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Facilitation of noradrenaline release from sympathetic nerves through activation of ACTH receptors, β-adrenoceptors and angiotensin II receptors

[1]Mary Costa & Henryk Majewski

Department of Pharmacology, University of Melbourne, Victoria, 3052, Australia

1 In rabbit pulmonary artery and left atrial strips previously incubated with [^{3}H]-noradrenaline, the active fragment of adrenocorticotropic hormone ($ACTH_{1-24}$, 0.1 μM) significantly enhanced the stimulation-induced (S-I) outflow of radioactivity when a cocktail containing corticosterone (40 μM), cocaine (30 μM) and propranolol (4 μM) was present, but not in the absence of these drugs. In rabbit pulmonary artery a facilitatory effect of $ACTH_{1-24}$ (0.1 μM) was also observed when only cocaine (30 μM) was present.

2 $ACTH_{1-24}$ (0.1 μM) did not affect the S-I outflow of radioactivity from rat atria, rat pulmonary artery or guinea-pig pulmonary artery, either in the presence or in the absence of the cocktail containing corticosterone (40 μM), cocaine (30 μM) and propranolol (4 μM). These results suggest that the presence of facilitatory prejunctional ACTH receptors may be restricted to rabbit sympathetic nerve endings.

3 Angiotensin II (0.01 μM), but not isoprenaline (0.1 μM) or $ACTH_{1-24}$ (0.1 μM), significantly enhanced the S-I outflow of radioactivity from rabbit pulmonary artery. In the presence of phentolamine (1 μM) to block inhibitory α_2-adrenoceptors, the facilitatory effect of angiotensin II (0.01 μM) was significantly enhanced, and a significant facilitatory effect of isoprenaline (0.1 μM) and of $ACTH_{1-24}$ (0.1 μM) was then revealed. These results suggest that feedback inhibition of noradrenaline release, mediated through the prejunctional α_2-adrenoceptor mechanism, buffers increases in noradrenaline release during activation of facilitatory prejunctional receptors.

4 In rabbit pulmonary artery, two concentrations of 8-Br-cyclic AMP, (270 or 540 μM), enhanced the S-I outflow of radioactivity in the presence of phentolamine (1 μM) to a similar extent. In the presence of 8-Br-cyclic AMP (270 μM) and phentolamine, the facilitatory effects of isoprenaline (0.1 μM) and of $ACTH_{1-24}$ (0.1 μM) were blocked, whereas that of angiotensin II (0.01 μM) was not changed. These results suggest that both prejunctional β-adrenoceptors and ACTH receptors enhance noradrenaline release by generating cyclic AMP. The mechanism by which angiotensin II facilitates noradrenaline release is probably independent of the cyclic AMP second messenger pathway.

Introduction

Noradrenaline release from sympathetic nerve endings can be modulated through a variety of prejunctional receptor-mediated processes (see Starke, 1977; 1981; Westfall, 1977; Rand *et al.*, 1980; 1987; Langer, 1981). Prejunctional α-adrenoceptors are activated by neuronally-released noradrenaline to inhibit subsequent noradrenaline release during a train of nerve impulses (autoinhibition), and this is likely to be the principal receptor-mediated modulatory influence on transmitter release (see Starke, 1987). However, there are in addition to this mechanism several other inhibitory receptor-mediated processes which can be activated by agents such as prostaglandins, acetylcholine, adenosine, 5-hydroxytryptamine and various peptides (see above reviews).

In contrast to the many inhibitory mechanisms, very few prejunctional receptor mechanisms which can enhance noradrenaline release have been identified. One such facilitatory mechanism is that mediated through prejunctional β-adrenoceptors, which has now been demonstrated in a wide variety of

[1] Author for correspondence.

tissues and species both *in vitro* (see Majewski, 1983) and *in vivo* (Schmidt *et al.*, 1984). Another facilitatory mechanism which has been relatively well characterized is that mediated through prejunctional angiotensin II receptors. This mechanism has been demonstrated both *in vitro* (see Zimmerman, 1981), and *in vivo* (Majewski *et al.*, 1984). A third facilitatory mechanism, which is activated by adrenocorticotropic hormone (ACTH), has been described recently. ACTH and fragments of ACTH (such as $ACTH_{1-24}$) enhanced noradrenaline release in rabbit pulmonary artery and rabbit aorta (Göthert, 1981; Göthert & Hentrich, 1984). The ACTH partial agonist, $ACTH_{7-39}$ significantly inhibited this effect in the rabbit pulmonary artery (Göthert, 1984). There is some evidence that prejunctional ACTH receptors may have physiological significance, since $ACTH_{1-24}$ also enhanced noradrenaline release *in vivo* in the pithed rabbit with electrically stimulated sympathetic outflow (Szabo *et al.*, 1987).

The facilitatory mechanism mediated through ACTH receptors has not been widely investigated, therefore the first part of this study examined whether or not prejunctional facilitatory ACTH receptors are present in sympathetically innervated tissues from various species.

The second part of the study determined the extent to which activation of inhibitory prejunctional α_2-adrenoceptors by neuronally-released noradrenaline could buffer the facilitatory effects mediated through ACTH receptors, as well as those mediated through β-adrenoceptors and angiotensin II receptors. The final part of the investigation examined whether these three facilitatory mechanisms enhanced noradrenaline release by increasing intraneuronal adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation. The tissue chosen for this investigation was the rabbit pulmonary artery.

Methods

Preparation of tissues

Rabbit atria, rat atria Rats (180–370 g), or rabbits (2.5–3.5 kg), of either sex were stunned by a blow to the head and then decapitated. The chest was opened and the heart was rapidly removed and placed in warm Krebs-Henseleit solution bubbled with 95% O_2 plus 5% CO_2. The rat spontaneously beating whole atria were dissected free and suspended by a tissue hook between two platinum electrodes in an organ bath which contained Krebs-Henseleit solution. In the case of rabbits, the left atrium was removed and strips of tissue were dissected from the wall of the atrium. The solution bathing the atria was maintained at 37°C and was continuously bubbled with 95% O_2 plus 5% CO_2.

Pulmonary arteries: rat, guinea-pig, rabbit The animals (rat; 180–370 g, guinea-pig; 200–350 g, rabbit; 2.5–3.5 kg) were stunned by a blow to the head and then decapitated. The chest was opened and the heart and adjoining blood vessels were removed. The pulmonary artery was dissected free in warm Krebs-Henseleit solution bubbled with 95% O_2, 5% CO_2 and then cut in half transversely. Each half was then cut into a spiral strip, attached to a tissue hook and placed in an organ bath as described above. The intial tension applied to the arteries was 1 g.

Radiolabelling with [^3H]-noradrenaline

Rat atria The atria were incubated with (−)-(7,8) [^{3}H]-noradrenaline (2.5 μCi ml^{-1}; 0.2 μM) for 20 min. Following the incubation the atria were washed repeatedly with fresh Krebs-Henseleit solution every 30 s for 10 min, then every 2 min for a further 50 min. A priming stimulation consisting of square wave pulses (20 V cm^{-1} field gradient, 1 ms duration), at a frequency of 2 Hz for 60 s, was delivered to the atria via the platinum electrodes 45 min after the commencement of the washing procedure. This was to remove loosely bound radioactive compounds from the tissue.

Rat pulmonary artery, guinea-pig pulmonary artery, rabbit pulmonary artery, rabbit atria These tissues were incubated with (−)-(7,8)[^{3}H]-noradrenaline (6.7 μCi ml^{-1}; 0.4 μM) for 60 min then washed every 30 s for 10 min, then every 2 min for a further 80 min. A priming stimulation consisting of square wave pulses (20 V cm^{-1} field gradient, 1 ms duration, 2 Hz for 60 s), was delivered to the tissue via the platinum electrodes 30 min after the commencement of the washing procedure.

Measurement of radioactive outflow

All tissues Following the washing procedure, the organ bath volume was adjusted to 4 ml. After a 3 min period of contact with the tissue, the 4 ml sample of the solution bathing the tissue was collected in a plastic vial, and a further 4 ml of Krebs-Henseleit solution was added to the organ bath. Eighteen consecutive samples of the solution were taken for measurement of radioactive content. Two test stimulations (S_1 and S_2) were delivered to the tissue 30 min apart, at 9 min and at 39 min after the commencement of the sampling procedure. The stimulation parameters were square wave pulses (20 V cm^{-1} field gradient, 1 ms duration), at a fre-

quency of 2 Hz for 30 s for the rat atria, and 2 Hz for 2 min for all other tissues. When the effects of drugs were assessed they were in contact with the tissue from 15 min before the second stimulation period. In some experiments corticosterone, cocaine and propranolol, or cocaine alone were present throughout the experiment, starting from the beginning of the washing period. Bathing solution (4 ml) was added to 10 ml of Picofluor 30 (Packard Instruments, U.S.A.) and the radioactive content was estimated using a liquid scintillation counter. Corrections for counting efficiency were determined by automatic external standardization, and all measurements were expressed as disintegrations per min (d.p.m.).

Calculation of radioactive outflow

Rat atria, rabbit atria and rat pulmonary artery The spontaneous, or resting outflow of radioactivity from the tissue was calculated as the mean value of the radioactive content of the sample taken immediately before the stimulation period and the sample taken 9 min following the stimulation period. The stimulation-induced (S-I) outflow of radioactivity was calculated by adding the radioactive contents of the sample from the period during which stimulation occurred, with that of the sample taken immediately following stimulation (total of 2 samples), after subtracting the mean resting outflow of radioactivity from each sample.

Rabbit pulmonary artery and guinea-pig pulmonary artery Four samples after stimulation were used to calculate the S-I outflow and the resting outflow of radioactivity was calculated as the mean radioactive content of the sample taken immediately before the stimulation period and the sample taken 15 min later.

Statistical analysis of results

All data are expressed as mean ± s.e.mean. The data were analysed by use of unpaired 2-tailed Student's *t* tests. Where appropriate, two-way analysis of variance was also performed. Probability levels of less than 0.05 were taken to indicate statistical significance in all cases.

Materials

The following drugs were used: (−)-7,8-[^{3}H]-noradrenaline (13–14 Ci mmol^{-1}; Amersham, U.K.), $ACTH_{1-24}$ (corticotropin$_{1-24}$; Sigma, U.S.A.), (±)-isoprenaline HCl (Sterling Pharmaceuticals, Australia), phentolamine mesylate (Ciba, Australia), (±)-propranolol HCl (ICI, Australia), corticosterone (Sigma, U.S.A.), cocaine HCl (MacFarlan-Smith, Australia), Val^5-angiotensin II (Sigma, U.S.A.), 8-bromo-3′:5′-cyclic-adenosine monophosphate (Boehringer Mannheim, W. Germany).

The modified Krebs-Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, KH_2PO_4 1.03, $MgSO_4$ 0.45, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14.

Results

Effect of $ACTH_{1-24}$ on the stimulation-induced outflow of radioactivity in rat and rabbit atria

Rat or rabbit atria were incubated with [^{3}H]-noradrenaline. Two stimulations were delivered (S_1 and S_2) and the absolute S-I outflows of radioactivity for the first stimulation are given in Table 1. $ACTH_{1-24}$ (0.1 μM) present only during the second stimulation, had no effect on the S-I outflow of radioactivity from either rat atria or rabbit atria (Figure 1). When the cocktail containing corticosterone (40 μM), cocaine (30 μM) and propranolol (4 μM) was present throughout the experiment, $ACTH_{1-24}$ (0.1 μM) significantly

Table 1 The absolute stimulation-induced (S-I) outflow of radioactive compounds in the first stimulation period (S_1)

Drug	S_1 (d.p.m.)	n
Rat atria		
Control	19000 ± 685	6
Cort, Coc, Pro	11679 ± 1808	13
Rat pulmonary artery		
Cort, Coc, Pro	14316 ± 2664	9
Guinea-pig pulmonary artery		
Cort, Coc, Pro	41083 ± 8653	8
Rabbit atria		
Control	27743 ± 3937	10
Cort, Coc, Pro	18461 ± 1650	10
Rabbit pulmonary artery		
Control	25940 ± 1423	86
Cort, Coc, Pro	10913 ± 1085	8
Coc	31287 ± 1752	10

The tissues were preincubated with [^{3}H]-noradrenaline and the absolute S-I outflow of radioactivity (mean ± s.e.mean) during the first period of stimulation (S_1) is shown. In some experiments the tissues were exposed to drugs; corticosterone (Cort, 40 μM), cocaine (Coc, 30 μM) or propranolol (Pro, 4 μM) from 75 min prior to S_1. It is difficult to attribute changes in these absolute S-I outflow data to drug effects as the absolute values are dependent on tissue size which varied.

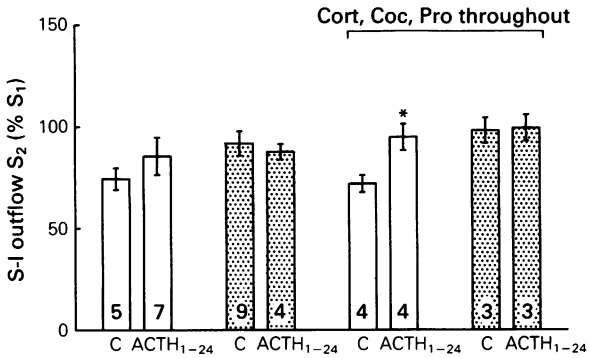

Figure 1 The effect of $ACTH_{1-24}$ (0.1 μM) on the stimulation-induced (S-I) outflow of radioactivity from rabbit and rat (stippled columns) isolated atria which were incubated with [^{3}H]-noradrenaline. There were two stimulation periods at 2 Hz for 2 min (rabbit) or 30 s (rat). $ACTH_{1-24}$ (0.1 μM) was present only during the second stimulation period. In some experiments a cocktail containing corticosterone (Cort, 40 μM), cocaine (Coc, 30 μM) and propranolol (Pro, 4 μM) was present during both stimulation periods. The S-I outflow in the second period of stimulation (S_2) is expressed as a percentage of that in the first (S_1). The vertical lines represent s.e.mean. * Represents a significant difference from control (C), $P < 0.05$, Student's t test. The number of experiments is shown at the base of each column.

enhanced the S-I outflow of radioactivity from rabbit atria but not from rat atria (Figure 1). $ACTH_{1-24}$ (0.1 μM) had no significant effect on the resting outflow of radioactivity from these tissues (not shown).

In rat atria, phentolamine (1 μM) enhanced the S-I outflow of radioactivity (mean $S_2/S_1 = 91.9\%$, s.e.mean = 6.0, $n = 9$ for control and 232.0%, s.e.mean = 11.1, $n = 8$ for phentolamine). However, the combination of phentolamine (1 μM) and $ACTH_{1-24}$ (0.1 μM) did not enhance the S-I outflow (mean $S_2/S_1 = 207.0\%$, s.e.mean = 22.0, $n = 4$) any more than phentolamine alone ($P > 0.05$, Student's t test).

Effect of $ACTH_{1-24}$ on the stimulation-induced outflow of radioactivity in rat and guinea-pig pulmonary artery

Rat or guinea-pig pulmonary arteries were incubated with [^{3}H]-noradrenaline. Two stimulations were delivered (S_1 and S_2) and the absolute S-I outflows of radioactivity for the first stimulation are given in Table 1. When the cocktail containing corticosterone (40 μM), cocaine (30 μM) and propranolol (4 μM) was present throughout the experiment, $ACTH_{1-24}$ (0.1 μM) present only during the second stimulation, had no effect ($P > 0.05$, Student's t test) on the S-I outflow of radioactivity in rat pulmonary artery (mean $S_2/S_1 = 69.9\%$, s.e.mean = 3.9, $n = 5$ for control and 67.4%, s.e.mean = 4.6, $n = 4$ for $ACTH_{1-24}$) or guinea-pig pulmonary artery (mean $S_2/S_1 = 91.3\%$, s.e.mean = 8.2, $n = 4$ for control and 81.0%, s.e.mean = 1.9, $n = 4$ for $ACTH_{1-24}$). $ACTH_{1-24}$ (0.1 μM) had no effect on the resting outflow in either tissue (not shown).

Effect of $ACTH_{1-24}$ on the stimulation-induced outflow of radioactivity in rabbit pulmonary artery

Rabbit pulmonary arteries were incubated with [^{3}H]-noradrenaline. Two stimulations were delivered to the tissues, and the absolute S-I outflow of radioactivity is given in Table 1. $ACTH_{1-24}$ (0.1 μM) had no effect on the S-I outflow of radioactivity (Figure 2). When the cocktail of corticosterone (40 μM), cocaine (30 μM) and propranolol (4 μM), or cocaine (30 μM) alone was present throughout the experiment, $ACTH_{1-24}$ (0.1 μM) significantly enhanced the S-I outflow of radioactivity (Figure 2).

Another series of experiments was performed in which phentolamine (1 μM) was present during the second stimulation period. Phentolamine (1 μM) had

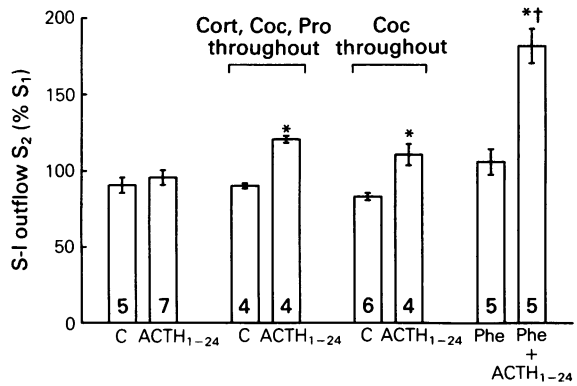

Figure 2 The influence of drugs on the effect of $ACTH_{1-24}$ (0.1 μM) on the stimulation-induced (S-I) outflow of radioactivity from rabbit pulmonary artery. The arteries were incubated with [^{3}H]-noradrenaline and stimulated twice (2 Hz, 2 min). In some experiments a cocktail of corticosterone (Cort, 40 μM), cocaine (Coc, 30 μM) and propranolol (Pro, 4 μM) or cocaine alone, was present during both stimulation periods. $ACTH_{1-24}$ (0.1 μM) and phentolamine (Phe, 1 μM) were present only during the second period of stimulation. The S-I outflow in the second period of stimulation (S_2) is expressed as a percentage of that in the first (S_1). The vertical lines represent s.e.mean. * Represents a significant difference from control (C), $P < 0.05$, Student's t test. † Represents a significant difference from phentolamine alone $P < 0.05$, Student's t test. The number of experiments is shown at the base of each column.

no effect on the S-I outflow of radioactivity (Figure 2). In the presence of phentolamine (1 μM), $ACTH_{1-24}$ (0.1 μM) significantly enhanced the S-I outflow of radioactivity (Figure 2). This was not significantly different ($P > 0.05$, Student's t test) from the facilitatory effect of a lower concentration of $ACTH_{1-24}$ (0.03 μM) (mean $S_2/S_1 = 195.8\%$, s.e.mean = 13.6, $n = 3$), but was significantly greater ($P < 0.05$, Student's t test) than the effect of $ACTH_{1-24}$ (0.3 μM) (mean $S_2/S_1 = 137.1\%$, s.e.mean = 13.4, $n = 3$) (all in the presence of phentolamine in S_2). There were no significant effects of the drugs on the resting outflow of radioactivity (not shown).

Effects of angiotensin II and isoprenaline on the stimulation-induced outflow of radioactivity in rabbit pulmonary artery

In rabbit pulmonary artery, angiotensin II (0.01 μM) significantly enhanced the S-I outflow of radioactivity (Figure 3). When phentolamine (1 μM) was also present during the second stimulation period, then angiotensin II (0.01 μM) enhanced the S-I outflow of radioactivity to an even greater extent (Figure 3).

Isoprenaline (0.1 μM) had no significant effect on the S-I outflow of radioactivity (Figure 3). However, in the presence of phentolamine (1 μM), isoprenaline (0.1 μM) significantly enhanced the S-I outflow of radioactivity (Figure 3).

There were no significant effects of the drugs on the resting outflow of radioactivity (not shown).

Effect of isoprenaline, angiotensin II and $ACTH_{1-24}$ on the stimulation-induced outflow of radioactivity in the presence of 8-Br-cyclic AMP

Since the facilitatory effects of isoprenaline and of $ACTH_{1-24}$ on the S-I outflow of radioactivity were clearly demonstrated in the presence of phentolamine the following experiments were carried out with phentolamine (1 μM) present during the second stimulation period.

The cell permeable cyclic AMP analogue, 8-bromo-3':5' cyclic adenosine monophosphate (8-Br-cyclic AMP, 270 or 540 μM) significantly enhanced the S-I outflow of radioactivity (Figure 4). The effects of the two concentrations were not significantly different ($P > 0.05$, Student's t test) and thus likely to

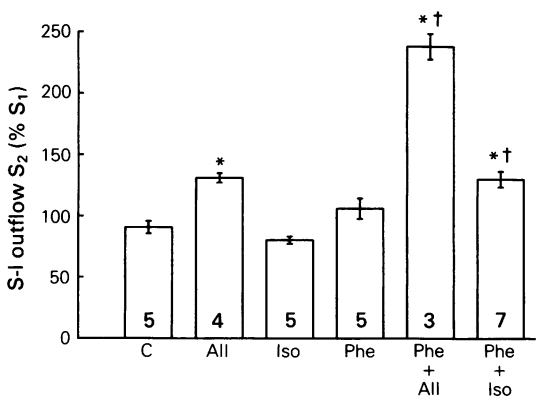

Figure 3 The effect of angiotensin II (AII, 0.01 μM) and isoprenaline (Iso, 0.1 μM) alone and in the presence of phentolamine (Phe, 1 μM) on the stimulation-induced (S-I) outflow of radioactivity from rabbit pulmonary artery. The arteries were preincubated with [^{3}H]-noradrenaline and stimulated twice (2 Hz, 2 min). The drugs (AII, Iso and Phe) were present only during the second period of stimulation. The S-I outflow in the second period of stimulation (S_2) is expressed as a percentage of that in the first (S_1). The vertical lines represent s.e.mean. * Represents a significant difference from control (C), $P < 0.05$, Student's t test. † Indicates that the effects of AII and isoprenaline in the presence of phentolamine differed significantly from the simple addition of the individual effects of the drugs $P < 0.05$, two-way analysis of variance. The number of experiments is shown at the base of each column.

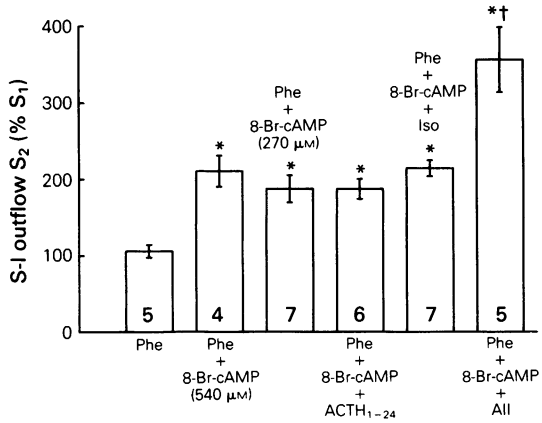

Figure 4 The influence of 8-Br-cyclic AMP on the effects of $ACTH_{1-24}$ (0.1 μM), isoprenaline (Iso, 0.1 μM) and angiotensin II (AII, 0.01 μM) on the stimulation-induced (S-I) outflow of radioactivity from rabbit pulmonary artery preincubated with [^{3}H]-noradrenaline. There were two stimulation periods (2 Hz, 2 min). All drugs: phentolamine (Phe, 1 μM), 8-Br-cyclic AMP (8-Br-cAMP, 270 and 540 μM), $ACTH_{1-24}$, AII and isoprenaline, were present only during the second stimulation period (S_2). The S-I outflow in S_2 was expressed as a percentage of that in the first stimulation (S_1). Vertical lines represent s.e.mean. * Indicates a significant difference from phentolamine alone $P < 0.05$. † Indicates a significant difference from phentolamine combined with 8-Br-cyclic AMP (270 μM) $P < 0.05$, Student's t test. The number of experiments is shown at the base of each column.

be maximal (Figure 4). In the presence of 8-Br-cyclic AMP (270 μM), $ACTH_{1-24}$ (0.1 μM) did not significantly affect the S-I outflow of radioactivity, nor did isoprenaline (0.1 μM) (Figure 4). This is in contrast to the facilitatory effects of the drugs in the absence of 8-Br-cyclic AMP (Figure 3). However, angiotensin II (0.01 μM) significantly enhanced the S-I outflow of radioactivity in the presence of 8-Br-cyclic AMP (270 μM) (Figure 4) as well as in its absence (Figure 3).

Effects of $ACTH_{1-24}$ in combination with isoprenaline or angiotensin II on the stimulation-induced outflow of radioactivity in the rabbit pulmonary artery

The following experiments were conducted with phentolamine (1 μM) present during the second stimulation period. $ACTH_{1-24}$ (0.1 μM) significantly enhanced the S-I outflow of radioactivity (Figure 2) and this was the maximally effective concentration (see above). In the presence of $ACTH_{1-24}$ (0.1 μM), isoprenaline (0.1 μM) did not further enhance the S-I outflow of radioactivity (Figure 5). In contrast, angiotensin II (0.01 μM) did significantly enhance the S-I outflow of radioactivity in the presence of $ACTH_{1-24}$ (0.1 μM) (Figure 5).

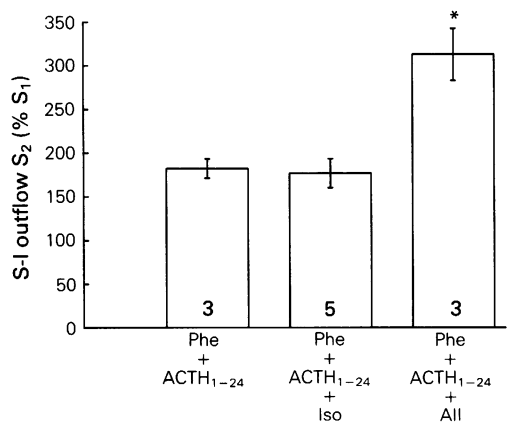

Figure 5 The interaction between $ACTH_{1-24}$ and isoprenaline or angiotensin II on the stimulation-induced (S-I) outflow of radioactivity from rabbit pulmonary artery which had been incubated with [^{3}H]-noradrenaline. There were two stimulation periods (2 Hz, 2 min). Drugs: phentolamine (Phe, 1 μM), $ACTH_{1-24}$ (0.1 μM), isoprenaline (Iso, 0.1 μM) and angiotensin II (AII, 0.01 μM) were present during the second stimulation period (S_2). The S-I outflow of radioactivity in S_2 is expressed as a percentage of that in the first stimulation (S_1). Vertical lines represent s.e.mean. * Indicates a significant difference from phentolamine plus $ACTH_{1-24}$, $P < 0.05$, Student's t test. The number of experiments is shown at the base of each column.

Discussion

The effect of $ACTH_{1-24}$ on noradrenaline release (S-I outflow of radioactivity) was assessed from a variety of sympathetically innervated tissues which had been preincubated with [^{3}H]-noradrenaline. The S-I outflow of radioactivity rather than [^{3}H]-noradrenaline outflow was used as an index of noradrenaline release, since this measure includes the metabolites that are formed subsequent to the release of noradrenaline from the nerves as well as unmetabolized noradrenaline (see Langer, 1974b). By itself, $ACTH_{1-24}$ had no effect on noradrenaline release in response to electrical stimulation, from either rabbit pulmonary artery, rabbit atria or rat atria. However, in the presence of a cocktail containing cocaine, corticosterone and propranolol a facilitatory effect of $ACTH_{1-24}$ on noradrenaline release was observed in both rabbit atria and pulmonary artery but not in rat atria, rat pulmonary artery or guinea-pig pulmonary artery. Thus, it appears that facilitation of noradrenaline release by $ACTH_{1-24}$ is restricted to rabbit tissues. It is interesting to note that the initial studies showing facilitatory prejunctional ACTH receptors on sympathetic nerve endings were limited to either rabbit pulmonary artery (Göthert, 1981; 1984), rabbit aorta (Göthert, 1984) or the pithed rabbit with stimulated sympathetic outflow (Szabo *et al.*, 1987), and to our knowledge there are no accounts of these effects being observed in other species. Nevertheless, the presence of prejunctional ACTH receptors in the rabbit tissues suggests that ACTH may have a role to play in the modulation of noradrenaline release, especially when ACTH levels are raised under conditions such as stress.

In the present study, the drug cocktail containing cocaine, corticosterone and propranolol revealed a facilitatory effect of $ACTH_{1-24}$ in both rabbit atria and rabbit pulmonary artery. Further experiments in rabbit pulmonary artery demonstrated that the presence of cocaine alone was sufficient to reveal the facilitatory effect of $ACTH_{1-24}$ on noradrenaline release. We can offer no explanation for this effect of cocaine. Earlier studies on prejunctional ACTH receptors (Göthert, 1984) found a facilitatory effect on $ACTH_{1-24}$ on noradrenaline release in rabbit pulmonary artery without using this cocktail of cocaine, corticosterone and propranolol, although the effect was much more pronounced in the presence of this cocktail of drugs.

The sympathetic nerves of rabbit pulmonary artery are endowed with three receptor-mediated facilitatory systems involving prejunctional ACTH receptors (Göthert, 1981; 1984), prejunctional β-adrenoceptors (Johnston & Majewski, 1986) and prejunctional angiotensin II receptors (Endo *et al.*,

1977). An important limitation to the effects of these facilitatory systems may be the inhibitory prejunctional α_2-adrenoceptor mechanism which is activated by neuronally released noradrenaline to complete an 'inhibitory feedback loop' (see Langer, 1974a; Starke, 1987). In the present study in the absence of the α-adrenoceptor blocking drug phentolamine, angiotensin II, but neither $ACTH_{1-24}$ nor isoprenaline, significantly enhanced noradrenaline release. However, when prejunctional α-adrenoceptors were blocked by phentolamine, the facilitatory effect of angiotensin II was much greater. Furthermore, $ACTH_{1-24}$ and isoprenaline then also enhanced noradrenaline release. These results suggest that there was indeed a buffering action being exerted by the inhibitory prejunctional α-adrenoceptor mechanism leading to the masking of the facilitatory effect of these drugs.

The interaction of phentolamine with β-adrenoceptor-mediated facilitation of noradrenaline release has previously been observed in rabbit pulmonary artery (Johnston & Majewski, 1986), as well as in a variety of other sympathetically innervated tissues including rat atria (Majewski & Rand, 1981), mouse atria (Johnston & Majewski, 1986) and rabbit ear artery (Majewski & Rand, 1981). In contrast, Nedergaard (1987) found no facilitatory effect of isoprenaline in the presence of α-adrenoceptor blockade in the rabbit pulmonary artery. The reason for the discrepancy between the results of Nedergaard (1987) in rabbit pulmonary artery with the present study and that of Johnston & Majewski (1986) are unclear. There has been another study on the influence of α-adrenoceptor blockade on facilitatory prejunctional systems. In rabbit heart a high concentration of phenoxybenzamine inhibited the facilitatory effect of angiotensin II on noradrenaline release (Starke & Schumann, 1972), but this may have been due to noradrenaline release being near maximally enhanced by phenoxybenzamine alone (Starke & Schumann, 1972).

Despite the interaction between phentolamine and $ACTH_{1-24}$ in rabbit pulmonary artery, in rat atria the presence of phentolamine failed to reveal a facilitatory effect of $ACTH_{1-24}$ on the S-I outflow of radioactivity. This further supports the contention (see above) that rat atria does not possess prejunctional ACTH receptors.

In various cell systems it appears that β-adrenoceptors are linked to adenylate cyclase and that cyclic AMP is involved in the signal transduction process (see Levitski, 1986). Similarly, the effect of ACTH in steroid synthesis in the adrenal cortex is also due to increased adenylate cyclase activity and increased cyclic AMP production (Grahame-Smith *et al.*, 1967). It is possible that facilitation of noradrenaline release through the prejunctional β-adrenoceptor and ACTH receptor systems involves cyclic AMP. The participation of cyclic AMP in these mechanisms is possible because cell permeable analogues of cyclic AMP enhance the action-potential induced release of noradrenaline from the sympathetic nerves of tissues (see Johnston *et al.*, 1987). Furthermore, inhibition of cyclic AMP phosphodiesterase which is responsible for the breakdown of cyclic AMP enhanced noradrenaline release in a wide variety of tissues (see Johnston *et al.*, 1987), presumably due to an increase in the intraneuronal levels of endogenous cyclic AMP.

In the present study in rabbit pulmonary artery, 8-Br-cyclic AMP, a cell permeable cyclic AMP analogue (Beebe *et al.*, 1984), enhanced the S-I release of noradrenaline in the presence of phentolamine. 8-Br-cyclic AMP was used to elevate total neuronal cyclic AMP levels (8-Br-cyclic AMP + endogenous cyclic AMP), such that changes in endogenous cyclic AMP production would not have an effect on noradrenaline release. In the presence of phentolamine, the concentration of 8-Br-cyclic AMP used (270 μM) was maximal for enhancing noradrenaline release. Under this condition of maximal noradrenaline release through the cyclic AMP mediated pathway, the facilitatory effects of $ACTH_{1-24}$ and of isoprenaline were no longer apparent. This suggests that the facilitatory effects of both $ACTH_{1-24}$ and isoprenaline are due to stimulation of cyclic AMP production.

Similar conclusions to the above have been made in other studies. Phosphodiesterase inhibitors potentiated the facilitatory effect of isoprenaline on noradrenaline release in cat spleen (Cubeddu *et al.*, 1975), guinea-pig myenteric plexus (Alberts *et al.*, 1985), rabbit pulmonary artery and mouse atria (Johnston & Majewski, 1986). Furthermore, saturation of mouse atria with 8-Br-cyclic AMP prevented the facilitatory effect of isoprenaline on noradrenaline release (Johnston *et al.*, 1987). Göthert & Hentrich (1984) also suggested that the effect of ACTH on noradrenaline release was due to activation of adenylate cyclase, since the facilitatory effect of $ACTH_{1-24}$ was potentiated in the presence of a phosphodiesterase inhibitor (Göthert & Hentrich, 1984).

To our knowledge there is no evidence that the effects of angiotensin II are due to activation of adenylate cyclase. Indeed, it has been shown that angiotensin II has either no effect or an inhibitory effect on adenylate cyclase in other cell systems (see Berridge, 1980). Thus in the present study in the presence of a maximal concentration of 8-Br-cyclic AMP for enhancing noradrenaline release (in the presence of phentolamine), angiotensin II still enhanced noradrenaline release suggesting that its effect was indeed independent of the adenylate cyclase-cyclic AMP generating system.

It is possible that prejunctional ACTH receptors and prejunctional β-adrenoceptors are linked to adenylate cyclase and that prejunctional angiotensin II receptors utilize a different pathway. This was further investigated by examining the facilitatory effects of isoprenaline and angiotensin II on the S-I release of noradrenaline in the presence of a maximally effective concentration of $ACTH_{1-24}$ (0.1 μM). In this case, in the presence of $ACTH_{1-24}$ (0.1 μM) the facilitatory effect of isoprenaline was no longer apparent, whereas that of angiotensin II was additive with $ACTH_{1-24}$, suggesting that isoprenaline but not angiotensin II was enhancing S-I release of noradrenaline by a similar pathway to $ACTH_{1-24}$. This is in accord with our earlier findings with 8-Br-cyclic AMP. It is therefore likely that the coupling of prejunctional modulatory systems to different second messenger pathways may help explain interactions between the various prejunctional receptors.

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