Br. J. Pharmac. (1987), **91**, 773–781

Involvement of cyclic nucleotides in prejunctional modulation of noradrenaline release in mouse atria

H. Johnston, [1]H. Majewski & I.F. Musgrave

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

1 In mouse isolated atria previously incubated with [^{3}H]-noradrenaline, 8-bromo-cyclic AMP (3–270 μM) produced a concentration-dependent increase in the fractional stimulation-induced outflow of radioactivity. 8-Bromo-cyclic GMP induced a lesser increase in the stimulation-induced outflow.

2 The phosphodiesterase inhibitors: M&B 22948 (90 μM); ICI 63197 (30 and 90 μM) and 3-isobutyl-1-methylxanthine (90 μM) increased the fractional stimulation-induced outflow. Together these results indicate that cyclic AMP may have a modulatory effect on noradrenaline release.

3 The inhibition of the stimulation-induced outflow produced by clonidine (0.03 μM) and its facilitation produced by phentolamine (1 μM) were unaltered in the presence of 8-bromo-cyclic AMP (90 μM). However, in the presence of 8-bromo-cyclic AMP (270 μM), the facilitatory effect of phentolamine was enhanced, but the inhibitory effect of clonidine (0.03 μM) was unaltered. In the presence of ICI 63197 (30 μM) the inhibitory effect of clonidine (0.03 μM) was unaltered, but the facilitatory effect of phentolamine (1 μM) was slightly enhanced.

4 Isoprenaline (0.003–0.1 μM) enhanced the fractional stimulation-induced outflow, an effect blocked by propranolol (0.1 μM). In the presence of 8-bromo-cyclic AMP (90 μM), the facilitatory effect of isoprenaline (0.01 μM) was blocked. In the presence of ICI 63197 (30 μM) the facilitatory effect of isoprenaline (0.003 μM) was potentiated.

5 These results suggest that whereas β-adrenoceptor-mediated enhancement of noradrenaline release is linked to the stimulation of adenylate cyclase and enhanced formation of cyclic AMP, α-adrenoceptor-mediated inhibition of noradrenaline release is not linked to inhibition of adenylate cyclase activity.

Introduction

There are two adrenoceptor-mediated modulatory systems at sympathetic nerve endings: an α_2-adrenoceptor-mediated system, activation of which leads to inhibition of noradrenaline release, and a β_2-adrenoceptor-mediated system, activation of which leads to facilitation of noradrenaline release (see reviews by Starke, 1977; 1981; Majewski, 1983). The inhibitory prejunctional α_2-adrenoceptor mechanism is tonically activated by neuronally-released noradrenaline during a train of nerve impulses, thus completing an 'inhibitory feedback loop' (Starke, 1977; 1981). The facilitatory prejunctional β_2-adrenoceptor mechanism appears not to be activated by neuronally-released noradrenaline, but may be activated by adrenaline either in the circulation or after its uptake into, and release from sympathetic nerve endings (Majewski, 1983).

The intraneuronal events that occur between activation of these receptors and alterations in noradrenaline release are unknown. An attractive hypothesis is that these adrenoceptor-mediated effects are due to alterations in adenylate cyclase activity and cyclic 3',5'-adenosine monophosphate (cyclic AMP) formation. In support of this, the stimulation induced (S-I) release of noradrenaline is enhanced by cell permeable analogues of cyclic AMP (dibutyryl-cyclic AMP, 8-bromo-cyclic AMP) as well as phosphodiesterase inhibitors which inhibit the breakdown of cyclic AMP (Langer, 1973; Wooten *et al.*, 1973; Cubeddu *et al.*, 1975; Celuch *et al.*, 1978; Stjärne *et al.*, 1979; Göthert & Hentrich, 1984; Hentrich *et al.*, 1985; Alberts *et al.*, 1985) and the adenylate cyclase activator forskolin (Hovevei-Sion *et al.*, 1983; Hentrich *et al.*, 1985; Alberts *et al.*, 1985). It is therefore possible that alterations in endogenous cyclic AMP production may explain the modulatory effects of α- and β-adrenoceptor agonists on noradrenaline release, especially in view of findings in non-neuronal tissues where β-adrenoceptor agonists enhance adenylate cyclase activity leading to increased cyclic AMP

[1] Author for correspondence.

production (Perkins, 1973) and α_2-adrenoceptor agonists inhibit adenylate cyclase and decrease cyclic AMP production (Jakobs *et al.*, 1981).

The aim of the present study was to investigate the possible role of cyclic AMP in the modulation of noradrenaline release in mouse isolated atria and, furthermore, to determine if changes in adenylate cyclase activity and the formation of cyclic AMP are an integral part of the mechanisms of prejunctional β-adrenoceptor facilitation and prejunctional α-adrenoceptor inhibition of noradrenaline release.

Methods

Female mice (20–32 g) were decapitated and the heart removed. The atria were dissected free of the surrounding tissue and suspended between two platinum electrodes in an organ bath containing 4 ml of Krebs-Henseleit solution aerated with a mixture of 5% CO_2 plus 95% O_2 and maintained at 37°C. The atria were incubated with (−)-7,8-[^{3}H]-noradrenaline (2.5 μCi ml^{-1}; 0.2 μM) for 20 min and then repeatedly washed with Krebs-Henseleit solution for 1 h. A priming stimulation (5 Hz for 60 s) was delivered 40 min after commencement of the washing procedure to assist in the removal of loosely bound radioactive compounds from the tissue. In some experiments 8-bromo-cyclic 3',5'-adenosine monophosphate was added immediately after the primary stimulation and was present for the duration of the experiment.

To assess the effects of drugs on the S-I outflow of radioactive compounds, the atria were stimulated twice at a frequency of 5 Hz for 60 s with square wave pulses (1 ms duration, 15 V cm^{-1}). These two test stimulations were applied 69 and 99 min after the commencement of the washing procedure, respectively. The effects of drugs on the S-I outflow of radioactivity were determined by adding the drugs to the Krebs-Henseleit bathing solution 15 min before the second stimulation.

The Krebs-Henseleit solution was collected after 3 min periods of contact. The spontaneous outflow of radioactivity was taken as the mean radioactive content of the bathing solution during the 3 min period immediately before stimulation and the 3 min period commencing 6 min after the onset of stimulation. The stimulation-induced (S-I) component of the radioactive outflow was calculated by subtracting the resting outflow from the mean radioactivity in the two 3 min samples collected immediately after stimulation. The S-I outflow was expressed as a ratio of (the total radioactivity present in the tissue at the onset of stimulation)/(the fractional stimulation-induced outflow). The fractional stimulation-induced outflow of the second stimulation was expressed as a percentage of the fractional outflow for the first.

The radioactivity present in the atria was determined by dissolving the atria in 2 ml of Soluene 350 (Packard Instruments) and mixing the resulting solution with Picofluor 30 (Packard Instruments), followed by liquid scintillation counting. Radioactivity in the bathing solution was also determined in Picofluor. Corrections were made for counting efficiency using automatic external standardization.

Statistical analysis of results

Data are expressed as mean ± s.e.mean. Unless otherwise indicated, differences between groups of data were analysed by Student's unpaired 2 tailed *t* test. Where appropriate, a two-way analysis of variance was performed on some groups of data to test for interactions. Probability levels of less than 0.05 were taken to indicate a significant difference. All tests were performed using the Statistical Package for Social Sciences (SPSS) (Nie *et al.*, 1975).

Materials

The following drugs were used: (−)-7,8-[^{3}H]-noradrenaline (specific activity 13–14 Ci mmol^{-1}; the Radiochemical Centre, Amersham); 8-bromo-cyclic 3',5'-guanosine monophosphate, 8-bromo-cyclic 3',5'-adenosine monophosphate, 3-isobutyl-1-methylxanthine, adenosine hemisulphate (Sigma, U.S.A.). The following drugs were generously donated: M&B 22948 (2-*o*-propoxyphenyl-8-azapurin-6-one; May and Baker, U.K.); (±)-propranolol HCl, ICI 63197 (2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-5-triazolo /1,5-a/ pyrimidine) (ICI, U.K. and Australia); phentolamine mesylate (Ciba-Geigy, Australia); (±)-isoprenaline HCl (Winthrop, Australia). Stock solutions were prepared by dissolving the drugs in Krebs-Henseleit solution, with the exception of M&B 22948 which was initially dissolved in 0.2 M NaOH (pH of final Krebs-Henseleit solution was 7.4).

The Krebs-Henseleit solution has the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, KH_2PO_3 1.03, $MgSO_4$ 0.45, D-(+)-glucose 11.1; disodium edetate 0.067 and ascorbic acid 0.14.

Results

Effects of cyclic nucleotide analogues

Mouse atria were incubated in [^{3}H]-noradrenaline and the fractional S-I outflow of radioactivity was increased in a concentration-dependent manner by 8-bromo-cyclic AMP (3–270 μM) (Figure 1). 8-Bromo-cyclic GMP (3–270 μM) also enhanced the fractional S-I outflow of radioactivity; however, the effect was

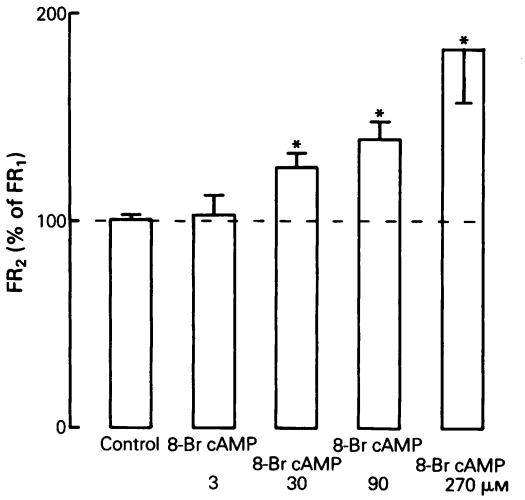

Figure 1 The effect of 8-bromo-cyclic AMP on the fractional S-I outflow of radioactivity from mouse isolated atria pre-incubated with [^{3}H]-noradrenaline. Two periods of electrical stimulation were applied (5 Hz for 60 s). The fractional S-I outflow of radioactivity in the second stimulation period (FR_2) is expressed as a percentage of that in the first (FR_1). 8-Bromo-cyclic AMP (8-Br cAMP; 3–270 μM) was present from 15 min before the second stimulation period. Each column represents the mean and the vertical bar the s.e.mean. See Table 1 for values of *n*. *Indicates a significant difference from control (*t* test).

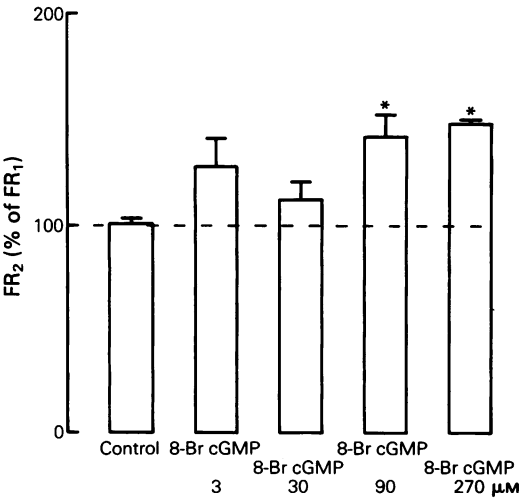

Figure 2 The effect of 8-bromo-cyclic GMP on the fractional S-I outflow of radioactivity from mouse isolated atria pre-incubated with [^{3}H]-noradrenaline. 8-Bromo-cyclic GMP (8-Br cGMP; 3–270 μM) was present from 15 min before the second stimulation period. Other details as in Figure 1.

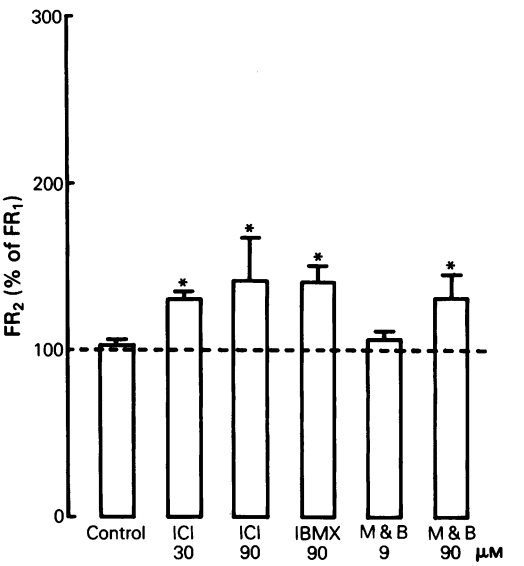

Figure 3 The effect of phosphodiesterase inhibitors on the fractional S-I outflow of radioactivity from mouse isolated atria preincubated with [^{3}H]-noradrenaline. M&B 22948 (M&B 9 and 90 μM), ICI 63197 (ICI 30 and 90 μM) and isobutylmethylxanthine (IBMX, 90 μM) were present from 15 min before the second stimulation period. Other details as in Figure 1.

not as pronounced as that of 8-bromo-cyclic AMP (Figure 2). Neither drug affected the spontaneous outflow of radioactivity (Table 1).

Effects of phosphodiesterase inhibitors

The relatively selective cyclic GMP phosphodiesterase inhibitor, M&B 22948, the relatively selective cyclic AMP phosphodiesterase inhibitor, ICI 63197 and 3-isobutyl-1-methylxanthine (IBMX), a non-selective phosphodiesterase inhibitor (Fredholm *et al.*, 1979) all increased the fractional S-I outflow of radioactivity (Figure 3). There were only slight effects on the spontaneous outflow of radioactivity (Table 1).

Effects of clonidine and phentolamine

Clonidine (0.03–1 μM) significantly decreased the fractional S-I outflow of radioactivity. The reduction was 38 ± 3% (mean ± s.e.mean; $n = 3$) for 0.3 μM clonidine and 55 ± 9% ($n = 3$) for 1 μM clonidine. The values for 0.03 μM clonidine are presented in Figure 4.

Conversely, phentolamine (1 μM) markedly increased the fractional S-I outflow of radioactivity (Figure 4). The spontaneous outflow of radioactivity was unaltered by clonidine (0.03 and 1 μM) and

phentolamine (1 μM), but was slightly reduced by clonidine (0.3 μM) (Table 1).

In the presence of ICI 63197 (30 μM), which by itself produced a significant increase in the fractional S-I outflow of radioactivity (Figure 4), the inhibitory effect of clonidine (0.03 μM) was unaltered (two-way analysis of variance) (Figure 4). In contrast, the enhancement of the fractional S-I outflow of radioactivity produced by the combination of ICI 63197 (30 μM) and phentolamine (1 μM) was greater than the simple addition of the individual effects of the drugs (Figure 4).

In some experiments 8-bromo-cyclic AMP (90 μM) was present for both stimulation periods to saturate the sympathetic nerve endings with cyclic AMP analogue. In this situation the decrease in the fractional S-I outflow of radioactivity produced by clonidine (0.03 μM) and the enhancement produced by phentolamine (1 μM) did not differ significantly from their effects in the absence of 8-bromo-cyclic AMP (cf. Figures 4 and 6) (two-way analysis of variance). The absolute fractional S-I outflow of radioactivity in the first stimulation period (FR_1) in the presence of 8-bromo-cyclic AMP (0.0107 ± 0.0015, mean ± s.e.mean, $n = 15$) was not significantly different from that in its absence (0.0103 ± 0.0068, $n = 146$). In the presence of a higher concentration of 8-bromo-cyclic AMP (270 μM) present for only the second stimulation period, the decrease in the fractional S-I outflow of radioactivity produced by clonidine (0.03 μM) was unaltered (two-way analysis of variance), but the enhancement in the fractional S-I outflow produced by phentolamine (1 μM) was significantly greater than the simple addition of the individual effects of the drugs (Figure 4).

Effect of isoprenaline

Isoprenaline (0.003–0.1 μM) produced a concentration-dependent increase in the fractional S-I outflow of radioactivity (Figure 5). The enhancing effect of isoprenaline was blocked by propranolol (0.1 μM), which itself produced no significant change in the fractional S-I outflow of radioactivity (Figure 5). Neither drug affected the spontaneous outflow of radioactivity (Table 1).

When ICI 63197 (30 μM) was present during the second stimulation period, it significantly enhanced the fractional S-I outflow of radioactivity (Figure 5). The combination of both ICI 63197 (30 μM) and isoprenaline (0.003 μM) produced an enhancement of the fractional S-I outflow of radioactivity greater than the simple addition of the individual effects of each drug (Figure 5).

When 8-bromo-cyclic AMP (90 μM) was present for both stimulation periods, isoprenaline (0.01 μM) present in the second stimulation period failed to

Table 1 Effect of drugs on the spontaneous (resting) outflow of radioactivity from mouse atria preincubated with [^{3}H]-noradrenaline

Drug treatment	*Conc.* (μM)	R_2 (*% of* R_1)	n
Control		76.7 ± 2.7	22
8-Br-cyclic AMP	3	82.0 ± 1.2	4
	30	82.3 ± 12.4	5
	90	76.6 ± 2.6	8
	270	74.3 ± 3.3	6
8-Br-cyclic GMP	3	74.9 ± 4.5	6
	30	71.2 ± 4.5	5
	90	75.9 ± 7.7	6
	270	64.0 ± 4.4	3
M&B 22948	9	75.9 ± 7.7	6
	90	60.8* ± 5.0	3
ICI 63197	30	78.3 ± 1.9	4
	90	65.4* ± 3.8	6
IBMX	90	68.9 ± 3.8	4
Clonidine	0.03	71.8 ± 2.8	6
	0.3	60.4* ± 4.2	3
	1.0	70.7 ± 8.5	3
Phe	1.0	78.6 ± 3.1	9
Iso	0.003	76.3 ± 2.9	4
	0.01	75.1 ± 2.5	6
	0.1	69.7 ± 3.3	6
Pro	0.1	71.9 ± 4.9	7
Pro + Iso	0.1 + 0.1	65.3 ± 6.3	5
ICI 63197 + Iso	30 + 0.003	78.4 ± 5.8	4
ICI 63197 + clonidine	30 + 0.03	76.9 ± 1.3	4
ICI 63197 + Phe	30 + 1.0	84.7 ± 1.7	4
8-Br-cyclic AMP + clonidine	270 + 0.03	72.2 ± 3.2	4
8-Br-cyclic AMP + Phe	270 + 1.0	78 ± 5.3	5
8-Br-cyclic AMP throughout			
Control		77.7 ± 2.1	7
Clonidine	0.03	74.3 ± 6.2	4
Phe	1.0	77.9 ± 3.9	4
Iso	0.01	72.6 ± 3.3	4

Resting outflow for the second stimulation measurement period (R_2) was expressed as a percentage of the resting outflow for the first measurement period (R_1) and the values shown are means ± s.e.mean. Drugs: 8-bromo-cyclic AMP (8-Br-cyclic AMP), 8-bromo-cyclic GMP (8-Br-cyclic GMP), ICI 63197, M&B 22948, clonidine, phentolamine (Phe), isoprenaline (Iso), isobutylmethylxanthine (IBMX) and propranolol (Pro) were present only for the second stimulation cycle. In some experiments 8-bromo-cyclic AMP (90 μM) was present for both stimulation cycles. *Indicates a significant difference ($P < 0.05$, t test) from the corresponding control.

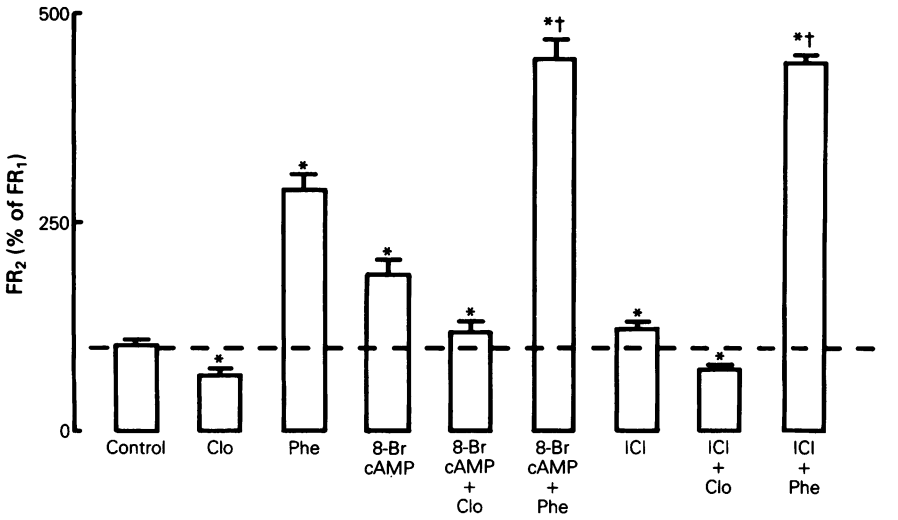

Figure 4 Effect of clonidine (Clo, 0.03 μM) and phentolamine (Phe, 1 μM) on the fractional S-I outflow of radioactivity from mouse isolated atria pre-incubated with [^{3}H]-noradrenaline. All drugs were present from 15 min before the second stimulation period. Other details as in Figure 1. †Indicates that the effect of phentolamine in the presence of ICI 63197 (ICI, 30 μM) or 8-bromo-cyclic AMP (8-BrcAMP, 270 μM) differed significantly from the simple addition of the individual effects of the drugs ($P < 0.05$, two-way analysis of variance).

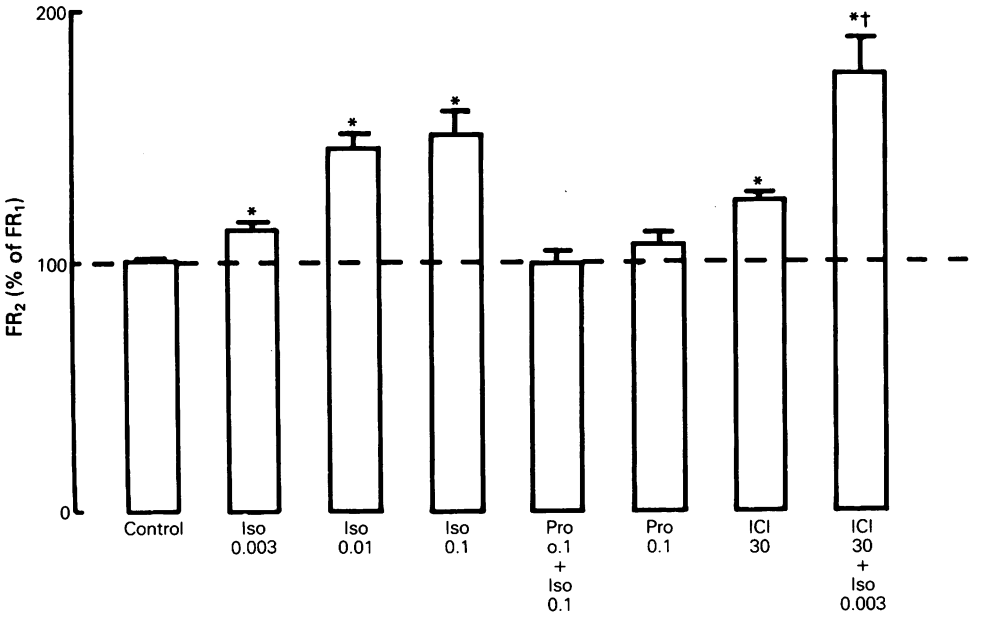

Figure 5 Effect of isoprenaline (Iso, 0.003–0.1 μM) on the fractional S-I outflow of radioactivity from mouse isolated atria pre-incubated with [^{3}H]-noradrenaline. Isoprenaline or propranolol (Pro, 0.1 μM) or ICI 63197 (ICI, 30 μM) were present from 15 min before the second stimulation period. Other details as in Figure 1. † Indicates that the effect of isoprenaline in the presence of ICI 63197 differed significantly from the simple addition of the individual effects of the drugs ($P < 0.05$, two-way analysis of variance).

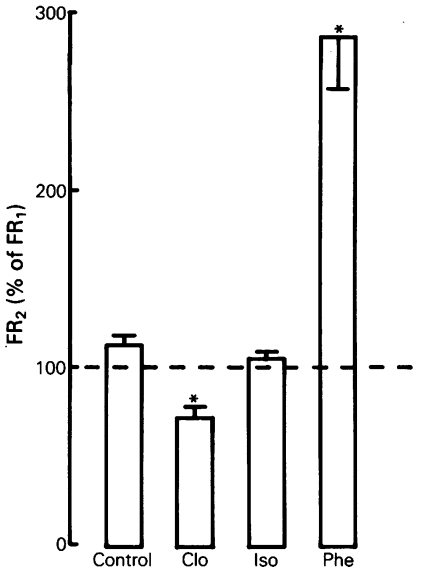

Figure 6 The influence of 8-bromo-cyclic AMP (90 μM) on the effects of clonidine (Clo, 0.03 μM); phentolamine (Phe 1.0 μM) and isoprenaline (Iso, 0.01 μM) on the fractional S-I outflow of radioactivity from mouse isolated atria pre-incubated with [^{3}H]-noradrenaline. 8-Bromo-cyclic AMP was present throughout both stimulation periods. The other drugs (Clo, Phe, Iso) were present from 15 min before the second stimulation. Other details as in Figure 1. *Represents a significant difference ($P<0.05$, t test) from control (8-bromo-cyclic AMP throughout).

produce an enhancement of the fractional stimulation-induced outflow of radioactivity (Figure 6), in contrast to its effects in the absence of 8-bromo-cyclic AMP (Figure 5).

Effect of adenosine

In a separate series of experiments, adenosine (10 μM) was added during the second period of stimulation. It had no significant effect on the fractional S-I outflow of radioactivity, which was 122 ± 11% in the presence of adenosine compared to the control value of 96 ± 7%. The spontaneous outflow of radioactivity was not altered by adenosine.

Discussion

In the present study mouse atria were incubated in [^{3}H]-noradrenaline and the stimulation-induced outflow of radioactivity measured. It has been well established that the radioactivity retained in tissues is almost exclusively unmetabolized [^{3}H]-noradrenaline and its release from the storage vesicles by nerve stimulation is similar to that of endogenous noradrenaline (see Langer, 1974; Starke, 1977; Starke *et al.*, 1984). It has also been established that the metabolism of noradrenaline ([^{3}H]-noradrenaline) released by electrical stimulation from isolated tissues (stimulation-induced outflow) occurs subsequent to its release from the nerve ending (although most of the radioactivity is accounted for by unmetabolized [^{3}H]-noradrenaline). Thus for the calculation of an index of actual output of transmitter, it is important to include these subsequently formed metabolites and not to rely on the determination of noradrenaline alone (Langer, 1970; 1974; Starke *et al.*, 1984). Therefore, in this study the stimulation-induced component of the release of radioactivity from the tissue was taken as an index of actual transmitter release since this measurement includes noradrenaline and its subsequently formed metabolites (see Langer, 1974). The spontaneous outflow of radioactivity consists mainly of noradrenaline metabolites and in most tissues probably is the result of leakage of noradrenaline from the storage vesicles into the nerve cytoplasm (see reviews above).

The cell permeable analogue of cyclic AMP, 8-bromo-cyclic AMP, produced a concentration-dependent increase in the S-I outflow of radioactivity from mouse atria pre-incubated with [^{3}H]-noradrenaline, without altering the spontaneous outflow. Similar findings with cell permeable cyclic AMP analogues have been obtained previously in peripheral tissues, e.g. guinea-pig vas deferens (Wooten *et al.*, 1973; Stjärne *et al.*, 1979), cat spleen (Cubeddu *et al.*, 1975; Celuch *et al.*, 1978), rabbit pulmonary artery (Göthert & Hentrich, 1984), human pulmonary artery (Hentrich *et al.*, 1985) and guinea-pig ileum myenteric plexus (Alberts *et al.*, 1985). It has been suggested that cyclic AMP may by itself induce noradrenaline release, since in guinea-pig vas deferens incubated with [^{3}H]-noradrenaline, cell permeable cyclic AMP analogues were found to enhance the spontaneous outflow of radioactivity (Stjärne *et al.*, 1979). This has not been observed in other tissues where only the stimulation-induced component of the release was enhanced (Alberts *et al.*, 1985; Hentrich *et al.*, 1985; present study). It is therefore more likely that cyclic AMP may only be involved in the modulation of noradrenaline release, as postulated by Cubeddu *et al.* (1975), and not in the initiation of the release process.

Another cyclic nucleotide which may be of importance is cyclic GMP, which in many systems may have effects opposite to those of cyclic AMP (Goldberg *et al.*, 1975). However, in the present study 8-bromo-cyclic GMP enhanced the fractional S-I outflow. The effect was much less pronounced than that produced by 8-bromo-cyclic AMP and was apparently not concentration-dependent. In the cat isolated spleen a

facilitatory effect of 8-bromo-cyclic GMP on [^{3}H]-noradrenaline release has also been observed (Cubeddu *et al.*, 1975). The importance of this small effect is unclear, but it might be due to inhibition of cyclic AMP phosphodiesterase activity (Cubeddu *et al.*, 1975). In the guinea-pig myenteric plexus, 8-bromo-cyclic GMP and dibutyryl cyclic GMP were without effect on noradrenaline release (Alberts *et al.*, 1985) and in guinea-pig vas deferens, 8-bromo-cyclic GMP also had no effect on noradrenaline release (Stjärne *et al.*, 1979).

The role of cyclic nucleotides in noradrenaline release may also be elucidated by using inhibitors of the phosphodiesterase enzymes which degrade endogenous cyclic AMP and cyclic GMP. In the present study, the selective cyclic AMP phosphodiesterase inhibitor, ICI 63197, and the non-selective phosphodiesterase inhibitor, IBMX (Fredholm *et al.*, 1979), both enhanced the fractional S-I outflow of radioactivity in mouse atria, presumably due to an increase in the intraneuronal levels of endogenous cyclic AMP. These results are entirely consistent with the facilitatory effect of cyclic AMP analogues. Similar findings with phosphodiesterase inhibitors have been observed in other tissues (Langer, 1973; Wooten *et al.*, 1973; Cubeddu *et al.*, 1975; Celuch *et al.*, 1978; Stjärne *et al.*, 1979; Göthert & Hentrich, 1984; Hentrich *et al.*, 1985; Alberts *et al.*, 1985; Johnston & Majewski, 1986). In mouse atria, these effects appear unlikely to be due to blockade of inhibitory prejunctional adenosine receptors at sympathetic nerve endings since adenosine (10 μM) had no inhibitory effect on the fractional S-I outflow of radioactivity in mouse atria and ICI 63197, even at 90 μM, has only weak blocking effects on prejunctional adenosine receptors (Mian & Majewski, unpublished observations). The facilitatory effect of phosphodiesterase inhibitors is an indication that endogenous cyclic AMP can modulate noradrenaline release.

The selective cyclic GMP phosphodiesterase inhibitor, M&B 22948 (9 μM), failed to enhance the fractional S-I outflow of radioactivity in mouse atria. At a concentration of 90 μM an enhancement was observed, but at this concentration it is likely that selectivity for cyclic GMP phosphodiesterase is lost (Fredholm *et al.*, 1979). Taken together with the effects of 8-bromo-cyclic GMP, these results suggest that cyclic GMP does not have an important role in the modulation of noradrenaline release.

In a previous study in mouse atria (Johnston & Majewski, 1986), it was suggested that at least part of the α-adrenoceptor-mediated inhibition of noradrenaline release may be due to an inhibition of adenylate cyclase activity and decreased neuronal cyclic AMP levels. In the present study, the facilitatory effects of 8-bromo-cyclic AMP and phosphodiesterase inhibitors on noradrenaline release provide the basic prerequisites for the hypothesis. Further circumstantial evidence comes from studies in other systems, where α_2-adrenoceptor agonists have been shown to exert their effects through inhibition of adenylate cyclase (Jakobs *et al.*, 1981). In mouse atria the prejunctional α-adrenoceptors are of the α_2-subtype, since the inhibitory effect of clonidine was blocked by the selective α_2-adrenoceptor blocking drug, idazoxan, but not by the α_1-selective prazosin (Musgrave & Majewski, unpublished observations). Despite this evidence, when the hypothesis is more closely tested the results appear negative. Firstly, the mouse atria were incubated with 8-bromo-cyclic AMP (90 μM) to elevate total neuronal cyclic AMP levels (cyclic AMP + 8-bromo-cyclic AMP), such that changes in endogenous cyclic AMP production would not appreciably alter total neuronal cyclic AMP levels, and would not affect noradrenaline release. Under these conditions, the inhibitory effect of clonidine (0.03 μM) and the facilitatory effect of phentolamine (1 μM) were unaltered. This suggests that the effects of these two drugs are independent of cyclic AMP, unless the 8-bromo-cyclic AMP levels were not high enough to saturate the system. However, even when 8-bromo-cyclic AMP (270 μM) was used, the inhibitory effect of clonidine on the fractional S-I outflow of radioactivity was maintained, whereas the facilitatory effect of phentolamine (1 μM) was enhanced. This latter result is most probably due to increased noradrenaline release evoked by 8-bromo-cyclic AMP producing greater feedback inhibition through prejunctional α_2-adrenoceptors. The facilitatory effect of phentolamine should have been attenuated by high concentrations of 8-bromo-cyclic AMP, if the effect were due to blockade of α-adrenoceptor-mediated inhibition of adenylate cyclase.

In the presence of the phosphodiesterase inhibitor, ICI 63197 (30 μM), the inhibitory effect of clonidine was unaltered and the facilitatory effect of phentolamine on the fractional S-I outflow of radioactivity was slightly enhanced. Again, this effect of phentolamine may be an indication of greater feedback inhibition through prejunctional α-adrenoceptors when ICI 63197 enhanced release. In cat spleen, the facilitatory effect of phentolamine on noradrenaline release was unaltered by IBMX (Cubeddu *et al.*, 1975). Similarly, in the guinea-pig myenteric plexus, the facilitatory effect of IBMX was unaltered by the α-adrenoceptor blocking drug yohimbine (Alberts *et al.*, 1985). The slight potentiation of the effects of phentolamine in the present study by ICI 63197 is probably only apparent because of the method of calculation of results. Our results are expressed as a percentage of a first stimulation in the absence of drugs, whereas Cubeddu *et al.* (1975) and Alberts *et al.* (1985) expressed their results as a percentage of a first stimulation which was already enhanced by IBMX

and yohimbine, respectively. In these cases alterations in drug effects may be masked by differing baseline values. Taken together, these results do not support the concept that α-adrenoceptor-mediated inhibition of noradrenaline release is due to inhibition of adenylate cyclase. Nor does a study in mouse atria treated with *pertussis* toxin where the inhibitory effect of clonidine and the facilitatory effect of phentolamine on noradrenaline release were unaltered (Musgrave *et al.*, 1986). *Pertussis* toxin inactivates the inhibitory guanyl nucleotide binding protein (Murayama & Ui, 1983) which mediates α_2-adrenoceptor inhibition of adenylate cyclase (see Jakobs *et al.*, 1981).

Studies in brain tissue appear to conflict with the results obtained for peripheral noradrenergic sympathetic nerves. In rat cortex, the modulating effects of clonidine and phentolamine on the electrically evoked release of [^{3}H]-noradrenaline were strongly diminished by 8-bromo-cyclic AMP and the adenylate cyclase activator forskolin (Mulder & Schoffelmeer, 1985). Furthermore, in rabbit hippocampal slices, *pertussis* toxin diminished α-adrenoceptor-mediated inhibition of noradrenaline release (Allgaier *et al.*, 1985). These results suggest that there may be fundamental differences between central and peripheral noradrenergic neurones which remain to be elucidated.

β-Adrenoceptor facilitation of noradrenaline release was first proposed by Adler-Graschinsky & Langer (1975) and has been demonstrated in a wide variety of tissues (see review by Majewski, 1983). In mouse atria, we previously showed that isoprenaline enhanced noradrenaline release by activating prejunctional β-adrenoceptors (Johnston & Majewski, 1986). This was confirmed in the present study when the stimulation frequency was 5 Hz. Stimulation of β-adrenoceptors has been frequently linked to the activation of adenylate cyclase (Perkins, 1973) and, in view of the facilitatory effects of cyclic AMP analogues and phosphodiesterase inhibitors on noradrenaline release (see above), it is possible that the facilitation of noradrenaline release by β-adrenoceptor agonists may be due to activation of adenylate cyclase. In the present study, when 8-bromo-cyclic AMP (90 μM) was present, isoprenaline (0.01 μM) failed to enhance noradrenaline release, suggesting that the two agents were acting through a common mechanism. Similarly, the facilitatory effect of isoprenaline (0.003 μM) was potentiated by the phosphodiesterase inhibitor ICI 63197, presumably because any cyclic AMP generated by isoprenaline was not rapidly degraded by phosphodiesterase. A similar interaction between phosphodiesterase inhibitors and isoprenaline has been observed in a number of tissues, including cat spleen (Cubeddu *et al.*, 1975), guinea-pig myenteric plexus (Alberts *et al.*, 1985), rabbit pulmonary artery and mouse atria (Johnston & Majewski, 1986). These interactions strongly suggest that β-adrenoceptor facilitation of noradrenaline release is the result of adenylate cyclase activation and increased neuronal levels of cyclic AMP.

It is concluded that cyclic AMP may have a modulatory role on noradrenaline release in mouse atria. Whereas prejunctional β-adrenoceptor effects may be mediated through adenylate cyclase, the inhibitory effects of the prejunctional α-adrenoceptor system are not mediated through adenylate cyclase.

This work was supported by the National Health and Medical Research Council of Australia. H.M. is a Wellcome Australian Senior Research Fellow. I.M. holds a University of Melbourne Postgraduate Award. The technical assistance of Mrs Heather Reekie is gratefully acknowledged.

References

ADLER-GRASCHINSKY, E. & LANGER, S.Z. (1975). Possible role of a β-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feedback mechanism. *Br. J. Pharmac.*, **53**, 43–50.

ALBERTS, P., ÖGREN, V.R. & SELLSTRÖM, Å.I. (1985). Role of adenosine 3',5'-cyclic monophosphate in adrenoceptor mediated control of ^{3}H-noradrenaline secretion in guinea-pig ileum myenteric nerve terminals. *Naunyn-Schmiedebergs Arch. Pharmac.*, **330**, 114–120.

ALLGAIER, C., FEUERSTEIN, T.J., JACKISCH, R. & HERTTING, G. (1985). Islet activating protein (pertussis toxin) diminishes α_2-adrenoceptor-mediated effects on noradrenaline release. *Naunyn-Schmiedebergs Arch. Pharmac.*, **331**, 235–239.

CELUCH, S.M., DUBOCOVICH, M.L. & LANGER, S.Z. (1978). Stimulation of presynaptic β-adrenoceptors enhances [^{3}H]-noradrenaline release during nerve stimulation in the perfused cat spleen. *Br. J. Pharmac.*, **63**, 97–109.

CUBEDDU, L.X., BARNES, E. & WEINER, N. (1975). Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation. IV. An evaluation of a role for cyclic adenosine monophosphate. *J. Pharmac. exp. Ther.*, **193**, 105–127.

FREDHOLM, B.B., BRODIN, K. & STRANDBERG, K. (1979). On the mechanism of relaxation of tracheal smooth muscle by theophylline and cyclic nucleotide phosphodiesterase inhibitors. *Acta pharmac. tox.*, **45**, 336–344.

GOLDBERG, N.D., HADDOX, M.K., NICOL, S.E., GLASS, D.B., SANFORD, C.H., KUEHL, F.A. & EXTENSEN, N.R. (1975). Biologic regulation through opposing influences of cyclic GMP and cyclic AMP. The Yin-yang hypothesis. *Adv. cyclic nucleotide Res.*, **5**, 307–330.

GÖTHERT, M. & HENTRICH, F. (1984). Role of cAMP for regulation of impulse-evoked noradrenaline release from the rabbit pulmonary artery and its possible relationship

to presynaptic ACTH receptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **328**, 127–134.

HENTRICH, F., GÖTHERT, M. & GRESCHUCHNA, D. (1985). Involvement of cAMP in modulation of noradrenaline release in the human pulmonary artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **330**, 245–247.

HOVEVEI-SION, D., FINBERG, J.P.M., BOMZON, A. & YOUDIM, M.B.H. (1983). Effect of forskolin in rat vas deferens – evidence for facilitatory β-adrenoceptors. *Eur. J. Pharmac.*, **95**, 295–299.

JAKOBS, K.H., AKTORIES, K. & SCHULTZ, G. (1981). Inhibition of adenylate cyclase by hormones and neurotransmitters. *Adv. cyclic nucleotide Res.*, **14**, 173–186.

JOHNSTON, H. & MAJEWSKI, H. (1986). Prejunctional β-adrenoceptors in rabbit pulmonary artery and mouse atria: effect of α-adrenoceptor blockade and phosphodiesterase inhibition. *Br. J. Pharmac.*, **87**, 553–562.

LANGER, S.Z. (1970). The metabolism of (^{3}H)-noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens of the rat. *J. Physiol.*, **208**, 515–546.

LANGER, S.Z. (1973). The regulation of transmitter release elicited by nerve stimulation through a presynaptic feedback mechanism. In *Frontiers in Catecholamine Research*, ed. Usdin, E. & Snyder, S.H. pp. 543–549. New York: Pergamon Press.

LANGER, S.Z. (1974). Selective metabolic pathways for noradrenaline in the peripheral and in the central nervous system. *Med. Biol.*, **52**, 372–383.

MAJEWSKI, H. (1983). Modulation of noradrenaline release through activation of presynaptic β-adrenoreceptors. *J. Autonom. Pharmac.*, **3**, 47–60 (Corrigenda, p. 155).

MULDER, A.H. & SCHOFFELMEER, A.N.M. (1985). Catecholamine and opioid receptors, presynaptic inhibition of CNS neurotransmitter release and adenylate cyclase. *Adv. cyclic nucleotide & protein phosphorylation Res.*, **18**, 273–286.

MURAYAMA, T. & UI, M. (1983). Loss of inhibitory function of the guanine nucleotide regulatory component of adenylate cyclase due to its ADP ribosylation by islet activating protein, pertussis toxin, in adipocyte membranes. *J. biol. Chem.*, **258**, 3319–3326.

MUSGRAVE, I.F., MAJEWSKI, H. & MARLEY, P. (1986). Pertussis toxin does not affect α-adrenoceptor mediated inhibition of noradrenaline release in mouse atria. *Proc. Aust. Soc. clin. exp. Pharmac. Melbourne*, 1986.

NIE, N.H., HADLAI-HULL, C., JENKINS, J.G., STEINBRENNER, K. & BENT, D.H. (1975). *Statistical Package For the Social Sciences*. New York: Mcgraw-Hill Book Company.

PERKINS, J.P. (1973). Adenyl cyclase. *Adv. cyclic nucleotide Res.*, **5**, 641–660.

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

STARKE, K. (1981). Presynaptic receptors. *A. Rev. Pharmac. Tox.*, **21**, 7–30.

STARKE, K., HEDLER, L. & MAJEWSKI, H. (1984). Formation of deaminated catechols from noradrenaline in guinea pig atria and rabbit hearts. In *Neuronal and Extraneuronal Events in Autonomic Pharmacology*. ed. Fleming, W.W., Graefe, K.-H., Langer, S.Z. & Weiner, N. pp. 13–21. New York: Raven Press.

STJÄRNE, L., BARTFAI, T. & ALBERTS, P. (1979). The influence of 8-Br 3',5'-cyclic nucleotide analogs and of inhibitors of 3',5'-cyclic nucleotide phosphodiesterase, on noradrenaline secretion and neuromuscular transmission in the guinea-pig vas deferens. *Naunyn-Schmiedebergs Arch. Pharmac.*, **308**, 99–105.

WOOTEN, G.F., THOA, N.B., KOPIN, I.J. & AXELROD, J. (1973). Enhanced release of dopamine-β-hydroxylase and norepinephrine from sympathetic nerves by dibutyryl cyclic adenosine 3',5'-monophosphate and theophylline. *Molec. Pharmac.*, **9**, 178–183.

(Received December 10, 1986.
Revised March 11, 1987.
Accepted March 24, 1987.)