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Angiotensin II receptors involved in the enhancement of noradrenergic transmission in the caudal artery of the spontaneously hypertensive rat

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1 The effects of the AT_1 receptor antagonist losartan and the AT_2 receptor antagonist PD 123319, on actions of angiotensin II in isolated caudal arteries of spontaneously hypertensive (SH) and age-matched normotensive (Wistar-Kyoto) rats were compared.

2 Angiotensin II (0.1-3 μM) produced concentration-dependent increases in perfusion pressure in artery preparations from both SH and Wistar-Kyoto (WKY) rats, the maximal increase in the SH rat being significantly greater than the increase in WKY rats. The increase in perfusion pressure in preparations from both strains of rats was prevented by losartan (0.1 μM) and unaffected by PD 123319 (0.1 μM), indicating that the vasoconstrictor action of angiotensin II is subserved by AT_1 receptors.

3 Angiotensin II (0.1-3 μM) produced concentration-dependent enhancement of both stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline and stimulation-evoked vasoconstrictor responses in isolated preparations of caudal artery from both SH and WKY rats, in which the noradrenergic transmitter stores had been labelled with [^{3}H]-noradrenaline. The maximum enhancement of S-I efflux produced by angiotensin II (1 μM) was significantly greater in artery preparations from WKY rats than in preparations from SH rats, whereas the maximum enhancement of stimulation-evoked vasoconstrictor responses was greater in preparations from SH rats than in those from WKY rats.

4 In artery preparations from both WKY and SH rats, the AT_1 angiotensin II receptor antagonist, losartan (0.01 and 0.1 μM), reduced or abolished the enhancement of both S-I efflux and vasoconstrictor responses by 1 μM angiotensin II.

5 The combination of 0.01 μM losartan and 0.1 μM angiotensin II enhanced both the S-I efflux and stimulation-evoked vasoconstrictor response in caudal artery preparations from WKY rats, whereas 0.1 μM angiotensin alone was ineffective. The AT_2 receptor antagonist PD 123319 (0.01 and 0.1 μM) prevented the enhancement of both S-I efflux and stimulation-evoked vasoconstrictor responses by the combination of angiotensin II and losartan.

6 In contrast to findings in WKY preparations and those previously obtained for arteries from another normotensive strain (Sprague-Dawley), in artery preparations from SH rats there was no synergistic interaction between losartan and angiotensin II. Rather, combinations of 0.1 μM angiotensin II and PD 123319 (both 0.01 and 0.1 μM) enhanced S-I [^{3}H]-noradrenaline efflux, whereas 0.1 μM angiotensin II alone was without effect. Moreover, losartan (0.1 μM) prevented the enhancement of S-I efflux by the combination of angiotensin II and PD 123319.

7 The present findings indicate that in the caudal artery of WKY and SH rats, and as previously found in Sprague-Dawley preparations, angiotensin II receptors similar to the AT_{1B} subtype subserve enhancement of transmitter noradrenaline release.

8 As previously suggested for Sprague-Dawley caudal artery preparations, the synergistic prejunctional interaction of losartan and 0.1 μM angiotensin II in caudal artery preparations from WKY rats may be due to either the unmasking by losartan of a latent population of angiotensin II receptors subserving facilitation of transmitter noradrenaline release, or blockade by losartan of an inhibitory action of angiotensin II on transmitter release.

9 The synergistic interaction of PD 123319 and 0.1 μM angiotensin II in caudal arteries of SH rats may also be explained by either of the mechanisms proposed for the normotensive strains, but the involvement of different receptor subtypes would need to be postulated for each of the proposed mechanisms.

Keywords: Angiotensin II; angiotensin II receptors; losartan; PD 123319; rat caudal artery; spontaneously hypertensive rat; noradrenergic transmission

Introduction

Apparently all of the numerous actions of angiotensin II are initiated by the binding of the peptide to specific receptors located on the membranes of its target cells. At least two pharmacologically distinct classes of angiotensin II receptors have been identified, AT_1 and AT_2. This classification has been based upon the differential binding affinities of selective compounds; namely the nonpeptide receptor antagonists losartan (AT_1) and PD 123319 (AT_2) (Chiu *et al.*, 1989; Dudley *et al.*, 1990). Further subdivision of the angiotensin II receptor subtypes has been proposed on the basis of findings from both cloning and receptor binding studies (Ernsberger *et al.*, 1992).

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Thus, two populations of AT_1 sites on mesangial cells were shown to bind losartan and PD 123319 with different relative affinities (Zhou *et al.*, 1993; Madhun *et al.*, 1993). The sites for which losartan had the higher affinity were classified AT_{1A} and those for which PD 123319 had the higher affinity were termed AT_{1B} (Zhou *et al.*, 1993).

Angiotensin II-mediated effects such as vascular smooth muscle contraction (Cox *et al.*, 1995), aldosterone release (Wong *et al.*, 1990a) and the regulation of fluid and electrolyte balance (Barbella *et al.*, 1993) are all blocked by losartan and unaffected by AT_2 receptor antagonists. Hence, the major physiological actions of angiotensin II appear to involve the AT_1 receptor subtype and a functional role of the AT_2 receptor remains to be determined.

We have recently found that the enhancement of transmitter noradrenaline release by angiotensin II in the caudal artery of Sprague-Dawley rats is blocked by both losartan and PD 123319 (Cox *et al.*, 1995). Thus, we suggested that the prejunctional receptor subserving the enhancement of transmitter noradrenaline release in this tissue had characteristics similar to the AT_{1B} binding site described by Zhou *et al.* (1993). In contrast, the receptor subtype subserving the direct vasoconstrictor actions of angiotensin II in the rat caudal artery was found to be sensitive to losartan but not PD 123319 (Cox *et al.*, 1995), which is consistent with results from other studies (Rhaleb *et al.*, 1991).

In our studies with isolated caudal artery preparations from a normotensive (Sprague-Dawley) strain of rat, we found that, compared to published findings in other tissues, angiotensin II had a relatively low potency in respect of its ability to enhance stimulation-induced (S-I) [^{3}H]-noradrenaline efflux (Cox *et al.*, 1996). In addition, a striking and unexpected finding of those studies was that the enhancement of S-I [^{3}H]-noradrenaline efflux and of the associated vasoconstrictor responses produced by angiotensin II, in a concentration just above threshold (0.1 μM), was greatly increased in the presence of 0.01 μM losartan. Furthermore, when, in addition to losartan, the AT_2 receptor antagonist PD 123319 was also present in the artery perfusion/superfusion solution in low concentrations (0.01 or 0.1 μM), angiotensin II (0.1 μM) failed to enhance the S-I efflux. We suggested that the synergistic interaction between losartan and angiotensin II was due either to the unmasking by losartan of a latent population of angiotensin II receptors, possibly of the AT_2 subtype which also subserve facilitation of noradrenaline release, or alternatively, to the blockade by losartan of an inhibitory action of angiotensin II on transmitter release, possibly involving AT_{1A} receptors which normally opposes its facilitatory action (Cox *et al.*, 1996).

Alterations in the activity of the sympathetic nervous system have been implicated in the development and/or maintenance of hypertension. In some experimental models of hypertension, enhanced facilitation by angiotensin II of transmitter noradrenaline release at sympathetic neuroeffector sites, compared with that in normotensive animals, has been demonstrated (Eikenburg *et al.*, 1981; Westfall *et al.*, 1984). In addition to enhanced facilitation of transmitter release by angiotensin II, enhanced smooth muscle responsiveness to exogenous angiotensin II and other vasoconstrictor stimuli have been obtained in spontaneously hypertensive (SH) rats (Lais & Brody, 1978; Collis *et al.*, 1980). In SH rats, the receptors involved in the prejunctional facilitatory effects of angiotensin II at noradrenaline receptor sites in the heart and some blood vessels have been shown to be blocked by AT_1 but not AT_2 receptor antagonists (Nagase *et al.*, 1994; Foucart *et al.*, 1994). However, the angiotensin II receptor(s) involved in the direct vasoconstrictor action of angiotensin II in hypertensive rats have not been well documented. Losartan has been demonstrated to be effective in reducing blood pressure in human hypertensive patients (Weber, 1992) and in spontaneously hypertensive dogs (Bovee *et al.*, 1991) and SH rats (Wong *et al.*, 1990b).

The aim of the present study was to characterize the angiotensin II receptors subserving modulation of sympathetic noradrenergic transmission in SH rats. In view of the findings of our study in caudal artery preparations from Sprague-Dawley rats (Cox *et al.*, 1996), we were particularly interested in the possibility of interactions between low concentrations of subtype-selective antagonists and angiotensin II leading to increased enhancement of transmitter moradrenaline release, compared to that produced by angiotensin II alone. The non-peptide angiotensin II receptor antagonists losartan and PD 123319 were used to characterize and compare the receptor subtype(s) through which angiotensin II mediates its effects on sympathetic neuroeffector function in isolated caudal arteries from spontaneously hypertensive and normotensive (Wistar Kyoto) rats. In addition, the responsiveness of the artery preparations of the two strains of rats to nerve stimulation and angiotensin II were compared.

Methods

Experiments were performed with caudal arteries isolated from spontaneously hypertensive (SH) (Yamori strain, 180–240 g body weight, 12–16 weeks of age) and age-matched normotensive Wistar-Kyoto (WKY) rats obtained from the Austin Hospital (Melbourne, Australia). Both strains of rats were held under the same conditions, receiving normal laboratory diet (GR2 Barastock) and tap water *ad libitum*. Systolic arterial blood pressure was routinely measured by tail-cuff plethysmography several times before rats were used. The average of the final 3 measurements in each rat was taken as the mean systolic blood pressure. The mean systolic blood pressure for the SH rats was 190 ± 5 ($n = 27$) and that for the WKY rats was 137 ± 5 mmHg ($n = 20$).

Caudal artery preparation

Caudal artery preparations were set up for perfusion and superfusion with physiological salt solution (PSS), as described previously (Cox *et al.*, 1996).

In the first series of experiments, vasoconstrictor responses to angiotensin II (0.1–3 μM) were established by introducing angiotensin II into the PSS perfusing/superfusing the artery preparations. To avoid problems with tachyphylaxis, each artery preparation was exposed only once to angiotensin II. In experiments in which the effect of losartan and PD 123319 on vasoconstrictor responses to angiotensin II were investigated, the antagonists (0.1 μM) were introduced into the perfusion solution 20 min before the introduction of angiotensin II.

In the second series of experiments, the noradrenergic transmitter stores of the artery preparations were radiolabelled with [^{3}H]-noradrenaline (52.3 Ci mmol^{-1}, 0.1 μM) and perfused and superfused with PSS. The periarterial sympathetic nerves of the artery preparations were subjected to two periods of electrical field stimulation (5 Hz, 30 s), delivered 30 min apart. For each period of stimulation, 6 consecutive 1-min collections (4 ml volume) of the perfusate/superfusate solution were taken for measurement of the efflux of [^{3}H]-noradrenaline from the artery preparations, the train of stimulation being delivered after the second collection in each case. In experiments in which the effects of angiotensin II and the non-peptide angiotensin II antagonists were investigated, these drugs were introduced to the perfusion/superfusion fluid 20 min before the second period of stimulation. All of these procedures have been described previously (Cox *et al.*, 1996).

[^{3}H]-noradrenaline present in the perfusate/superfusate collection was separated chromatographically from its tritiated metabolites and measured by liquid scintillation counting (Cox *et al.*, 1996). The values of resting and stimulation-induced effluxes of [^{3}H]-noradrenaline given have not been corrected for recovery ($75 \pm 3\%$, $n = 16$).

In each experiment, the resting and S-I effluxes of [^{3}H]-

noradrenaline were determined, again, as previously described (Cox *et al.*, 1996), the second period of stimulation in each case being expressed as a percentage of the corresponding value for the first period of stimulation (% R_2/R_1 and % S_2/S_1, respectively).

Drugs and radiochemicals

The physiological salt solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.03 and D-(+)-glucose 11.1. Ethylenediaminetetraacetic acid disodium salt (0.067 mM) and ascorbic acid (0.14 mM) were also present to minimise the oxidation of noradrenaline.

The following drugs were used: angiotensin II (synthetic, human sequence, Sigma, U.S.A.); losartan (gift from Du Pont Merck Pharmaceuticals, Wilmington, Delaware); and 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, ditrifluoroacetate, dihydrate, gift from Parke Davis, Ann Arbor, Michigan).

Angiotensin II stock solutions (1 mM) were prepared in 5% acetic acid and stored in 50 μl aliquots at 0°C. Stock solutions of losartan and PD 123319 (1 mM) were prepared in deionised water and stored at 5°C. All stock solutions were diluted in PSS and added to the reservoirs supplying the organ bath.

Tritiated noradrenaline ([2,5,6-^{3}H]-(−)-noradrenaline) was supplied by the Radiochemical Centre, Amersham, U.K., with a specific activity of 52.3 Ci $mmol^{-1}$ and a radioactive concentration of 1 mCi ml^{-1}.

Statistical analysis

Data are expressed as means ± s.e.mean; *n* denotes the number of experiments. The statistical significance of differences between means was determined by unpaired, two-tailed Student's *t* test or, in the case of multiple (more than two) groups, by first testing for global differences between experimental means by analysis of variance (ANOVA). Student-Newman-Keuls (SNK) test was used to test for differences between predetermined pairs of means. The significance of differences between the effects of individual concentrations of angiotensin II and the relevant control value was determined by Dunnett's test. All statistical analyses were performed using the computer programme Sigmastat for Windows (Version 1.0, Jandel Scientific). In all cases the probability levels less than 0.05 ($P<0.05$) were taken to indicate significant differences.

Results

Vasoconstrictor responses to angiotensin II

When isolated caudal artery preparations from either SH or WKY rats were perfused and superfused at a constant flow rate of 4 ml min^{-1}, the luminal perfusion pressure stabilized within 10–15 min and remained stable for the duration of the experiment. The stable perfusion pressure was significantly (*t* test) greater in arteries of SH rats (45.2 ± 2.0 mmHg, $n=24$) compared to arteries from WKY rats (31.1 ± 4.0 mmHg, $n=8$).

Introduction of angiotensin II (0.1–3 μM) into the perfusion/superfusion solution produced concentration-dependent increases in perfusion pressure in preparations from both strains of rats. Increases in perfusion pressure were taken as an index of vasoconstriction. As shown in Figure 1, except with 0.1 μM angiotensin II, the means of the vasoconstrictor responses were greater in artery preparations from SH rats than in preparations from WKY rats. With preparations from both strains of rats, the peak increases in perfusion pressure occurred within 20–30 s and, in the continued presence of angiotensin II, the pressure returned to basal levels over the next 2 to 3 min.

Neither losartan (0.1 μM) nor PD 123319 (0.1 μM), when present alone, significantly altered basal perfusion pressure in preparations from either SH or WKY rats (*t* test, data not

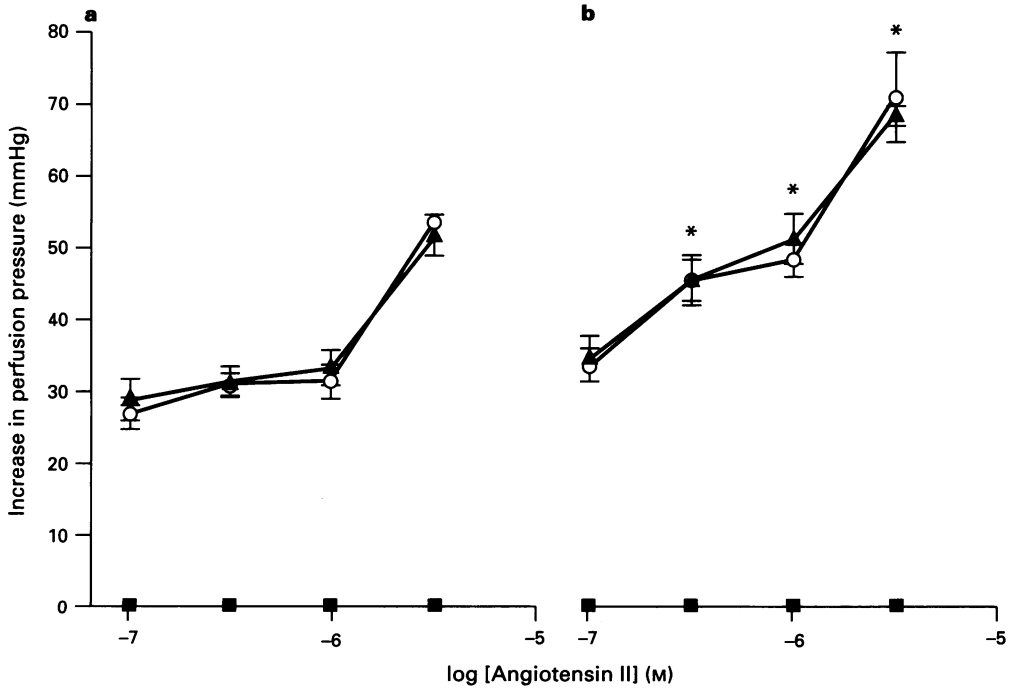

Figure 1 Effects of losartan and PD 123319 on the vasoconstrictor response to angiotensin II in isolated perfused and superfused caudal artery preparations from WKY (a) and SH (b) rats. The antagonists were introduced 20 min before the introduction of angiotensin II. The vertical axis represents the increase in perfusion pressure in mmHg. The points plotted show the mean increases in perfusion pressure produced by angiotensin II (0.1–3 μM) in the absence (○) and presence of the antagonists losartan (■) and PD 123319 (▲) (each 0.1 μM). The vertical lines represent the s.e.means from 4–11 preparations (note that in some cases these were smaller than the symbol representing the mean). Losartan abolished the vasoconstrictor response to angiotensin II. The asterisks indicate significant differences in the mean response to angiotensin II alone, between preparations from WKY and SH rats (SNK test).

shown). The AT_1 receptor antagonist losartan (0.1 μM), when introduced 20 min before angiotensin II (0.1–3 μM), abolished the vasoconstrictor action of angiotensin II in preparations from both strains of rats (Figure 1). In contrast, as shown in Figure 1, the vasoconstrictor responses to angiotensin II (0.1–3 μM) were unaltered by the presence of the AT_2 receptor antagonist PD 123319 (0.1 μM).

Effects of angiotensin II on S-I efflux and stimulation-evoked vasoconstrictor responses

Noradrenergic nerves of caudal artery preparations from both WKY and SH rats were loaded with [^{3}H]-noradrenaline and subjected to two periods of electrical field stimulation (5 Hz, 30 s), delivered 30 min apart. The absolute resting and S-I effluxes of [^{3}H]-noradrenaline for the first period of stimulation (d.p.m.) and the resting and S-I effluxes for the second period, expressed as percentages of their corresponding values for the first period, are given in Table 1. The resting efflux of [^{3}H]-noradrenaline from artery preparations from SH rats, preceding the first period of stimulation, was about 50% greater than that from artery preparations from WKY rats. However, there was no significant difference in the absolute S-I efflux of [^{3}H]-noradrenaline evoked by the first period of stimulation between the two strains (Table 1). There were also no significant differences between preparations from the two strains in the mean values of either resting or S-I effluxes for the second period of stimulation, expressed as percentages of the corresponding effluxes for the first period (Table 1).

Introduction of angiotensin II (0.1–3 μM) into the perfusion/superfusion fluid, 20 min before the second period of stimulation, did not significantly alter the resting efflux of [^{3}H]-noradrenaline from artery preparations of either WKY or SH rats (ANOVA-SNK, data not shown). In the lowest concentration tested (0.1 μM) angiotensin II did not significantly alter the S-I efflux from arteries of WKY or SH rats (Figure 2). However, angiotensin II in concentrations of 0.3 and 1 μM (WKY) and 1 μM (SH), enhanced the S-I efflux (Figure 2). The nature of the relationship between the concentration of angiotensin II and the magnitude of the increase in S-I efflux differed between artery preparations of the two strains, such that the maximal enhancement of S-I efflux (with 1 μM) was greater with WKY preparations than with SH preparations (Figure 2). With artery preparations from both strains, angiotensin II, in the highest concentration tested (3 μM), did not significantly alter S-I efflux.

Table 1 Resting and stimulation-induced (S-I) effluxes from [^{3}H]-noradrenaline-loaded caudal arteries of Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats

	WKY (*n*=5)	*SH* (*n*=8)
First period of stimulation		
Resting efflux (d.p.m.)	229±15	361±28*
S-I efflux (d.p.m.)	1371±247	1029±151
Second period of stimulation		
Resting efflux (% R_2/R_1)	86±8	88±5
S-I efflux (% S_2/S_1)	98±6	95±6

Values are means±s.e.mean, the number of preparations (*n*) from each strain is indicated. The asterisk indicates a significant difference compared with preparations from WKY rats (*t* test).

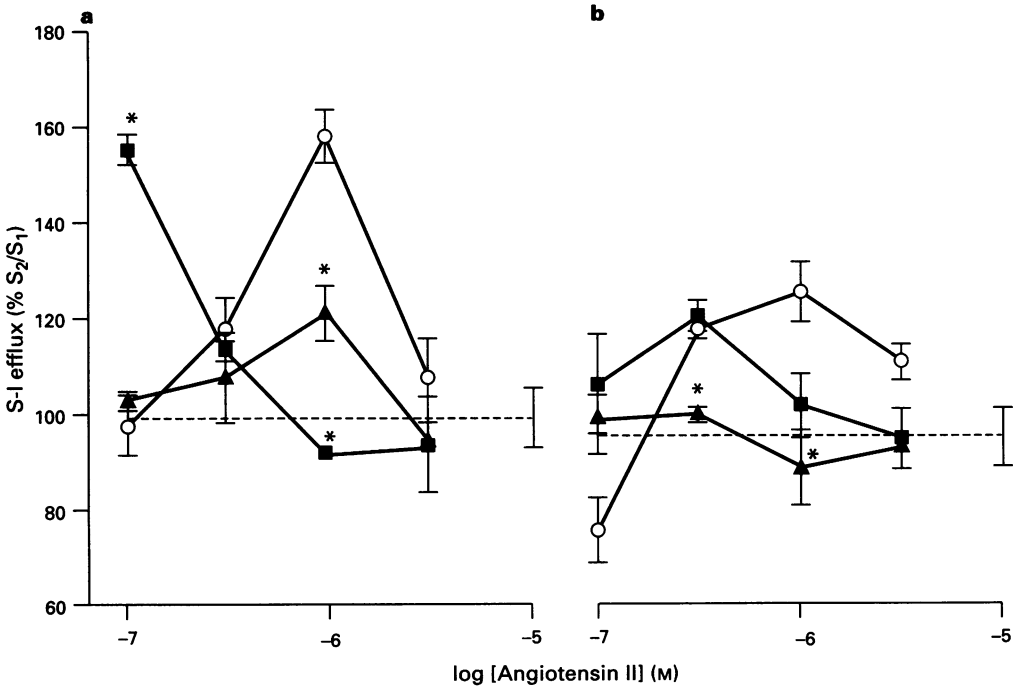

Figure 2 Effect of angiotensin II, in the absence and presence of losartan, on the stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline from WKY (a) and SH (b) rat isolated caudal artery preparations in which the noradrenergic transmitter stores had been radiolabelled with [^{3}H]-noradrenaline. The periarterial sympathetic nerves were stimulated for 2 periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the S-I efflux with the second period of stimulation was expressed as a percentage of that with the first period (% S_2/S_1). Angiotensin II (0.1–3 μM, ○), or the combination of angiotensin II and losartan (0.01, ■ or 0.1 μM, ▲), were introduced into the PSS 20 min before the second period of stimulation. The points plotted represent the means and vertical lines the s.e.mean from 4–11 preparations (note that in one case the s.e. was smaller than the symbol representing the mean). The broken horizontal lines in each panel represent the mean S-I effluxes in the absence of angiotensin II, the s.e.means being shown at the right hand end of the lines. Note that the data for losartan alone are given in Table 2. S-I efflux was significantly enhanced by 0.3 and 1 μM angiotensin II in WKY preparations and by 1 μM angiotensin II in SH preparations (Dunnett's test). The asterisks indicate that the effect of a combination of losartan and angiotensin II was significantly different from that of angiotensin II alone (SNK test).

Changes in perfusion pressure in response to electrical field stimulation of the periarterial sympathetic nerves were also measured. In artery preparations from both SH and WKY rats, each of the two periods of field stimulation (5 Hz, 30 s) produced increases in perfusion pressure. The mean peak increase in perfusion pressure with the first period of stimulation was significantly (t tests) greater in preparations from SH rats (146.6±11.4 mmHg, $n=24$) than that in preparations from WKY rats (75.3±14.5 mmHg, $n=10$). In each experiment the vasoconstrictor response to the second period of stimulation was expressed as a percentage of that with the first period (% V_2/V_1). The mean control values of % V_2/V_1 for preparations from WKY and SH rats were 99.2±4.8% ($n=5$) and 101.1±2.4% ($n=8$), respectively, these values not differing significantly (t tests).

When introduced into the PSS 20 min before the second period of stimulation, angiotensin II produced concentration-dependent enhancement of the stimulation-evoked vasoconstrictor responses in artery preparations from both SH and WKY rats (Figure 3). Angiotensin II significantly enhanced S-I efflux in the concentration range 0.3–3 μM, in preparations from both WKY and SH rats. In preparations from both strains, the maximum enhancement of stimulation-evoked vasoconstrictor response was observed with 1 μM angiotensin II, the effect with this concentration being significantly greater in preparations from SH rats than in preparations from WKY rats (SNK test). In preparations from both strains of rats, a further increase in concentration of angiotensin II to 3 μM produced a smaller enhancement (Figure 3).

Effects of losartan on the enhancement of S-I efflux and vasoconstrictor responses by angiotensin II

Losartan in concentrations of 0.01 and 0.1 μM, added to the PSS 20 min before the second period of stimulation, did not significantly alter either the resting (data not shown) or S-I (Table 2) effluxes of [^{3}H]-noradrenaline from artery preparations from either SH or WKY rats (t tests).

Table 2 Effects of antagonists on the stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline from isolated caudal artery preparations from Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats in which the noradrenergic transmitter stores had been radiolabelled with [^{3}H]-noradrenaline

Antagonist		*Mean % S_2/S_1* WKY	SH
Losartan	0.01 μM	80.4±10.0	101.5±1.6
	0.1 μM	90.1±1.8	93.1±2.1
PD 123319	0.01 μM	98.6±5.8	96.2±0.8
	0.1 μM	93.9±8.5	94.1±4.4

The S-I efflux of [^{3}H]-noradrenaline for the second period of stimulation (S_2) was expressed as a percentage of that for the first period (% S_2/S_1). Values are mean±s.e.mean from 4 preparations in each case. Antagonists were present 20 min before the second period of stimulation.

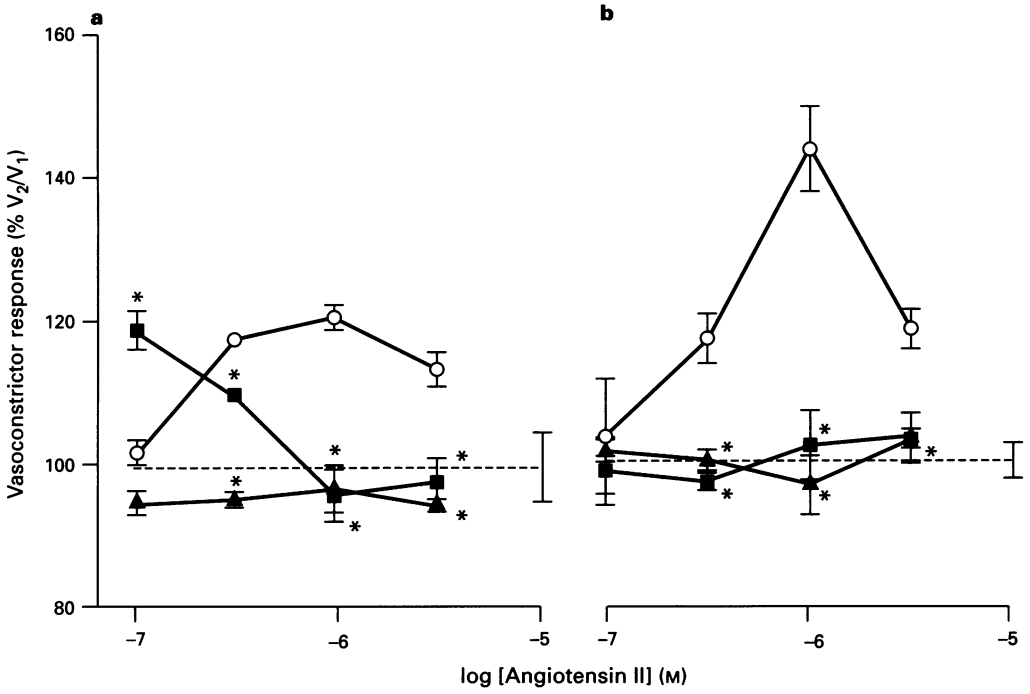

Figure 3 Effect of angiotensin II, in the absence and presence of losartan, on the stimulation-evoked vasoconstrictor responses from WKY (a) and SH (b) rat isolated caudal artery preparations. The periarterial sympathetic nerves were stimulated for 2 periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the stimulation-evoked vasoconstrictor response with the second period of stimulation was expressed as a percentage of that with the first period (% V_2/V_1). Angiotensin II (0.1–3 μM, ○), or the combination of angiotensin II and losartan (0.01, ■ or 0.1 μM, ▲), were introduced into the PSS 20 min before the second period of stimulation. The points plotted represent the means and vertical lines the s.e.mean from 4–11 preparations (note that in one case the s.e. was smaller than the symbol representing the mean). The broken horizontal lines represent the mean stimulation-evoked vasoconstrictor responses in the absence of angiotensin II, the s.e.means being shown at the right hand end of the lines. Note that the data for losartan alone are given in the text. Angiotensin II significantly enhanced stimulation-evoked vasoconstriction in the concentration range 0.3–3 μM in preparations from both strains of rats (ANOVA-SNK). The asterisks indicate that the effect of a combination of losartan and angiotensin II was significantly different from that of angiotensin II alone (SNK test). In SH preparations, with 3 μM angiotensin II the asterisk refers to the difference with 0.1 μM losartan.

WKY artery preparations In preparations from WKY rats, the effect of losartan on the enhancement of S-I efflux by angiotensin II was dependent upon the concentration of both losartan and angiotensin II. As shown in Figure 2a, the enhancing effect of angiotensin II in its maximally effective concentration (1 μM) was reduced or abolished by losartan (0.01 and 0.1 μM). Angiotensin II in the lowest concentration tested (0.1 μM), was still without effect on S-I efflux in the presence of 0.1 μM losartan. However, in the presence of the lower concentration of losartan (0.01 μM), angiotensin II markedly enhanced S-I efflux (Figure 2a).

Losartan alone, in concentrations of 0.01 and 0.1 μM, was also without significant effect (t tests) on the stimulation-evoked vasoconstrictor responses of the artery preparations, the values of % V_2/V_1, in the presence of 0.01 and 0.1 μM losartan being $103.1 \pm 2.0\%$ ($n=4$) and $100.4 \pm 0.4\%$ ($n=4$), respectively.

As shown in Figure 3a the enhancing effects of angiotensin II in the two highest concentrations tested (1 and 3 μM) were abolished by 0.01 and 0.1 μM losartan. The enhancing effect of 0.3 μM angiotensin II was reduced by 0.01 μM losartan and abolished by 0.1 μM losartan. Angiotensin II in the lowest concentration tested (0.1 μM) was again without effect on the vasoconstrictor response to stimulation in the presence of 0.1 μM losartan but, when angiotensin II (0.1 μM) was present in combination with the lower concentration of losartan (0.01 μM), the vasoconstrictor response to stimulation was enhanced (Figure 3).

SH artery preparations As shown in Figure 2b, in preparations from SH rats, losartan prevented (0.1 μM) or reduced (0.01 μM) the enhancement of S-I efflux by 1 μM angiotensin II. In contrast to the finding with WKY preparations, the combination of 0.01 μM losartan with 0.1 μM angiotensin II failed to alter S-I efflux (Figure 2b).

Losartan alone, in concentrations of 0.01 and 0.1 μM, was also without significant effect (t tests) on the stimulation-evoked vasoconstrictor responses of the artery preparations, the values of % V_2/V_1 in the presence of 0.01 and 0.1 μM losartan being $101.4 \pm 3.0\%$ ($n=4$) and $98.6 \pm 2.1\%$ ($n=4$), respectively.

As shown in Figure 3b, the enhancements of % V_2/V_1 by angiotensin II (0.3–3 μM) were abolished by losartan (0.01 and 0.1 μM).

Effects of PD 123319 on the enhancement of S-I effluxes and vasoconstrictor responses by angiotensin II

The angiotensin AT_2 antagonist PD 123319 (0.01 and 0.1 μM), added alone to the perfusion/superfusion fluid 20 min before the second period of stimulation, did not significantly alter either the resting (data not shown) or S-I (Table 2) effluxes of [^{3}H]-noradrenaline from artery preparations of WKY or SH rats (t tests).

WKY artery preparations PD 123319 in concentrations of 0.01 and 0.1 μM abolished the enhancement of S-I efflux by 0.3 and 1 μM angiotensin II in preparations from WKY rats (Figure 4a).

PD 123319 alone, in concentrations of 0.01 and 0.1 μM, was also without significant effect (t tests) on the stimulation-evoked vasoconstrictor responses of the artery preparations, the values of % V_2/V_1, in the presence of 0.01 and 0.1 μM PD 123319 being $103.4 \pm 1.5\%$ ($n=4$) and $99.8 \pm 2.7\%$ ($n=4$), respectively.

The effects of PD 123319 on the enhancement of stimulation-evoked vasoconstrictor responses by angiotensin II were

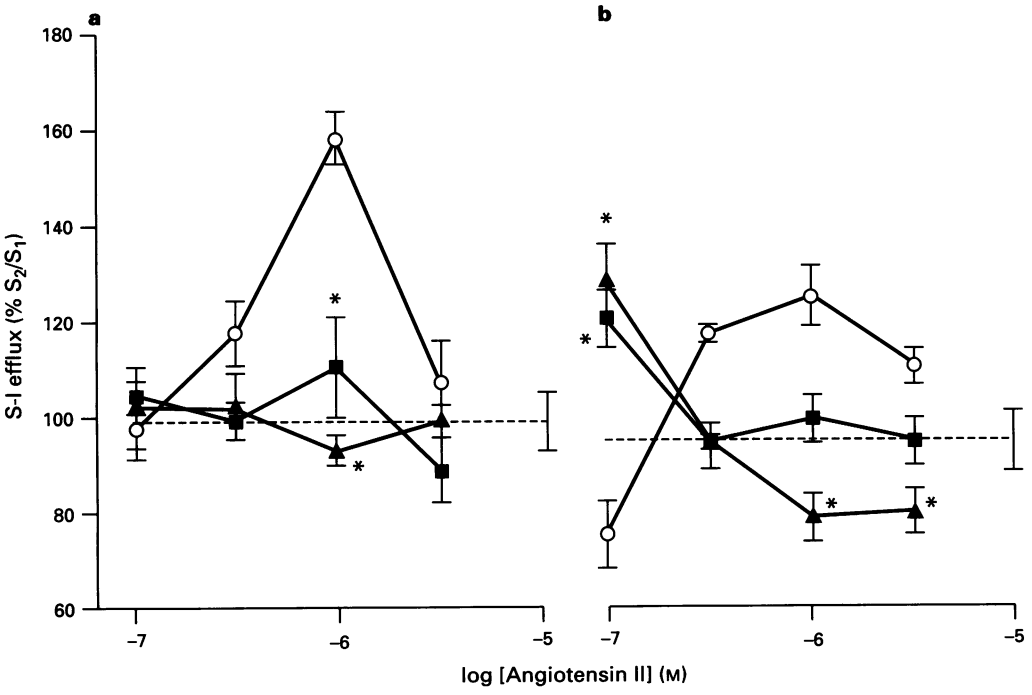

Figure 4 Effect of angiotensin II, in the absence and presence of PD 123319, on the stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline from WKY (a) and SH (b) rat isolated caudal artery preparations in which the noradrenergic transmitter stores had been radiolabelled with [^{3}H]-noradrenaline. The periarterial sympathetic nerves were stimulated for 2 periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the S-I efflux with the second period of stimulation was expressed as a percentage of that with the first period (% S_2/S_1). Angiotensin II (0.1–3 μM, ○), or the combination of angiotensin II and PD 123319 (0.01, ■ or 0.1 μM, ▲), were introduced into the PSS 20 min before the second period of stimulation. The points plotted represent the means and vertical lines the s.e.mean from 4–11 preparations (note that with the combination of 0.1 μM PD 123319 and 0.3 μM angiotensin II in SH preparations, the s.e. is smaller than the symbol representing the mean). The broken horizontal lines in each panel represent the mean S-I effluxes in the absence of angiotensin II, the s.e.means being shown at the right hand end of the lines. Note that the data for PD 123319 alone are given in Table 2. The asterisks indicate that the effect of a combination of PD 123319 and angiotensin II was significantly different from that of angiotensin II alone (SNK test).

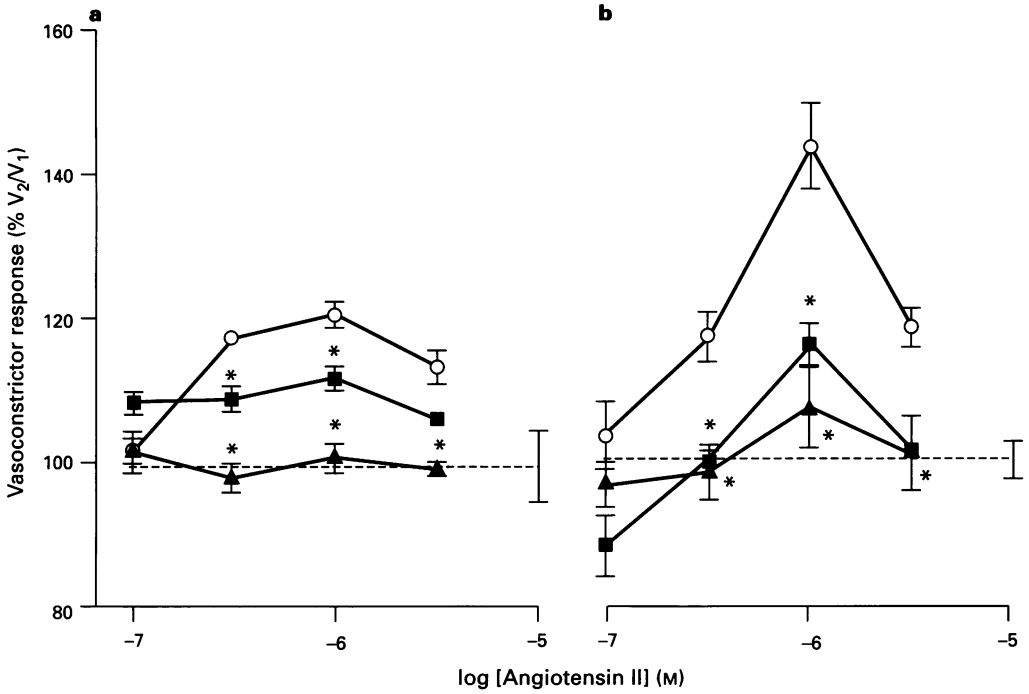

Figure 5 Effect of angiotensin II, in the absence and presence of PD 123319, on the stimulation-evoked vasoconstrictor responses from WKY (a) and SH (b) rat isolated caudal artery preparations. The periarterial sympathetic nerves were stimulated for 2 periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the stimulation-evoked vasoconstrictor response with the second period of stimulation was expressed as a percentage of that with the first period (% V_2/V_1). Angiotensin II (0.1–3 μM, ○), or the combination of angiotensin II and PD 123319 (0.01, ■ or 0.1 μM, ▲) were introduced into the PSS 20 min before the second period of stimulation. The points plotted represent the means and vertical lines the s.e.mean from 4–11 preparations (note that in some cases the s.e. are smaller than the symbols representing the mean). The broken horizontal lines represent the mean stimulation-evoked vasoconstrictor responses in the absence of angiotensin II, the s.e.means being shown at the right hand end of the lines. Note that the data for PD 123319 alone are given in the text. The asterisks indicate that the effect of a combination of PD 123319 and angiotensin II was significantly different from that of angiotensin II alone (SNK test).

similar to the findings for S-I [^{3}H]-noradrenaline efflux. Thus, as shown in Figure 5, the enhancement of % V_2/V_1 by angiotensin II (0.3–3 μM) were reduced or abolished by PD 123319 (0.01 and 0.1 μM) (Figure 5a).

SH artery preparations In preparations from SH rats, the effect of PD 123319 on the enhancement of S-I efflux by angiotensin II was dependent upon the concentrations of both angiotensin II and PD 123319. Thus, the enhancing effect of angiotensin II in a concentration of 1 μM, was reduced or abolished by both 0.01 and 0.1 μM PD 123319. However, the combination of 0.1 μM angiotensin II and PD 123319 (0.01 or 0.1 μM), significantly increased S-I efflux in contrast to angiotensin II alone (Figure 4b).

PD 123319 alone, in concentrations of 0.01 and 0.1 μM, was also without significant effect (t tests) on the stimulation-evoked vasoconstrictor responses of the artery preparations, the values of % V_2/V_1 in the presence of 0.01 and 0.1 μM PD 123319 being 98.5±2.7% (n=4) and 103.2±2.0% (n=4), respectively.

The enhancement of stimulation-evoked vasoconstrictor responses by angiotensin II (0.3–3 μM) were reduced or abolished by PD 123319 (0.01 and 0.1 μM). In contrast to the findings with S-I [^{3}H]-noradrenaline efflux, the effects of combinations of PD 123319 (0.01 or 0.1 μM) with 0.1 μM angiotensin II on vasoconstrictor responses were not significantly different from that of 0.1 μM angiotensin II alone (Figure 5b).

Effects of PD 123319 on the interaction of angiotensin II and losartan on S-I efflux and vasoconstrictor responses in preparations from WKY rats

Introduction of PD 123319 (0.01 or 0.1 μM), 20 min before the second period of stimulation, prevented the enhancement of S-I [^{3}H]-noradrenaline efflux produced by the combination of 0.01 μM losartan and 0.1 μM angiotensin II (Figure 6a). Moreover, in the presence of PD 123319 in the lower concentration (0.01 μM), the combination of 0.01 μM losartan and 0.1 μM angiotensin II, did not enhance S-I efflux, but produced a small reduction (Figure 6a).

PD 123319 (0.01 and 0.1 μM) prevented the enhancement of stimulation-evoked vasoconstriction produced by the combination of 0.1 μM angiotensin II and 0.01 μM losartan (Figure 6b).

Effects of losartan on the interaction of angiotensin II and PD 123319 on S-I efflux in preparations from SH rats

The additional presence of losartan (0.1 μM) with the combination of PD 123319 (0.01 or 0.1 μM) and 0.1 μM angiotensin II, from 20 min before the second period of stimulation, prevented the enhancement of S-I efflux produced by the combination of angiotensin II (0.1 μM) and PD 123319 (0.01 (a) and 0.1 μM (b)). These findings are summarized in Figure 7.

Discussion

Interactions between the renin-angiotensin system and the sympathetic nervous system in the development and/or maintenance of high blood pressure in spontaneously hypertensive rats have been widely studied (see Lokhandwala & Eikenburg, 1983). The significantly greater basal luminal perfusion pressure observed in the present study in caudal artery preparations from SH rats compared to that in preparations from WKY rats is consistent with findings with the mesenteric vasculature (Lais & Brody, 1978; Ekas & Lokhandwala, 1981).

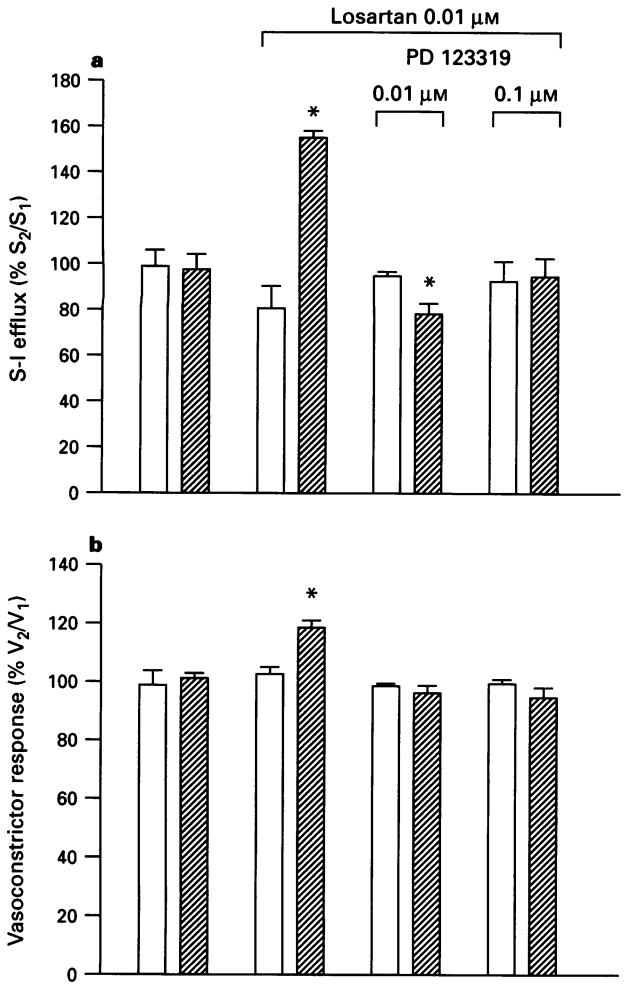

Figure 6 Effect of angiotensin II alone, in the presence of losartan and in the presence of losartan plus PD 123319 on the stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline (a) and on the associated stimulation-evoked vasoconstrictor response (b) from WKY rat caudal artery preparations. The periarterial sympathetic nerves were stimulated for two periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the S-I efflux and vasoconstriction with the second period of stimulation was expressed as a percentage of that with the first period (% S_2/S_1 and % V_2/V_1, respectively). Angiotensin II (0.1 μM, hatched columns) was introduced into the PSS perfusing and superfusing the artery preparations alone, or together with losartan (0.01 μM), or losartan plus PD 123319 (0.01 or 0.1 μM), 20 min before the second period of stimulation. The columns represent the means and the vertical lines the s.e.means from 4–6 experiments. The asterisks indicate significant differences between pairs of means (SNK test).

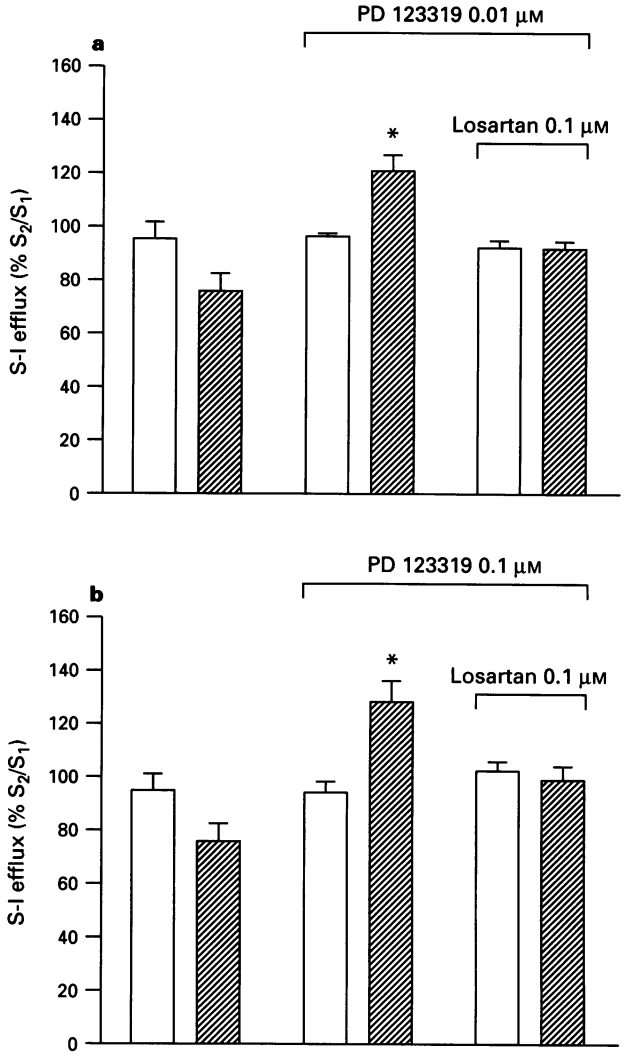

Figure 7 Effect of angiotensin II alone and in the presence of PD 123319 and combination of PD 123319 plus losartan, on the stimulation-induced (S-I) efflux of [^{3}H]-noradrenaline from SH rat caudal artery preparations. The periarterial sympathetic nerves were stimulated for two periods (5 Hz, 30 s), at 30 min intervals. In each experiment, the S-I with the second period of stimulation was expressed as a percentage of that with the first period (% S_2/S_1). Angiotensin II (0.1 μM, hatched columns) was introduced into the PSS perfusing and superfusing the artery preparations alone, or together with PD 123319 (0.01 μM, a, or 0.1 μM, b), or PD 123319 plus losartan (0.1 μM), 20 min before the second period of stimulation. The columns represent the means and the vertical lines the s.e.means from 4–8 experiments. The asterisks indicate significant differences between pairs of means (SNK test).

Previous studies have also demonstrated greater responsiveness to sympathetic nerve stimulation, to exogenous angiotensin II and also greater enhancement of vasoconstrictor responses to sympathetic nerve stimulation by angiotensin II in vascular preparations from SH rats compared to those from WKY (Collis *et al.*, 1980; Eikenburg *et al.*, 1981). Our findings are in accord with such observations. Thus, the responses induced by electrical field stimulation of the periarterial sympathetic nerves, the vasoconstrictor responses to angiotensin II and the enhancement by angiotensin II of the responses to sympathetic nerve stimulation were all greater in caudal artery preparations from SH rats than in those from WKY rats.

The postjunctional angiotensin II receptors involved in contraction of vascular smooth muscle from normotensive animals appears to be the AT_1 subtype as the responses are blocked by losartan but not by AT_2 receptor antagonists. Losartan has been observed to block the vasoconstrictor actions of angiotensin II in rabbit renal artery (Zhang *et al.*, 1994), rat portal vein, rabbit aorta and pulmonary artery (Rhaleb *et al.*, 1991). Likewise, we previously described blockade by losartan of the vasoconstrictor response to angiotensin II in the caudal artery of Sprague-Dawley rats (Cox *et al.*, 1995). The receptor subtype mediating vasoconstriction by angiotensin II in the SH rat has not been clearly established. In the present study, in arteries from both normotensive (WKY) and SH rats, losartan but not PD 123319 blocked the vasoconstrictor effect of angiotensin II. Thus, it appears that, in both normotensive and SH rats, AT_1 receptors subserve contraction of the vascular smooth muscle of the caudal artery.

In isolated caudal artery preparations from both WKY and SH rats, in which transmitter stores had been radiolabelled with [^{3}H]-noradrenaline, angiotensin II produced a concentration-dependent enhancement of both the S-I efflux of [^{3}H]-noradrenaline and the associated vasoconstrictor re-

sponse. In preparations from both strains of rats, the maximal enhancement of S-I [^{3}H]-noradrenaline efflux and of the vasoconstrictor response occurred with a concentration of 1 μM and a further increase in concentration to 3 μM produced less enhancement. We have previously shown this characteristic 'bell-shaped' concentration-effect relationship for the modulation of sympathetic transmission by angiotensin II in the caudal artery of another normotensive strain of rat (Sprague-Dawley; Cox *et al.*, 1995; 1996) and in guinea-pig atria (Ziogas *et al.*, 1984). The reason for the decline in the effects of angiotensin II when its concentration was increased from 1 to 3 μM was not investigated in the present study, but could possibly be explained by receptor desensitization.

The maximal enhancement of S-I [^{3}H]-noradrenaline efflux in artery preparations from SH rats was significantly less than that in arteries of WKY rats. This is in contrast to findings of other investigators, who have obtained greater enhancement of the S-I release of [^{3}H]-noradrenaline by angiotensin II in isolated preparations from SH rats than in those from WKY rats, such as perfused mesenteric vasculature (Eikenburg *et al.*, 1981) and perfused kidneys (Collis *et al.*, 1980). The findings with S-I efflux also stand in contrast to our findings on the stimulation-evoked vasoconstrictor response. Thus, as previously stated, angiotensin II (0.1–3 μM) produced greater enhancement of the vasoconstrictor responses of caudal artery preparations from SH rats than of those from WKY rats.

Previous workers have obtained no significant difference in the resting release of [^{3}H]-noradrenaline between artery preparations of WKY and SH rats, but noted greater S-I [^{3}H]-noradrenaline release in preparations from SH rats compared to those from WKY rats. This is in contrast to our findings of greater resting release of [^{3}H]-noradrenaline in preparations from SH rats than in those from WKY rats, and of no significant difference between the two strains of rats in the S-I release of [^{3}H]-noradrenaline. At present we are unable to explain these differences. However, it is worth noting that Head *et al.* (1983) found a greater noradrenaline content of mesenteric artery preparations from SH rats compared to WKY preparations.

In the present study, in artery preparations from WKY and SH rats, both the AT_1 and AT_2 receptor antagonists losartan and PD 123319 reduced or abolished the enhancements of S-I [^{3}H]-noradrenaline release and of the associated vasoconstrictor response produced by angiotensin II in the concentration range 0.3–3 μM. Previous studies in which the nature of the prejunctional angiotensin II receptors at noradrenergic neuroeffector sites have been investigated, have indicated an AT_1 subtype. Thus, the enhancement of S-I release of [^{3}H]-noradrenaline by angiotensin II in rat (Gironacci *et al.*, 1994) and guinea-pig isolated atria (Brasch *et al.*, 1993) was blocked by losartan and unaltered by PD 123319. In the rabbit iris ciliary body the enhancement of S-I release of [^{3}H]-noradrenaline by angiotensin II was also blocked by losartan and unaffected by PD 123177 (Ohio & Jumblatt, 1993). Likewise, in isolated preparations of mesenteric arteries (Nagase *et al.*, 1994) and atria (Foucart *et al.*, 1994) from hypertensive rats, AT_1 but not AT_2 receptor antagonists have been shown to block the prejunctional facilitatory effects of angiotensin II. However, there are data at variance with those cited above. For example, Trachte *et al.* (1990; 1991) showed that both losartan and the AT_2 antagonist PD 123177 failed to abolish the enhancement of noradrenergic transmission by angiotensin II in the rabbit isolated vas deferens and we have previously found that in caudal arteries from Sprague-Dawley rats, both losartan and PD 123319 blocked the enhancing effect of angiotensin II on noradrenergic transmission (Cox *et al.*, 1995; 1996). In addition, we observed that the enhancement of twitch responses to sympathetic nerve stimulation by angiotensin II in the rat isolated vas deferens was blocked by both losartan and PD 123319 (Cox *et al.*, 1995). The present study provides evidence for prejunctional angiotensin II receptors sensitive to losartan and PD 123319 in caudal arteries of both normotensive WKY and SH rats.

In the present study, angiotensin II in the lowest concentration tested (0.1 μM), did not significantly alter the S-I efflux of [^{3}H]-noradrenaline, or the stimulation-evoked vasoconstrictor response in artery preparations from WKY and SH rats. However, combinations of AT_1 or AT_2 receptor antagonists with 0.1 μM angiotensin II had surprising effects which differed between the two strains of rats. In artery preparations from WKY rats, the combination of 0.1 μM angiotensin II and the lower concentration of losartan (0.01 μM) produced marked enhancement of both S-I efflux and stimulation-evoked vasoconstrictor responses. The enhancement of radiolabelled transmitter efflux and responses by losartan and angiotensin II in combination, was dependent upon the concentration of both losartan and angiotensin II. Thus, the combination of 0.1 μM angiotensin II and a higher concentration of losartan (0.1 μM) was without effect on S-I efflux or stimulation-evoked vasoconstriction and the enhancement produced by higher concentrations of angiotensin II (0.3–3 μM) were not potentiated by losartan. The enhancement of noradrenergic transmission by the combination of angiotensin II (0.1 μM) and losartan (0.01 μM) in the preparations from WKY rats was prevented by PD 123319 in concentrations of both 0.01 and 0.1 μM. These findings in caudal artery preparations from WKY rats are essentially identical to those obtained for caudal artery preparations from another normotensive strain of rat, Sprague-Dawley (Cox *et al.*, 1996).

In contrast to the finding in preparations from normotensive rats (WKY and Sprague-Dawley), in preparations from SH rats, the combination of 0.1 μM angiotensin II and losartan (0.01 μM), like 0.1 μM angiotensin II alone, did not enhance either S-I [^{3}H]-noradrenaline efflux or the associated vasoconstrictor responses. However, in preparations from the hypertensive rats, the combination of 0.1 μM angiotensin II and PD 123319 (both 0.01 or 0.1 μM) enhanced the S-I efflux of [^{3}H]-noradrenaline, although, the combination of angiotensin II and PD 123319 did not alter the stimulation-evoked vasoconstrictor response. Losartan (0.1 μM) prevented the enhancement of S-I [^{3}H]-noradrenaline release by the combination of 0.1 μM angiotensin II and PD 123319 (0.01 and 0.1 μM).

The IC_{50} of PD 123319 for AT_2 binding sites, found by various workers, is of the order of 20 nM (Dudley *et al.*, 1990). However, evidence has accumulated to indicate that AT_2 receptor antagonists, such as PD 123319, are not entirely selective for the AT_2 receptor subtype. A recent study indicates that PD 123319 in concentrations greater than 0.5 μM cross-reacts with the AT_{1B} receptor subtype (De Gasparo *et al.*, 1995). In rat renal mesangial cells, Ernsberger *et al.* (1992) have described two distinct populations of AT_1 binding sites, designated as AT_{1A} and AT_{1B} which bind AT_2 receptor antagonists PD 123319 and CGP 42112. The inhibitor constant (K_i) of PD 123319 for the AT_{1A} subtype was 24 μM whereas the value for the AT_{1B} subtype was 2.2 nM. AT_2 receptor antagonists have also been shown to inhibit certain physiological actions of angiotensin II. Thus, the pressor and bradycardiac effects of intracerebroventricular administration of angiotensin II in anaesthetized rats (Widdop *et al.*, 1993a) and the angiotensin II-mediated drinking response in rats (Rowland *et al.*, 1992) are both blocked by PD 123319. In contrast, the structurally related AT_2 antagonist, PD 123177, was without effect on the intracerebroventricular administration of angiotensin II (Widdop *et al.*, 1993b; Rowland *et al.*, 1992). The angiotensin-induced excitation of inferior olivary neurones was blocked by both PD 12377 and CGP 42112 but was unaffected by losartan (Ambühl *et al.*, 1992). In anaesthetized rats, the release of vasopressin by central administration of angiotensin II was blocked by both losartan and PD 123177 (Hogarty *et al.*, 1992). In rat isolated caudal arteries, the enhancement by angiotensin II of the vasoconstrictor responses to noradrenaline was blocked by both losartan and PD 123319 (Vlahos & Story, 1994). In all of the above studies, the AT_2 receptor antagonists were administered in concentrations high enough to interact with AT_1 receptors. The results from such studies must

therefore be interpreted cautiously. However, based on the evidence from binding studies, in the lower concentration in which it was used in the present study to characterize the prejunctional angiotensin II receptor (0.01 μM), PD 123319 should be highly selective for the AT_2 receptor subtype.

Our present study has revealed a difference in the interactions of angiotensin II and non-peptide angiotensin receptor antagonists on noradrenergic transmission between caudal artery preparations of WKY rats and preparations of SH rats. The findings with preparations from WKY rats are essentially the same as those we obtained for caudal artery preparations from Sprague-Dawley rats (Cox *et al.*, 1996). In the artery preparations from the two normotensive strains there was a synergistic interaction between the AT_1 antagonist losartan and angiotensin II. In preparations from SH rats, there was an apparently similar synergistic interaction between the AT_2 antagonist PD 123319 and angiotensin II. Moreover, the enhancement of [^{3}H]-noradrenaline efflux and vasoconstrictor responses produced by the combination of losartan and angiotensin II in the WKY and Sprague-Dawley artery preparations was prevented by PD 123319, whilst the effect of the combination of PD 123319 and angiotensin II on [^{3}H]-noradrenaline efflux in SH rat artery preparations was prevented by losartan.

We previously suggested two possible mechanisms for the synergistic interaction between losartan and angiotensin II in caudal artery preparations of Sprague-Dawley rats (Cox *et al.*, 1996). We hypothesized that there might be a synergistic prejunctional interaction between 0.01 μM losartan and 0.1 μM angiotensin II at noradrenergic neuroeffector sites, involving either the 'unmasking' by losartan of a latent population of angiotensin II receptors subserving facilitation of transmitter release, or the block by losartan of an inhibitory action of angiotensin II on transmitter release (Cox *et al.*, 1996). Given the similar findings, the same two mechanisms could be advanced to explain the synergistic interaction between 0.1 μM angiotensin II and 0.01 μM losartan on noradrenergic transmission in caudal arteries from WKY rats. Moreover, by analogy with the first proposal, the synergistic interaction of PD 123319 and angiotensin II in caudal arteries of SH rats may be due to the 'unmasking' by PD 123319 of a latent population of AT_1 receptors which subserve facilitation of transmitter release. Such a proposal was advanced by Hong *et al.* (1994) to explain the ability of AT_2 antagonists to reveal a contractile effect of normally subthreshold concentrations of angiotensin II in rabbit abdominal aortic preparations. By analogy with our alternative proposal to explain our findings in caudal arteries from normotensive rats, the interaction of angiotensin II and PD 123319 in arteries from SH rats might be due to the existence of two opposing actions of angiotensin II on transmitter noradrenaline release. Thus, the well known prejunctional facilitatory action of angiotensin II could be offset by an inhibitory action. In caudal arteries of SH rats, the putative inhibitory effect might be subserved by AT_2 receptors, since there was greater enhancement of S-I [^{3}H]-noradrenaline efflux with the combination of PD 123319 (0.01 μM) and angiotensin II (0.1 μM) than with angiotensin II alone.

In conclusion, taking the present findings and those of our earlier study (Cox *et al.*, 1996) together, angiotensin II receptors similar to the AT_{1B} subtype appear to subserve enhancement of noradrenergic transmission by angiotensin II in caudal arteries from WKY, Sprague-Dawley and SH rats, whereas receptors of the AT_1 subtype appear to subserve the direct vasoconstrictor action of angiotensin II in caudal arteries of all three strains. We also observed synergistic interactions of angiotensin II and the receptor subtype antagonists losartan and PD 123319, in artery preparations from WKY and SH rats respectively. The receptors involved in the synergistic interaction between angiotensin II and the receptor antagonists appear to differ between caudal arteries from the two normotensive strains of rats and those from SH rats.

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