried out with selective AT_1 and AT_2 angiotensin receptor antagonists suggest that the effect is mediated by AT_1 receptors (Wong *et al*., 1991; Suzuki *et al*., 1992; Brasch *et al*., 1993; Ohia & Jumblatt, 1993; Gironacci *et al*., 1994; Rump *et al*., 1995; Boicos *et al*., 1998). However, there are also reports at variance with this view. In the rat caudal artery, the facilitatory effect of angiotensin II on noradrenaline release was reduced by both the selective AT_1 and AT_2 receptor antagonists losartan and PD 123319, respectively (Cox *et al*., 1995; 1996a,b). Conversely, the effect was not altered by selective concentrations of either antagonist in the rat left ventricle (Moura *et al*., 1997) or canine mesenteric and pulmonary arteries (Guimarães *et al*., 1998). Thus, the prejunctional receptor for angiotensin II is not clearly defined.

The smaller naturally occurring peptide fragments of angiotensin II, such as angiotensin III, angiotensin IV (angiotensin (3–8)) and angiotensin (1–7), are also now considered active hormones of the renin-angiotensin system (see Jackson & Garrison, 1996). However, little is known about their prejunctional effects. Angiotensin III has been shown to enhance the release of noradrenaline in the human pulmonary artery and saphenous vein (Molderings *et al*., 1988) and rabbit aorta (Storgaard & Nedergaard, 1997), but the receptor involved has not been investigated. Angiotensin IV and angiotensin-(1–7) failed to alter the overflow of noradrenaline in rabbit aorta (Storgaard & Nedergaard, 1997), while angiotensin-(1–7) increased the overflow in rat isolated atria (Gironacci *et al*., 1994).

A renin-angiotensin system, including all of the components, exists within the brain (Ganten *et al*., 1984). An effect of angiotensin on transmitter release from central noradrenergic neurons remains controversial; some reports show an increase while others show no effect (see Discussion).

In the present study, we have investigated the effect of angiotensin II, angiotensin III, angiotensin IV and angiotensin-(1–7) on the release of noradrenaline from peripheral (atria and spleen) as well as central (hippocampus, occipito-parietal cortex and hypothalamus) neurons of the mouse. The prejunctional angiotensin receptor was examined using the selective receptor antagonists losartan and PD 123319. The mouse was chosen because nothing is known about prejunctional angiotensin receptors in this species, except for the occurrence of a prejunctional facilitatory effect of angiotensin II in the atria (Musgrave & Majewski, 1989; Rajanayagam *et al*., 1989), and also because of the possibility of gene

*Author for correspondence;
E-mail: coxsandr@sun2.ruf.uni-freiburg.de

manipulation which has made the mouse a particularly important species to study.

Methods

Preparations and protocols

Adult male NMRI mice were killed by exsanguination. The heart, spleen or brain was removed and placed in physiological salt solution (PSS) which had been bubbled with 95% CO_2 and 5% O_2 and stored on ice. The wall of each atrium was cut into three to four pieces. The spleen was cut into 14 pieces. Round slices, 0.3 mm thick, 2 mm diameter, six to seven per animal, were prepared from the occipito-parietal cortex, parallel to the surface, after a superficial layer of 0.2 mm had been removed. Fourteen slices, 0.3 mm thick, were prepared from one hippocampus. The hypothalamus was prepared as described by Glowinski & Iversen (1966) and divided into four equal sized pieces. The tissue pieces (12–14) were incubated in 3 ml of PSS containing (−)-[2,5,6-^{3}H]-noradrenaline (46.8–56.1 Ci mmol^{-1}, 0.1 μM) for 30 min at 37°C. Twelve preparations were then superfused in parallel in 12 superfusion chambers with PSS at a constant flow rate of 1.2 ml min^{-1}. The preparations were subjected to seven periods of electrical field stimulation consisting of a train of square wave pulses of 1 ms width and 80 mA (peripheral tissues) or 60 mA (central tissues) current strength, yielding a voltage drop of 45 or 34 V cm^{-1}, respectively, between the electrodes of each chamber. After 30 min of superfusion ($t = 30$ min), a 'priming' stimulation period (peripheral tissues: 180 p, 3 Hz; central tissues: 36 p, 3 Hz) was applied. Beginning at $t = 54$ min, the preparations were subjected to six periods (S_1–S_6) of electrical field stimulation (peripheral tissues: 120 p, 3 Hz; central tissues: 36 p, 3 Hz), delivered 18 min apart. Consecutive 2-min samples of the superfusate were collected. At the end of the experiment the tissue was dissolved in 0.5 ml of Soluene (Packard, Frankfurt am Main, Germany) and tritium was determined in the superfusate samples and preparations.

Concentration-response curves to the angiotensin peptides were determined by introducing the peptide in increasing concentrations after S_1, 12 min before S_2, S_3, S_4, S_5 and S_6. To investigate the possibility of receptor desensitization, a single, maximally effective concentration of each peptide was introduced into the PSS 12 min before S_3 and remained present for the duration of the experiment. The antagonists were introduced from the beginning of superfusion ($t = 0$ min) and remained for the duration of the experiment. To determine an effect of antagonist alone, it was introduced into the PSS 12 min before S_3 and remained present for the duration of the experiment. In each experiment with 12 preparations, at least one preparation was superfused throughout with drug-free PSS. In experiments investigating the effects of the antagonists, at least one preparation was superfused with the antagonist alone and at least one with the agonist alone.

Drugs and radiochemicals

The PSS had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5 (peripheral tissues) or 1.3 (central tissues), $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, ethylenediaminetetraacetic acid disodium salt 0.03 and desipramine 0.001. The PSS for incubation with ^{3}H-noradrenaline contained 0.2 mM (peripheral tissues) or 1.3 mM (central tissues) $CaCl_2$ and no desipramine.

The following drugs were used: angiotensin II (human), angiotensin III (human), angiotensin IV (human), angiotensin II fragments 1–7 (human, Asp-Arg-Val-Tyr-Ile-His-Pro), saralasin, S(+)-1-[(4-dimethylamino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c] pyridine-6-carboxylic acid di-trifluoroacetate (PD 123319), desipramine hydrochloride (Sigma, Deisenhofen, Germany), losartan (gift from Merck, Darmstadt, Germany), phentolamine hydrochloride (gift from Ciba-Geigy, Basel, Switzerland). (−)-[2,5,6-^{3}H]-noradrenaline was supplied by New England Nuclear (Dreieich, Germany) with a specific activity of 46.8–56.1 Ci mmol^{-1} and a radioactive concentration of 1 mCi ml^{-1}.

Analysis of data

The outflow of tritium was calculated as a fraction of the tritium content of the tissue at the onset of the respective collection period, per min. The overflow of tritium evoked by electrical field stimulation was calculated as the total tritium outflow during the collection period in which stimulation was applied and during the two collection periods thereafter, minus the estimated basal outflow. Basal outflow was assumed to decline linearly from the collection period before stimulation to the second collection period

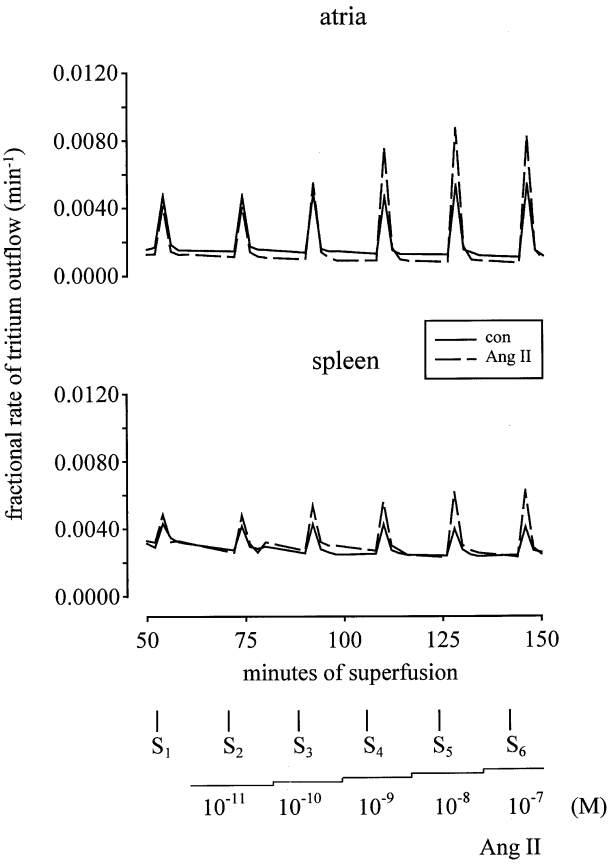

Figure 1 Tritium efflux from mouse atrial and splenic preparations in the absence and presence of angiotensin II (Ang II). Each preparation was stimulated for six periods (S_1–S_6, 120 p, 3 Hz), delivered 18 min apart. Solid lines represent the control (con) experiments, in the absence of drugs, and dashed lines, the effect of Ang II when introduced into the PSS in increasing concentrations (0.01 nM–0.1 μM), 12 min before S_2, S_3, S_4, S_5 and S_6. Each line represents the outflow of tritium from a single preparation.

after stimulation. The evoked overflow was expressed as a percentage of the tritium content of the tissue at the time of stimulation. Overflow ratios (S_n/S_1) were then determined for each period of stimulation. Percentage changes of S_n/S_1 ratios caused by a drug added after S_1 were calculated for each preparation, taking as reference value the average corresponding S_n/S_1 ratio in control experiments, in the absence of the drug. In experiments in which angiotensin antagonists (from $t=0$ min) were combined with agonists (after S_1), the percentage changes of S_n/S_1 ratios caused by the agonists added after S_1 were also calculated for each preparation, taking as reference value the average corresponding S_n/S_1 ratio in experiments in which the antagonist was present alone. Effects of drugs added after S_1 on basal tritium outflow were calculated in the same manner, based on samples collected immediately before stimulation.

Data are expressed as means ± s.e.mean; n denotes the number of preparations. The statistical significance of differences between groups was determined by Mann-Whitney test followed by Bonferroni correction. In all cases probability levels less than 0.05 ($P<0.05$) were taken to indicate significant differences.

Results

Basal outflow

In the absence of drugs, except for desipramine which was always present throughout superfusion, the basal outflow of tritium preceding the first period of stimulation averaged 0.0020 ± 0.0001 min^{-1} in atrial ($n=33$), 0.0032 ± 0.0001 min^{-1} in splenic ($n=29$), 0.0019 ± 0.0002 min^{-1} in hippocampal ($n=9$), 0.0019 ± 0.0002 min^{-1} in occipito-parietal cortex ($n=10$) and 0.0021 ± 0.0001 min^{-1} ($n=6$) in hypothalamic preparations. There was a small decline in the basal outflow of tritium during the course of the experiments. None of the agonists, nor any of the antagonists, caused any change of basal tritium efflux (data not shown).

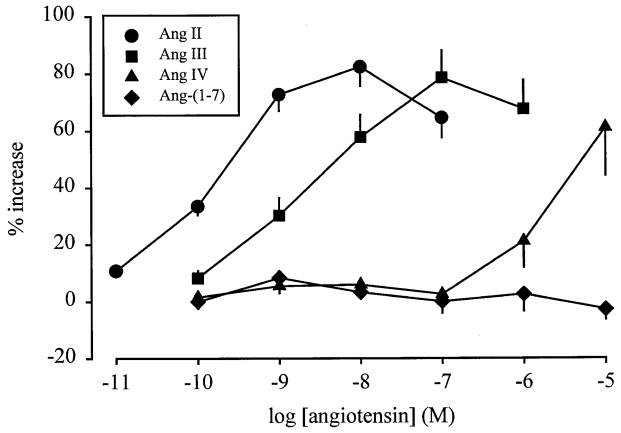

Figure 2 Effect of angiotensin II (Ang II), angiotensin III (Ang III), angiotensin IV (Ang IV) and angiotensin-(1–7) (Ang-(1–7)) on the stimulation-induced overflow of tritium from mouse atria. The preparations were stimulated for six periods (S_1-S_6, 120 p, 3 Hz), delivered 18 min apart. Angiotensin was introduced into the PSS in increasing concentrations, 12 min before S_2, S_3, S_4, S_5 and S_6. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of agonists. The vertical lines represent the s.e.mean from 4–27 preparations.

Evoked tritium overflow: atria

Electrical field stimulation produced an overflow of tritium which amounted to 0.979 ± 0.073% of total tissue tritium (S_1, $n=33$).

The effect of angiotensin II on tritium overflow from a single preparation is illustrated in Figure 1 (upper panel). Angiotensin II and angiotensin III increased the overflow of tritium with similar maximal enhancements of about 80% (Figure 2). The EC_{50} values were determined as the concentrations causing half-maximal enhancement, i.e. 40%, and amounted to 0.17 nM for angiotensin II and 2.2 nM for

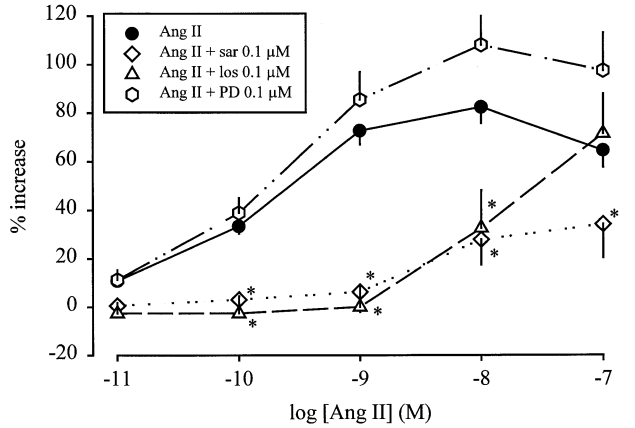

Figure 3 Effect of angiotensin II (Ang II), in the absence and presence of saralasin (sar), losartan (los) and PD 123319 (PD), on the stimulation-induced overflow of tritium from mouse atria. The preparations were stimulated for six periods (S_1-S_6, 120 p, 3 Hz), delivered 18 min apart. Ang II was administered either alone or in the presence of sar, los or PD. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of Ang II. The vertical lines represent the s.e.mean, from 4–27 preparations. Asterisks represent significant differences from Ang II given alone; $P<0.05$.

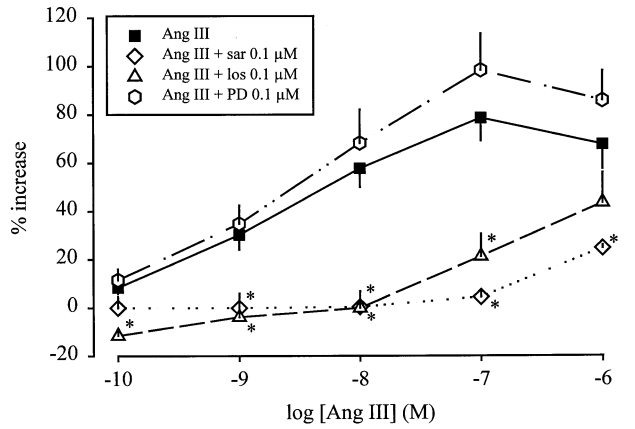

Figure 4 Effect of angiotensin III (Ang III), in the absence and presence of saralasin (sar), losartan (los) and PD 123319 (PD), on the stimulation-induced overflow of tritium from mouse atria. The preparations were stimulated for 6 periods (S_1-S_6, 120 p, 3 Hz), delivered 18 min apart. Ang III was administered either alone or in the presence of sar, los or PD. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of Ang III. The vertical lines represent the s.e.mean from 4–19 preparations. Asterisks represent significant differences from Ang III given alone; $P<0.05$.

Table 1 pK_B values of losartan at prejunctional angiotensin receptors in mouse atria and spleen

	Atria		*Spleen*	
Losartan (μM)	*Angiotensin II*	*Angiotensin III*	*Angiotensin II*	*Angiotensin III*
0.1	9.0	9.5	7.9	8.6
1.0			7.4	8.3

pK_B values of losartan against angiotensin II and angiotensin III were calculated from the antagonist-induced increase in EC_{50} values. Each pK_B value is based on 4–27 preparations for control agonist concentration-response curves and 4–14 preparations for agonist concentration-response curves determined in the presence of losartan.

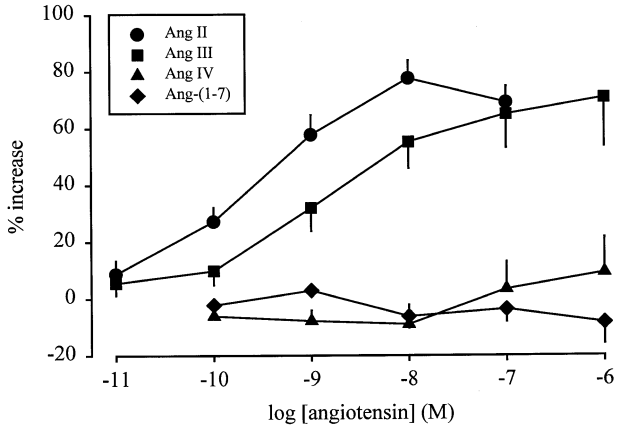

Figure 5 Effect of angiotensin II (Ang II), angiotensin III (Ang III), angiotensin IV (Ang IV) and angiotensin-(1–7) (Ang-(1–7)) on the stimulation-induced overflow of tritium from mouse spleen. The preparations were stimulated for six periods (S_1–S_6, 120 p, 3 Hz), delivered 18 min apart. Angiotensin was introduced into the PSS in increasing concentrations, 12 min before S_2, S_3, S_4, S_5 and S_6. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of agonists. The vertical lines represent the s.e.mean from 4–19 preparations.

angiotensin III (interpolated by hand from the nearest points of the concentration-response curves). A single concentration of angiotensin II or angiotensin III (0.1 μM), when introduced into the PSS 12 min before S_3, increased the evoked overflow, an effect which was maintained in the continued presence of the peptide and thus, was not subject to desensitization ($n=4$, data not shown). Angiotensin IV enhanced the stimulation-induced overflow of tritium only at concentrations of 1 and 10 μM ($P<0.05$, Figure 2). Angiotensin-(1–7) was without effect (Figure 2).

Little enhancement by angiotensin II (Figure 3) and angiotensin III (Figure 4) remained in the presence of the non-selective antagonist saralasin. The AT_1 selective receptor antagonist losartan shifted the concentration-response curve of angiotensin II (Figure 3) and angiotensin III (Figure 4) to the right. The pK_B values of losartan were calculated from the increase in EC_{50} concentrations, assuming that the maximal effect of the agonists, i.e. an 80% increase, was not changed by losartan (see Table 1). The AT_2 selective receptor antagonist PD 123319 was without effect on the concentration-response curves to angiotensin II and angiotensin III (Figures 3 and 4).

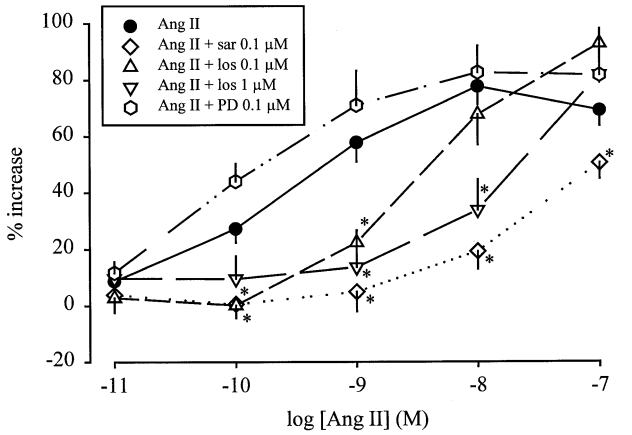

Figure 6 Effect of angiotensin II (Ang II), in the absence and presence of saralasin (sar), losartan (los) and PD 123319 (PD), on the stimulation-induced overflow of tritium from mouse spleen. The preparations were stimulated for six periods (S_1–S_6, 120 p, 3 Hz), delivered 18 min apart. Ang II was administered either alone or in the presence of sar, los or PD. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of Ang II. The vertical lines represent the s.e.mean from 4–27 preparations. Asterisks represent significant differences from Ang II given alone; $P<0.05$.

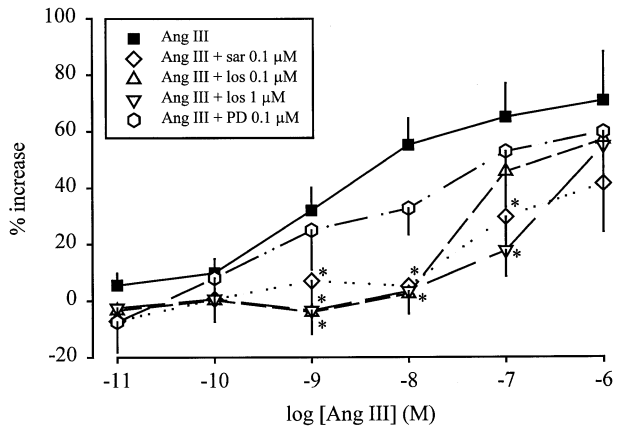

Figure 7 Effect of angiotensin III (Ang III), in the absence and presence of saralasin (sar), losartan (los) and PD 123319 (PD), on the stimulation-induced overflow of tritium from mouse spleen. The preparations were stimulated for six periods (S_1–S_6, 120 p, 3 Hz), delivered 18 min apart. Ang III was administered either alone or in the presence of sar, los or PD. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of Ang III. The vertical lines represent the s.e.mean from 4–19 preparations. Asterisks represent significant differences from Ang III given alone; $P<0.05$.

Evoked tritium overflow: spleen

Electrical field stimulation produced an increased overflow of tritium which amounted to 0.376 ± 0.035% of total tissue tritium (S_1, $n=29$).

Angiotensin II and angiotensin III increased the stimulation-induced overflow of tritium. The maximal enhancements again were about 80% and the EC_{50} values, calculated as described above, were 0.25 and 1.5 nM, respectively (Figure 5). Figure 1 (lower panel) illustrates

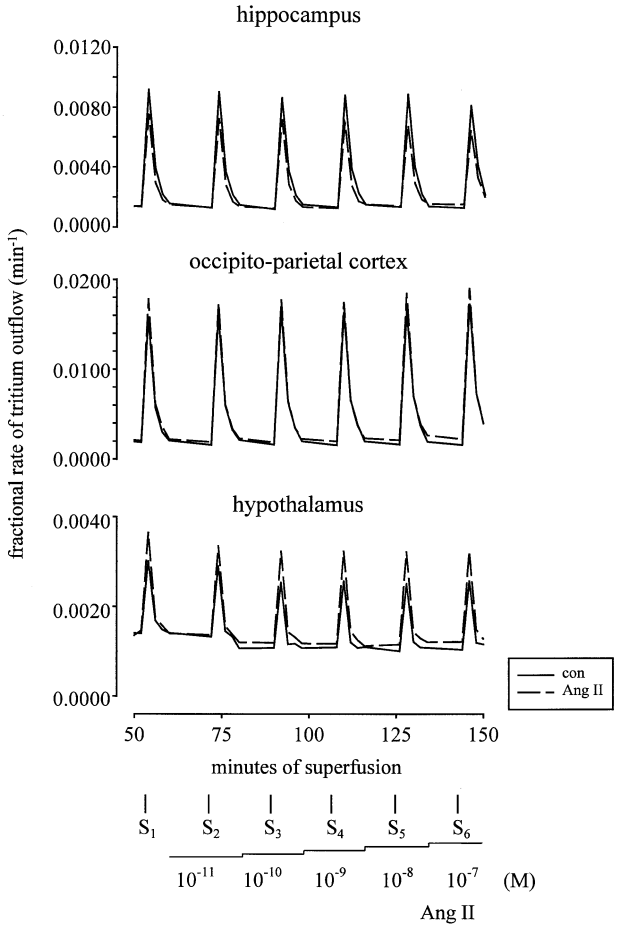

Figure 8 Tritium efflux from mouse hippocampal, occipito-parietal cortex and hypothalamic preparations in the absence and presence of angiotensin II (Ang II). Each preparation was stimulated for six periods (S_1-S_6, 36 p, 3 Hz), delivered 18 min apart. Solid lines represent the control (con) experiments, in the absence of drugs, and dashed lines, the effect of Ang II when introduced into the PSS in increasing concentrations (0.01 nM–0.1 μM), 12 min before S_2, S_3, S_4, S_5 and S_6. Each line represents the outflow of tritium from a single preparation.

the effect of angiotensin II on tritium overflow from a single preparation. Angiotensin IV and angiotensin-(1–7) were without effect (Figure 5).

Saralasin again strongly antagonized the effects of angiotensin II (Figure 6) and angiotensin III (Figure 7). Losartan produced a concentration-dependent rightward shift of the concentration-response curve of both angiotensin II (Figure 6) and angiotensin III (Figure 7). The pK_B values are given in Table 1. PD 123319 did not significantly alter the facilitatory effect of angiotensin II (Figure 6) or angiotensin III (Figure 7).

Evoked tritium overflow: hippocampus, occipito-parietal cortex and hypothalamus

The overflow of tritium evoked by the first stimulation period, S_1, amounted to 2.08 ± 0.17, 3.40 ± 0.49 and $0.44\pm0.05\%$ of total tissue tritium, in the hippocampus, occipito-parietal cortex and hypothalamus, respectively ($n=9$, 10 and 6). In the hypothalamus, the α adrenoceptor antagonist phentolamine, when added before S_3, at a concentration of 1 μM, increased the evoked overflow of noradrenaline by $397\pm26\%$ ($n=4$).

Angiotensin II, angiotensin III, angiotensin IV and angiotensin-(1–7) (each 0.1 nM–0.1 μM), when introduced in increasing concentrations, 12 min before S_2, S_3, S_4, S_5 and S_6, did not significantly alter the the stimulation-induced overflow of tritium (data not shown, $n=4-6$). Figure 8 illustrates the lack of effect of angiotensin II in a single preparation from the hippocampus, occipito-parietal cortex and hypothalamus.

Discussion

In mouse hippocampus, occipito-parietal cortex and hypothalamus, angiotensin II, angiotensin III, angiotensin IV and angiotensin-(1–7) all failed to alter the electrically-induced release of noradrenaline. Previous studies investigating the effects of angiotensin on noradrenaline release in central tissues have revealed mixed findings. In agreement with the present study, angiotensin II failed to alter the depolarization-evoked release of noradrenaline in rat occipital cortex (Taube *et al*., 1977), hypothalamus (Taube *et al*., 1977; Meldrum *et al*., 1984; Meldrum & Westfall, 1986) and locus coeruleus (Huang *et al*., 1987). Other authors have reported that angiotensin enhances depolarization-evoked noradrenaline release in rabbit (Garcia-Sevilla *et al*., 1979) and rat hypothalamus (Sato *et al*., 1993) and rat parietal cortex (Huang *et al*., 1987). The present negative findings with angiotensin contrast with the expected release-enhancing effect of phentolamine in the hypothalamus. In the mouse occipito-parietal cortex and hippocampus, the α_2 adrenoceptor antagonist rauwolscine also, in contrast to the angiotensins, increases release of noradrenaline (unpublished observations). In view of the findings to date, we are unable to identify the reason for the discrepant results on the effects of angiotensin in brain preparations.

Of the angiotensin peptide fragments studied, angiotensin IV and angiotensin-(1–7) had little or no effect in the peripheral tissues as well: only angiotensin IV, when given at high concentrations (1 and 10 μM), produced facilitation in the atria. Similarly, Storgaard & Nedergaard (1997) found that the two fragments did not alter the release of noradrenaline in the rabbit aorta, although angiotensin-(1–7) enhanced release in rat isolated atria (Gironacci *et al*., 1994). Overall, the effects of the two peptides on noradrenaline release seem to be minor.

Major, potent, and concentration-dependent facilitatory effects in both atria and spleen, however, were obtained with angiotensin II and III. In mouse atria, the observations with angiotensin II confirm previous findings by Musgrave & Majewski (1989) and Rajanayagam *et al*. (1989). Enhancement by angiotensin of noradrenaline release in the spleen apparently has not been shown previously. In the whole perfused cat spleen, angiotensin II did not alter the nerve stimulation-evoked overflow of noradrenaline (Hertting & Suko, 1966). This negative finding could be due to a species difference or to the vasoconstrictor effect of angiotensin II, which reduced flow through the spleen (Hertting & Suko, 1966) and in this way may have favoured retention of released noradrenaline (Hertting & Schiefthaler, 1963) and thus concealed the facilitatory effect of angiotensin.

Angiotensin III was approximately 13 and six times less potent than angiotensin II in the atria and spleen respectively. Previous studies investigating the pre- (Molderings *et al*., 1988; Trachte, 1988; Storgaard & Nedergaard, 1997) and postjunctional (Moore *et al*., 1976; Pendleton *et al*., 1991; Li *et al*., 1995; 1996) effects of angiotensin II and angiotensin III have generally observed a lower potency of angiotensin III compared to angiotensin II.

The facilitatory effect of angiotensin II on transmitter noradrenaline release in the mouse atria and spleen was blocked by the non selective angiotensin receptor antagonist saralasin and the selective AT_1 receptor antagonist losartan, but was not altered by the AT_2 receptor antagonist PD 123319. This finding is in accord with the majority of studies in a number of tissues from different species, including guinea-pig (Brasch *et al*., 1993) and rat atria (Gironacci *et al*., 1994), rat trachea (Boicos *et al*., 1998), rabbit iris ciliary body (Ohia & Jumblatt, 1993), and human (Rump *et al*., 1995) and dog (Wong *et al*., 1991; Suzuki *et al*., 1992) kidney. In most studies, full concentration-response curves to angiotensin in the presence of the receptor antagonists were not performed and hence, little is known about the pK_B values for losartan against the facilitatory effect of angiotensin. Guimarães *et al*. (1998) reported very low pK_B values for losartan against the facilitation of noradrenaline release by angiotensin II in canine mesenteric ($pK_B = 5.24$) and pulmonary ($pK_B = 5.40$) arteries. In the present study, the pK_B estimates for losartan against angiotensin II were similar to those previously reported for the antagonism by losartan of the contractile effects of angiotensin II (6.9 – 9.5; Zhang *et al*., 1994; Boulanger *et al*., 1995; Li *et al*., 1997; Guimarães *et al*., 1998; de Gasparo *et al*., 1998). The pK_B estimates for losartan in the present study are also similar to its pIC_{50} for inhibition of [^{125}I]-angiotensin II (0.05 nM) binding to AT_1 receptors (Chiu *et al*., 1989).

In addition to confirming the (predominant) AT_1 character of the prejunctional receptor for angiotensin II, our experiments show that the same receptor also mediates the effect of angiotensin III, both in the atria and in the spleen: in either tissue, the pK_B value of losartan against angiotensin III was similar to that against angiotensin II. Postjunctionally, angiotensin II and angiotensin III also act on the same AT_1 receptor (Li *et al*., 1997). The reason for the relatively large difference (greater than 1 log unit) in the pK_B values of losartan between the atria and spleen (Table 1) is not known, but could be due to tissue differences. Nevertheless, all values lie within the range of published pK_B values at the AT_1 receptor, as mentioned above.

In conclusion, apart from confirmation of the facilitatory effect of angiotensin II on action potential-evoked noradrenaline release in mouse atria, our study presents the following new findings: the analogous facilitatory effect of angiotensin II in mouse spleen and of angiotensin III in both peripheral tissues, the AT_1 character of the prejunctional receptor for angiotensin II and angiotensin III, the lack of, or at most weak, effect of angiotensin IV and angiotensin-(1 – 7) in atria and spleen, and the lack of any effect of all four peptides in the hippocampus, occipito-parietal cortex and hypothalamus.

S.L. Cox is a recipient of an Alexander von Humboldt-Stiftung and their support is greatly appreciated.

References

BOICOS, K.M., COX, S.L. & STORY, D.F. (1998). Modulation of noradrenergic transmission in rat isolated trachea by angiotensin I and angiotensin II. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **358** (supp 1): R293.

BOULANGER, C.M., CAPUTO, L. & LÉVY, B.I. (1995). Endothelial AT_1-mediated release of nitric oxide decreases angiotensin II contractions in rat carotid artery. *Hypertension,* **26,** 752 – 757.

BRASCH, H., SIEROSLAWSKI, L. & DOMINIAK, P. (1993). Angiotensin II increases norepinephrine release from atria by acting on angiotensin subtype 1 receptors. *Hypertension,* **22,** 699 – 704.

CHIU, A.T., HERBLIN, W.F., MCCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989). Identification of angiotensin II receptor subtypes. *Biochem. Biophys. Res. Comm.,* **165,** 196 – 203.

COX, S.L., BEN, A., STORY, D.F. & ZIOGAS, J. (1995). Evidence for the involvement of different receptor subtypes in the pre- and postjunctional actions of angiotensin II at rat sympathetic neuroeffector sites. *Br. J. Pharmacol.,* **114,** 1057 – 1063.

COX, S.L., STORY, D.F. & ZIOGAS, J. (1996a). Angiotensin II receptors involved in the enhancement of noradrenergic transmission in the caudal artery of the spontaneously hypertensive rat. *Br. J. Pharmacol.,* **119,** 965 – 975.

COX, S.L., STORY, D.F. & ZIOGAS, J. (1996b). Multiple prejunctional actions of angiotensin II on noradrenergic transmission in the caudal artery of the rat. *Br. J. Pharmacol.,* **119,** 976 – 984.

DE GASPARO, M., CATT, K.J. & INAGAMI, T. (1998). Angiotensin receptors. In *The IUPHAR Compendium of Receptor Characterization and Classification*, ed. Girdlestone, D. pp 80 – 86. Cambridge: London: IUPHAR Media.

GANTEN, D., LANG, R.E., LEHMANN, E. & UNGER, T. (1984). Brain angiotensin: on the way to becoming a well-studied neuropeptide system. *Biochem. Pharmacol.,* **33,** 3523 – 3528.

GARCIA-SEVILLA, J.A., DUBOCOVICH, M.L. & LANGER, S.Z. (1979). Angiotensin II facilitates the potassium-evoked release of ^{3}H-noradrenaline from the rabbit hypothalamus. *Eur. J. Pharmacol.,* **56,** 173 – 176.

GIRONACCI, M.M., ALDER-GRASCHINSKY, E., PEÑA, C. & ENERO, M.A. (1994). Effects of angiotensin II and angiotensin-(1-7) on the release of [^{3}H] norepinephrine from rat atria. *Hypertension,* **24,** 457 – 460.

GLOWINSKI, J. & IVERSEN, L. (1966). Regional studies of catecholamines in the rat brain-I The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. *J. Neurochem.,* **13,** 655 – 669.

GUIMARÃES, S., PAIVA, M.Q. & MOURA, D. (1998). Different receptors for angiotensin II at pre- and postjunctional level of the canine mesenteric and pulmonary arteries. *Br. J. Pharmacol.,* **124,** 1207 – 1212.

HERTTING, G. & SCHIEFTHALER, T. (1963). Beziehung zwischen Durchflussgrösse and Noradrenalinfreisetzung bei Nervenreizung der isolierten durchströmten Katzenmilz. *Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol.,* **246,** 13 – 14.

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

HUANG, Y., ROGERS, J. & HENDERSON, G. (1987). Effects of angiotensin II on [^{3}H]noradrenaline release and phosphatidylinositol hydrolysis in the parietal cortex and locus coeruleus of the rat. *J. Neurochem.,* **49,** 1541 – 1549.

JACKSON, E.K. & GARRISON, J.C. (1996). Renin and angiotensin. In *The Pharmacological Basis of Therapeutics*, Ed. 9, ed. Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. & Goodman Gilman, A. pp 733 – 758. New York: McGraw-Hill.

LI, Q., FEENSTRA, M., PFAFFENDORF, M., EIJSMAN, L. & VAN ZWIETEN, P.A. (1997). Comparative vasoconstrictor effects of angiotensin II, III, and IV in human isolated saphenous vein. *J. Cardiovasc. Pharmacol.,* **29,** 451 – 456.

LI, Q., ZHANG, J., PFAFFENDORF, M. & VAN ZWIETEN, P.A. (1995). Comparative effects of angiotensin II and its degradation products angiotensin III and angiotensin IV in rat aorta. *Br. J. Pharmacol.,* **116,** 2963 – 2970.

LI, Q., ZHANG, J., PFAFFENDORF, M. & VAN ZWIETEN, P.A. (1996). Direct positive chronotropic effects of angiotensin II and angiotensin III in pithed rats and in rat isolated atria. *Br. J. Pharmacol.,* **118,** 1653 – 1658.

MELDRUM, M.J. & WESTFALL, T.C. (1986). Angiotensin facilitation of ^{3}H-norepinephrine release in central tissue of spontaneously hypertensive rats but not in Wistar-Kyoto rats: effects of sodium depletion. *J. Cardiovasc. Pharmacol.,* **8,** 582 – 587.

MELDRUM, M.J., XUE, C.-S., BADINO, L. & WESTFALL, T.C. (1984). Angiotensin facilitation of noradrenergic neurotransmission in central tissues of the rat: Effects of sodium restriction. *J. Cardiovasc. Pharmacol.,* **6,** 989 – 995.

MOLDERINGS, G.J., LIKUNGU, J., HENTRICH, F. & GÖTHERT, M. (1988). Facilitatory prejunctional angiotensin receptors on the sympathetic nerves of the human saphenous vein and pulmonary artery. Potential involvement in β-adrenoceptor-mediated facilitation of noradrenaline release. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **338,** 228 – 233.

MOORE, A.F., HALL, M.H. & KHAIRALLAH, P.A. (1976). A comparison of the effects of angiotensin II and heptapeptide on smooth muscle (vascular and uterine). *Eur. J. Pharmacol.,* **39,** 101 – 107.

MOURA, D., VAZ-DA-SILVA, M., MAGINA, S., PINHEIRO, H. & GUIMARÀES, S. (1997). Involvement of angiotensin II receptors in the facilitatory effect of bradykinin on noradrenaline release from the rat left ventricle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** R51.

MUSGRAVE, I.F. & MAJEWSKI, H. (1989). Effect of phorbol ester and pertussis toxin on the enhancement of noradrenaline release by angiotensin II in mouse atria. *Br. J. Pharmacol.,* **96,** 609 – 616.

OHIA, S.E. & JUMBLATT, J.E. (1993). Prejunctional receptors and second messengers for angiotensin II in the rabbit iris-ciliary body. *Exp. Eye Res.,* **57,** 419 – 425.

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

PENDLETON, R.G., GESSNER, G. & HORNER, E. (1991). Comparative effects of angiotensin II and angiotensin III in rabbit adrenal and aortic tissues. *J. Pharmacol. Exp. Ther.,* **256,** 614 – 620.

RAJANAYAGAM, M.A.S., MUSGRAVE, I.F., RAND, M.J. & MAJEWSKI, H. (1989). Facilitation of noradrenaline release by isoprenaline is not mediated by angiotensin II in mouse atria and rat tail artery. *Arch. Int. Pharmacodyn.,* **299,** 185 – 199.

RUMP, L.C., BOHMANN, C., SCHAIBLE, U., SCHULTZE-SEEMAN, W. & SCHOLLMEYER, P.J. (1995). β-adrenergic, angiotensin II, and bradykinin receptors enhance neurotransmission in human kidney. *Hypertension,* **26,** 445 – 451.

SATO, K., OKUMURA, F., GOSHIMA, Y., MIYAMAE, T. & MISU, Y. (1993). L-Dopa potentiates presynaptic inhibitory α_2-adrenoceptor- but not facilitatory angiotensin II receptor-mediated modulation of noradrenaline release from rat hypothalamic slices. *Jap. J. Pharmacol.,* **62,** 119 – 122.

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

STORGAARD, T. & NEDERGAARD, O.A. (1997). Prejunctional modulation by angiotensins of noradrenaline release from sympathetic neurons in isolated rabbit aorta. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 706 – 711.

SUZUKI, Y., MATSUMURA, Y., EGI, Y. & MORIMOTO, S. (1992). Effects of losartan, a nonpeptide angiotensin II receptor antagonist, on norepinephrine overflow and antidiuresis by stimulation of renal nerves in anesthetized dogs. *J. Pharmacol. Exp. Ther.,* **263,** 956 – 963.

TAUBE, H.D., STARKE, K. & BOROWSKI, E. (1977). Presynaptic receptor systems on the noradrenergic neurones of rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **299,** 123 – 141.

TRACHTE, G.J. (1988). Angiotensin effects on vas deferens adrenergic and purinergic neurotransmission. *Eur. J. Pharmacol.,* **146,** 261 – 269.

WONG, P.C., HART, S.D. & TIMMERMANS, P.B.M.W.M. (1991). Effect of angiotensin II antagonism on canine renal sympathetic nerve function. *Hypertension,* **17,** 1127 – 1134.

ZHANG, J., PHAFFENDORF, M., ZHANG, J.S. & VAN ZWIETEN, P.A. (1994). A non-competitive type of angiotensin-receptor antagonism by losartan in renal artery preparations. *Eur. J. Pharmacol.,* **252,** 337 – 340.

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

(Received November 26, 1998
Revised April 9, 1999
Accepted April 13, 1999