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Facilitation of noradrenaline release from sympathetic nerves in rat anococcygeus muscle by activation of prejunctional β-adrenoceptors and angiotensin receptors

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1 Isolated preparations of rat anococcygeus muscle were incubated with [^{3}H]-noradrenaline and the efflux of radioactivity induced by stimulation of intramural sympathetic nerves was used as a measure of release of transmitter noradrenaline. Isometric contractile responses were also measured.

2 Angiotensin I (0.03 μM) and angiotensin II (0.03 μM) produced non-sustained contractile responses and enhanced the stimulation-induced (S-I) effluxes of radioactivity as well as the contractile responses to electrical stimulation. These effects were blocked by the angiotensin II receptor antagonist saralasin (0.03 μM), and the effect of angiotensin I, but not angiotensin II, was blocked by the angiotensin converting enzyme inhibitor captopril (0.1 μM).

3 The findings indicate that there are both pre- and postjunctional receptors for angiotensin II and that angiotensin I is converted to angiotensin II in the anococcygeus muscle preparation.

4 Isoprenaline (0.1 μM) slightly enhanced the S-I efflux of radioactivity, and produced a greater enhancement after neuronal uptake blockade with desipramine (0.03 μM) and α-adrenoceptor blockade with phentolamine (1 μM).

5 The facilitatory effect of isoprenaline on S-I efflux of radioactivity was abolished by propranolol (0.3 μM), but was not affected by low concentrations of saralasin (0.03 μM) or captopril (0.1 μM) which abolished the effect of angiotensin I. The findings suggest that isoprenaline acts directly on prejunctional β-adrenoceptors to enhance S-I noradrenaline release, rather than indirectly by releasing angiotensin II from within the tissue. Higher concentrations of saralasin (0.1 μM) or captopril (5 μM) did block the facilitatory effect of isoprenaline. The significance of this finding is not clear.

Introduction

The amount of noradrenaline released by stimulating postganglionic sympathetic nerves can be modulated by agents acting on receptors located at the nerve terminals, so called prejunctional or presynaptic receptors (see Starke, 1977; Westfall, 1977; Langer, 1980; Rand *et al.*, 1980). Activation of prejunctional receptors usually results in inhibition of noradrenaline release, but activation of two types, namely angiotensin II receptors and β-adrenoceptors, results in enhanced stimulation-induced release of noradrenaline.

Angiotensin II enhances the release of noradrenaline by activation of prejunctional receptors at postganglionic sympathetic nerve endings in a variety of isolated tissues (see Starke, 1977; Westfall, 1977) as well as *in vivo* (Majewski *et al.*, 1984). There is also evidence that angiotensin II, in addition to its role as a circulating hormone, may be synthesized locally in a number of sympathetically innervated tissues (Dzau, 1984; Campbell, 1985). It is likely that locally synthesized angiotensin II can enhance noradrenaline release since angiotensin II precursors (angiotensin I and the tetradecapeptide fragment of angiotensinogen) enhances noradrenergic transmission in isolated tissues (Malik & Nasjletti, 1976; Böke & Malik, 1983; Ziogas *et al.*, 1984; 1985; 1986).

Another mechanism by which noradrenaline release may be enhanced is by activation of prejunctional β-adrenoceptors (see Majewski, 1983). β-Adrenoceptor agonists have been found to enhance noradrenaline release from sympathetically innervated tissues *in vitro* and *in vivo* in animals and in man (see Majewski, 1983; Majewski & Rand, 1986). The physiological activator of the prejunctional β-adrenoceptor system appears to be adrenaline, which can enhance noradrenaline release both *in vitro* and *in vivo* (see Majewski, 1983; Schmidt *et al.*, 1984).

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Results from some vascular tissues, such as rat mesenteric arteries (Kawasaki et al., 1984) and rat vena cava (Göthert & Kollecker, 1986), suggest that at least part of the facilitation of noradrenergic transmission by β-adrenoceptor agonists involves stimulation of angiotensin II formation within the vascular wall and subsequent activation of prejunctional angiotensin II receptors.

The present study was conducted using the rat anococcygeus muscle, a non-vascular smooth muscle which is known to possess a noradrenergic motor innervation (Gillespie 1980). The aim was to determine whether noradrenaline release from the anococcygeus could be facilitated by activation of angiotensin II receptors and β-adrenoceptors. The possibility that facilitation of noradrenaline release produced by activation of β-adrenoceptors could be explained by local generation of angiotensin II in the anococcygeus smooth muscle was also investigated.

Methods

Male Wistar rats (300–400 g) were decapitated and the anococcygeus muscle removed as previously described by Gillespie (1972). The tissue was placed in 4 ml modified Krebs-Henseleit solution and incubated with (−)-[7,8-^{3}H]-noradrenaline (12 Ci mmol^{-1}; 0.25 μM) for 40 min. The incubation solution was bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C. After incubation, the anococcygeus muscle was mounted between two platinum wire electrodes in an organ bath containing 3 ml of noradrenaline-free modified Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C. The muscle was suspended under an initial tension of 1 g and isometric tension was continually monitored.

The solution in the organ bath was repeatedly exchanged with fresh modified Krebs-Henseleit solution every 30 s for 10 min, then every 1 min for 10 min and finally every 3 min for 40 min. After 60 min of washout, a 'priming stimulation' (2 Hz for 30 s with 1 ms square wave pulses, field gradient 17 V cm^{-1}) was applied in order to facilitate removal of loosely bound radioactive compounds. The washout in fresh modified Krebs-Henseleit solution was continued for a further 30 min, giving a total wash duration of 90 min.

Following the 90 min washout period, two periods of field stimulation (2 Hz for 30 s) were applied at 30 min intervals. When the effects of drugs were to be assessed they were in contact with the tissue from 20 min before the second period of stimulation. In some experiments drugs were present for both stimulation periods and in this case were added 28 min before the first period of stimulation and remained present throughout the experiment.

Samples of tissue bathing solution were collected every 3 min for measurement of efflux of radioactivity. For each stimulation period, the resting efflux of radioactivity was taken as the mean of the radioactivity in two 3 min samples of the bathing solution, one collected immediately before onset of stimulation, the other collected 9 min after commencement of stimulation. The stimulation-induced (S-I) efflux was determined by subtracting the resting efflux from the radioactive content of each of the two successive 3 min samples of the bathing solution collected during and immediately after stimulation.

The amount of radioactivity present in the tissue bathing solution was measured by liquid scintillation counting and was expressed as disintegrations per min (d.p.m.). Corrections for counting efficiency were made using an automatic external standard.

Materials

The modified Krebs-Henseleit solution was of the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose 11.1, disodium edetate 0.067, ascorbic acid 0.14.

The following drugs were purchased: (±)-isoprenaline hydrochloride (British Drug Houses, Australia); phentolamine mesylate, desipramine hydrochloride and [Val^5]angiotensin II amide (Ciba-Geigy, Australia); [Ile^5]angiotensin I (Sigma, U.S.A.); [Sar^1, Ala^8]angiotensin II (saralasin) (Peninsula Laboratories, U.S.A.). (±)-Propranolol hydrochloride was generously donated by ICI, Australia. Drugs were dissolved in distilled water. Stock solutions were subsequently diluted with modified Krebs-Henseleit solution to give the required concentrations of drugs. The (−)-[7,8-^{3}H]-noradrenaline hydrochloride (specific activity 12 Ci mmol^{-1}) was obtained from the Radiochemical Centre, Amersham, U.K.

Statistical analysis of results

The data were analysed by the unpaired 2-tailed Student's t test where indicated. Two-way analysis of variance was performed on some groups of data. In all cases, probability levels of less than 0.05 were taken to indicate statistical significance.

Results

Effects of angiotensin I and angiotensin II on resting and stimulation-induced (S-I) effluxes of radioactivity

In the presence of 0.03 μM of either angiotensin I or angiotensin II, the resting efflux of radioactivity was not significantly altered (not shown). However, each peptide significantly enhanced the S-I efflux

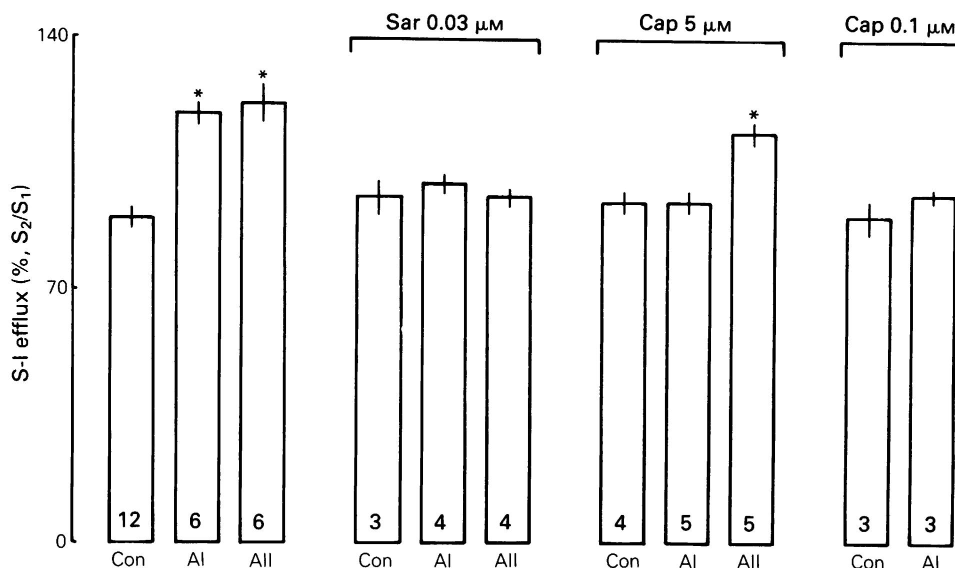

Figure 1 Effect of angiotensin I (AI, 0.03 μM) and angiotensin II (AII, 0.03 μM) on the stimulation-induced (S-I) efflux of radioactivity from rat anococcygeus muscle preincubated with [^{3}H]-noradrenaline. Two periods of electrical stimulation were applied (2 Hz, for 30 s). The S-I efflux of radioactivity in the second stimulation period (S_2) was expressed as a percentage of that in the first (S_1). AI and AII were present from 20 min before the second stimulation period. Saralasin (Sar) and captopril (Cap) were present for both stimulation periods. Each column represents the mean and the vertical bar the s.e.mean. * Indicates a significant difference from the appropriate control (Con) ($P < 0.05$, Student's t test). None of the drug treatments altered the resting efflux of radioactivity ($P > 0.05$, Student's t test).

(Figure 1). The absolute values for the S-I efflux are given in Table 1. When captopril (0.1 and 5 μM) or saralasin (0.03 μM) alone were present, the resting and S-I effluxes did not differ significantly from those in control experiments (Table 1). However, in the presence of either captopril (0.1 and 5 μM) or saralasin (0.03 μM), angiotensin I failed to enhance the S-I efflux (Figure 1). Furthermore, the facilitatory effect of angiotensin II was blocked by saralasin (0.03 μM) but not by captopril (5 μM) (Figure 1).

In another series of experiments the neuronal uptake inhibitor desipramine (0.03 μM) was present for both stimulation periods. When the α-adrenoceptor blocking drug phentolamine (1 μM) was present during the second stimulation period, the S-I efflux of radioactivity was markedly enhanced

Table 1 Absolute resting (R_1) or stimulation-induced (S_1) effluxes of radioactivity from rat anococcygeus muscle (preincubated with [^{3}H]-noradrenaline) in the first stimulation period in the absence and presence of various drugs

Pretreatment	R_1 (d.p.m.)	S_1 (d.p.m.)	n
Control	2553 ± 69	2341 ± 111	36
DMI 0.03 μM	2620 ± 52	3820 ± 133*	65
DMI 0.03 μM + Prop 0.3 μM	2429 ± 120	3349 ± 333*	10
DMI 0.03 μM + Cap 5 μM	2559 ± 133	4407 ± 405*	19
DMI 0.03 μM + Cap 0.1 μM	2819 ± 141	4276 ± 181*	15
DMI 0.03 μM + Sar 0.1 μM	2559 ± 84	3865 ± 270*	14
DMI 0.03 μM + Sar 0.03 μM	2411 ± 57	3365 ± 154	26
Cap 5 μM	2567 ± 97	2518 ± 156	18
Cap 0.1 μM	2388 ± 275	2297 ± 374	6
Sar 0.03 μM	2472 ± 108	2189 ± 129	11

DMI, desipramine; Prop, propranolol; Cap, captopril; Sar, saralasin. * Significant difference from control.

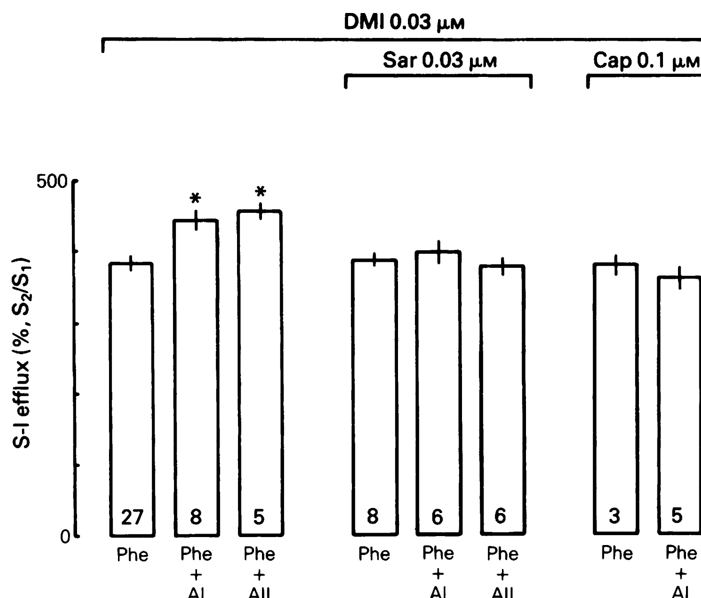

Figure 2 Effect of angiotensin I (AI, 0.03 μM) and angiotensin II (AII, 0.03 μM) in the presence of desipramine (DMI, 0.03 μm) and phentolamine (Phe, 1 μM) on the stimulation-induced (S-I) efflux of radioactivity from rat anococcygeus muscle preincubated with [^{3}H]-noradrenaline. AI, AII and phentolamine were present from 20 min before the second stimulation period. Other drugs: DMI, captopril (Cap) and saralasin (Sar) were present for both stimulation periods. * Indicates a significant difference from the effect in the appropriate phentolamine experiment ($P < 0.05$, Student's t test). Other details as in Figure 1. None of the drug treatments altered the resting efflux of radioactivity ($P > 0.05$, Student's t test).

(Figure 2). When either angiotensin I (0.03 μM) or angiotensin II (0.03 μM) was added together with phentolamine for the second stimulation period, the S-I efflux further increased (Figure 2). The facilitatory effects of angiotensin I and angiotensin II in the presence of phentolamine were blocked by saralasin (0.03 μM) and the effect of angiotensin I was also blocked by captopril (0.1 μM) (Figure 2).

Table 2 Effect of angiotensin I (AI) and angiotensin II (AII) on contractile responses to field stimulation

Response to S_1 (g)	*Drug present during* S_2	*Response to* S_2 (g)	S_2/S_1 (%)	n
2.5 ± 0.3	Control	2.0 ± 0.3	89.0 ± 4.4	12
4.6 ± 0.4	AI 0.03 μM	6.0 ± 0.5	137.5 ± 7.5*	6
3.3 ± 0.6	AII 0.03 μM	5.1 ± 0.6	161.2 ± 11.9*	6
Saralasin 0.03 μM throughout				
3.0 ± 0.7	Control	2.5 ± 0.9	81.2 ± 16.6	3
3.6 ± 0.4	AI 0.03 μM	3.7 ± 0.4	103.7 ± 4.1	3
2.8 ± 0.7	AII 0.03 μM	2.7 ± 0.8	95.1 ± 3.5	3
Captopril 0.1 μM throughout				
2.3 ± 0.6	Control	2.3 ± 0.6	103.3 ± 3.3	3
3.1 ± 0.3	AI 0.03 μM	3.3 ± 0.5	104.9 ± 4.6	3
Captopril 5 μM throughout				
2.8 ± 0.4	Control	2.6 ± 0.5	90.4 ± 8.8	4
3.3 ± 0.5	AI 0.03 μM	3.0 ± 0.4	95.4 ± 6.6	5
2.9 ± 0.4	AII 0.03 μM	5.2 ± 0.3	187.4 ± 21.5*	5

Anococcygeus muscles were placed under an initial tension of 1 g and the contractile responses to field stimulation (2 Hz, 30 s, 30 min apart, S_1 and S_2) measured. Angiotensin I and angiotensin II were present 20 min before the second stimulation period. In some experiments saralasin (0.03 μM) or captopril (0.1 and 5 μM) were present for both stimulation periods. * Indicates a significant difference from the appropriate control ($P < 0.05$, Student's t test).

Effects of angiotensin I and angiotensin II on contractile responses to field stimulation

Both angiotensin I and angiotensin II significantly increased the contractile responses of the anococcygeus to field stimulation (Table 2). The enhanced contractile response to stimulation in the presence of angiotensin I was abolished by both captopril (0.1 and 5 μM) and saralasin (0.03 μM), whereas the effect of angiotensin II was blocked only by saralasin (0.03 μM) (Table 2).

Direct contractile effects of angiotensin I and angiotensin II on the anococcygeus muscle

Both angiotensin I (0.03 μM) and angiotensin II (0.03 μM) caused an initial contraction of the rat anococcygeus muscle, but this was not maintained. Captopril and saralasin did not affect the baseline tone of the preparation. However, the contractile response to angiotensin I was completely abolished by captopril (0.1 and 5 μM) and also by saralasin (0.03 μM) (Table 3). The response to angiotensin II was unaltered by captopril (5 μM) (Table 3).

Effects of isoprenaline on the resting and S-I efflux of radioactivity

The β-adrenoceptor agonist isoprenaline (0.1 μM) did not significantly affect the resting efflux of radioactivity (not shown). However, the S-I efflux of radioactivity was slightly but significantly enhanced (Figure 3). The absolute enhancement of radioactivity was 354 d.p.m. (s.e.mean = 55 d.p.m., $n = 12$);

Table 3 Direct contractile effects of angiotensin I (AI) and angiotensin II (AII) on rat anococcygeus muscle

	Contractile responses (g)	n
AI 0.03 μM	1.0 ± 0.3	6
AI 0.03 μM + Sar 0.03 μM	0.0 ± 0.0	4
AI 0.03 μM + Cap 5 μM	0.0 ± 0.0	5
AI 0.03 μM ± Cap 0.1 μM	0.0 ± 0.0	3
AII 0.03 μM	1.5 ± 0.6	6
AII 0.03 μM + Sar 0.03 μM	0.0 ± 0.0	4
AII 0.03 μM + Cap 5 μM	1.4 ± 0.4	5

Anococcygeus muscles were placed under an initial tension of 1 g and the maximal contractile response produced by either angiotensin I or angiotensin II measured. In some experiments saralasin (Sar, 0.03 μM) or captopril (Cap, 0.1 and 5 μM) was added 48 min before the administration of angiotensin.

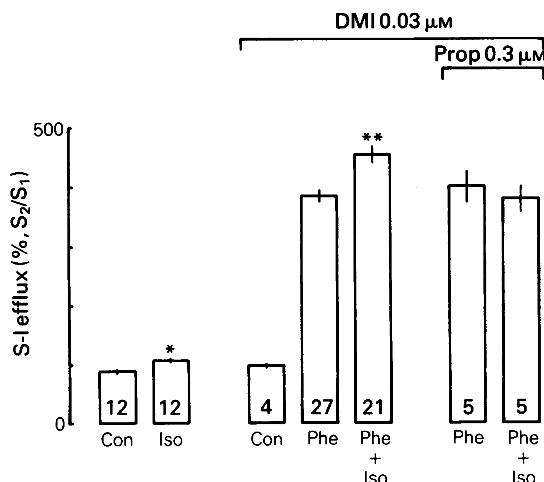

Figure 3 Effect of isoprenaline on the stimulation-induced (S-I) efflux of radioactivity from rat anococcygeus muscle preincubated with [^{3}H]-noradrenaline. Phentolamine (Phe, 1 μM) and isoprenaline (Iso, 0.1 μM) were present from 20 min before the second stimulation period. Desipramine (DMI, 0.03 μM) and propranolol (Prop, 0.3 μM) were present for both stimulation periods. * Indicates a significant difference from control (Con) and ** indicates a significant difference from phentolamine experiments ($P < 0.05$, Student's t test). Other details as in Figure 1. None of the drug treatments altered the resting efflux of radioactivity ($P < 0.05$, Student's t test).

thus the ratio S_2/S_1 with isoprenaline in S_2 was 109% compared to 90% in control experiments (Figure 3).

In further experiments, desipramine (0.03 μM) was present for both stimulation periods. Phentolamine (1 μM) added for the second stimulation period markedly enhanced the S-I efflux of radioactivity (Figure 3). When phentolamine (1 μM) plus isoprenaline (0.1 μM) were added for the second stimulation period the S-I efflux of radioactivity was even further enhanced. The absolute enhancement of radioactivity by isoprenaline in the presence of desipramine and phentolamine was 2793 d.p.m. (s.e.mean = 740 d.p.m., $n = 21$).

The facilitatory effect of isoprenaline in the presence of desipramine and phentolamine was abolished by propranolol (0.3 μM) (Figure 3). This concentration of propranolol did not significantly alter the resting or S-I effluxes of radioactivity (Table 1). Isoprenaline (0.1 μM) had no significant effect on either basal tone or on the contractile response to field stimulation under any of the conditions mentioned above (not shown).

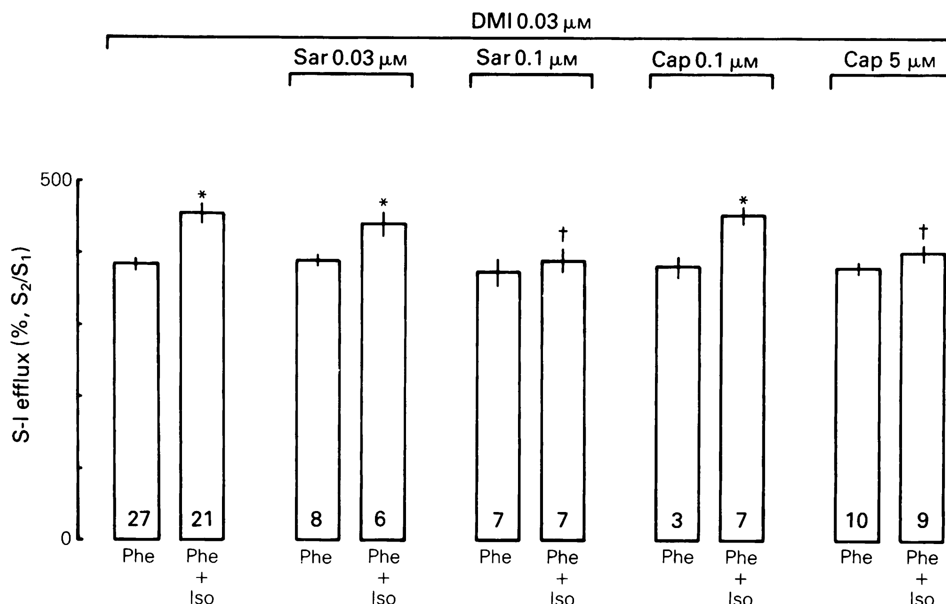

Figure 4 Effect of captopril and saralasin on the enhancement of the stimulation-induced (S-I) efflux of radioactivity from the rat anococcygeus muscle preincubated with [^{3}H]-noradrenaline. Phentolamine (Phe, 1 μM) and isoprenaline (Iso 0.1 μM) were present from 20 min before the second stimulation period. Desipramine (DMI, 0.03 μM), captopril (Cap 5 μM and 0.1 μM) and saralasin (Sar, 0.1 and 0.03 μM) were present for both stimulation periods. * Indicates a significant difference from the appropriate phentolamine experiment ($P < 0.05$, Students t test). † Indicates a significant difference from the effect of isoprenaline in the presence of phentolamine, $P < 0.05$, two-way analysis of variance. Other details as in Figure 1. None of the drug treatments altered the resting efflux of radioactivity ($P > 0.05$, Student's t test).

Effect of isoprenaline on the S-I efflux of radioactivity in the presence of captopril and saralasin

Since the effect of isoprenaline was much more pronounced in the presence of desipramine and phentolamine than when used alone, we chose to investigate further the mechanism of action of isoprenaline under these conditions.

In anococcygeus muscle treated with desipramine (0.03 μM) and phentolamine (0.1 μM), the facilitatory effect of isoprenaline (0.1 μM) on S-I efflux of radioactivity was significantly reduced by captopril (5 μM) and saralasin (0.1 μM). However, lower concentrations of captopril (0.1 μM) and saralasin (0.03 μM) failed to inhibit the facilitatory effect of isoprenaline (0.1 μM) (Figure 4).

Discussion

Angiotensin II enhanced the S-I noradrenaline efflux from rat anococcygeus muscle incubated with [^{3}H]-noradrenaline and this effect was blocked by the angiotensin II receptor antagonist saralasin. These findings are consistent with previous studies, which have demonstrated facilitatory effects of angiotensin II on sympathetic neurotransmission in a variety of other tissues (see Starke, 1977; Westfall, 1977), and suggest that sympathetic nerves of the anococcygeus muscle possess prejunctional angiotensin II receptors which subserve facilitation of noradrenaline release.

The decapeptide precursor of angiotensin II, angiotensin I, also enhanced S-I efflux of noradrenaline. Since this effect was blocked by both the angiotensin receptor antagonist saralasin and the angiotensin converting enzyme inhibitor captopril, it suggests that the rat anococcygeus muscle can generate angiotensin II from angiotensin I. A similar local conversion of angiotensin I to angiotensin II has been described in other tissues and also resulted in enhanced sympathetic neurotransmission; for example: rat mesenteric arteries (Malik & Nasjletti, 1976), rat kidney (Böke & Malik, 1983; Rump & Majewski, 1987), rat tail artery (Ziogas *et al.*, 1984) and guinea-pig atria (Ziogas *et al.*, 1985). It is possible that the local generation of angiotensin II may occur without the exogenous addition of a precursor

and thus noradrenergic transmission may be tonically under the influence of angiotensin II. In support of this, in perfused mesenteric arteries of the rat, a relatively high concentration of captopril (100 μM) inhibited responses to sympathetic nerve stimulation (Collis & Keddie, 1981). However, in the present study in rat anococcygeus neither captopril nor saralasin by themselves affected noradrenaline release, which does not support this contention.

In addition to enhancing the S-I efflux of noradrenaline, both angiotensin I and angiotensin II produced contractions of the rat anococcygeus muscle and these effects were abolished by saralasin and in the case of angiotensin I also by captopril. These results suggest that the smooth muscle of rat anococcygeus also possesses angiotensin II receptors, as has been previously suggested (Doggrell & Woodruff, 1978), and provides further evidence for the local conversion of angiotensin I to angiotensin II in this tissue.

Both angiotensin I and angiotensin II enhanced contractile responses to sympathetic nerve stimulation. Their effects were abolished by saralasin, and in the case of angiotensin I also by captopril. This enhancement of contractile responses to sympathetic nerve stimulation can be attributed to the release of a greater amount of noradrenaline, but may also involve postjunctional effects of angiotensin II, since it has been shown that angiotensin II enhances responses to noradrenaline in a variety of tissues (see Zimmerman, 1978).

In the present study, the β-adrenoceptor agonist isoprenaline (0.1 μM) significantly enhanced the S-I efflux of noradrenaline from the rat anococcygeus muscle. Since this effect of isoprenaline was blocked by the β-adrenoceptor antagonist propranolol it suggests that it was due to activation of prejunctional β-adrenoceptors at sympathetic nerve endings (see Majewski, 1983). The facilitatory effect of isoprenaline on S-I noradrenaline release was greater in the combined presence of the α-adrenoceptor antagonist phentolamine and the neuronal uptake inhibitor desipramine. It is likely that phentolamine was responsible for the increased facilitatory effect of isoprenaline since it has been observed that phentolamine increases the facilitatory effect of isoprenaline on noradrenergic transmission in other tissues (Majewski & Rand, 1981; Johnston & Majewski, 1986). This may be due to the blockade of inhibitory prejunctional α-adrenoceptors, thus preventing 'feedback inhibition' from buffering the enhanced noradrenaline release in the presence of isoprenaline.

In rat isolated mesenteric arteries (Kawasaki *et al.*, 1984) and vena cava (Göthert & Kollecker, 1986) it has been proposed that activation of β-adrenoceptors in the vascular wall may stimulate the local synthesis of angiotensin II, which then mediates the facilitation of noradrenergic transmission by acting on prejunctional angiotensin II receptors. In the present study in rat anococcygeus muscle, the facilitatory effect of isoprenaline on S-I noradrenaline release in the presence of desipramine and phentolamine was inhibited by captopril (5 μM) and saralasin (0.1 μM). These findings may be considered as evidence in support of the proposal that locally generated angiotensin II mediates the facilitatory effect of isoprenaline on noradrenergic transmission. However, when lower concentrations of captopril (0.1 μM) or saralasin (0.03 μM) were used they failed to block the facilitatory effect of isoprenaline. These lower concentrations of captopril and saralasin were sufficient to inhibit the facilitatory effect of angiotensin I.

There are several possibilities which may explain these results. Firstly, locally generated angiotensin II may not be involved in the facilitatory effect of isoprenaline, thus the inhibitory effects of captopril (5 μM) and saralasin (0.1 μM) on the enhancement of noradrenaline release by isoprenaline may be through non-specific mechanisms. However, since the higher concentrations of captopril and saralasin did not significantly affect the resting or S-I effluxes or the facilitatory effect of phentolamine, the effect of these drugs seems to be specific for the facilitatory effect of isoprenaline. Another explanation is that activation of β-adrenoceptors may result in the local generation of angiotensin II in the vicinity of the nerve endings, in such a way that higher concentrations of saralasin or captopril are required than those needed to block the effect of either exogenous angiotensin II or angiotensin II generated from angiotensin I. It is unclear at present which interpretation is correct.

Similar observations were made in a study using rat isolated kidney in which a concentration of captopril (0.1 μM) capable of blocking the facilitatory effect of angiotensin I on noradrenaline release failed to block the facilitatory effect of isoprenaline (Rump & Majewski, 1987). However, as in the present study on anococcygeus muscle, a high concentration of captopril (5 μM) did block the facilitatory effect of isoprenaline on S-I noradrenaline release in kidney. In the studies of Kawasaki *et al.* (1984) and Göthert & Kollecker (1986) captopril and saralasin reduced but did not abolish the effects of isoprenaline in enhancing noradrenaline release, and it may be that only part of the enhancement of noradrenaline release by isoprenaline was due to local angiotensin II formation. In some tissues such as mouse atria (Musgrave & Majewski, 1987) and rat tail artery (Rajanayagam *et al.*, 1987) a high concentration of captopril (5 μM) had no effect on the facilitatory effect of isoprenaline on noradrenaline release. Taken together these results suggest that there is consider-

able variation between tissues in the degree of involvement of the local generation of angiotensin II in the facilitatory effect of isoprenaline on the S-I release of noradrenaline.

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