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Prejunctional β-adrenoceptors in rabbit pulmonary artery and mouse atria: effect of α-adrenoceptor blockade and phosphodiesterase inhibition

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1 In rabbit isolated pulmonary artery previously incubated with [^{3}H]-noradrenaline, isoprenaline (0.3 μM) had no effect on the stimulation-induced outflow of radioactivity. However, if the phosphodiesterase inhibitor ICI 63,197 (30 μM) or the α-adrenoceptor blocker phentolamine (1 μM) was present, then isoprenaline significantly enhanced the stimulation-induced outflow, an effect blocked by propranolol (0.1 μM). ICI 63,197 (30 μM) but not phentolamine significantly enhanced the stimulation-induced outflow of radioactivity.

2 In mouse isolated atria previously incubated with [^{3}H]-noradrenaline and stimulated at a frequency of 10 Hz, isoprenaline had no effect on the stimulation-induced outflow of radioactivity; this is in contrast to its release-enhancing effects at stimulation frequencies of 4 Hz and 2 Hz. The facilitation of stimulation-induced outflow by isoprenaline at 4 Hz was blocked by propranolol (0.08 μM) which, by itself, had no effect on the stimulation-induced outflow.

3 At a stimulation frequency of 2 Hz in mouse atria the facilitatory effect of isoprenaline (0.01 μM) was significantly greater in the presence of ICI 63,197 (30 μM) which, by itself, had no effect on the stimulation-induced outflow. Similarly, the facilitatory effect of isoprenaline was significantly greater in the presence of phentolamine (1 μM) but, in this case, phentolamine significantly enhanced the stimulation-induced outflow.

4 These results suggest that facilitatory prejunctional β-adrenoceptors are present in both rabbit pulmonary artery and mouse atria. The effects of the phosphodiesterase inhibitor ICI 63,197 suggest that they are linked to adenylate cyclase in both tissues and we propose that the ability of phentolamine to facilitate the release and enhance the effect of isoprenaline may be due to the blockade of α-adrenoceptor inhibition of adenylate cyclase. This latter proposition needs further investigation.

Introduction

β-Adrenoceptor agonists enhance the stimulation-induced (S-I) outflow of noradrenaline in many sympathetically innervated tissues and this has been interpreted as evidence for a facilitatory mechanism mediated by prejunctional β-adrenoceptors at sympathetic nerve endings (see reviews by Langer, 1981; Majewski, 1983). Since the facilitatory effect of β-adrenoceptor agonists occurs in rat cultured postganglionic sympathetic nerve sprouts (Weinstock *et al.*, 1978), it is likely that the β-adrenoceptors are indeed topographically prejunctional receptors. The physiological activation of prejunctional β-adrenoceptors by endogenous catecholamines remains an area of controversy and despite earlier suggestions that neuronally-released noradrenaline may activate prejunctional β-adrenoceptors to form a 'positive feedback loop' (Adler-Graschinsky & Langer, 1975), recent evidence has suggested that adrenaline is more likely to be a physiological activator of the system (see review by Majewski, 1983). The neuronal accumulation of adrenaline and its subsequent release from sympathetic nerves also results in prejunctional β-adrenoceptor activation and enhanced noradrenaline release *in vitro* (Guimarães *et al.*, 1978; Majewski *et al.*, 1981b) and *in vivo* (Majewski *et al.*, 1981a; Schmidt *et al.*, 1984). This action of adrenaline has been proposed as a causative factor in the development of hypertension (Majewski & Rand, 1981a; 1984; Brown & Macquin, 1981) and suggests that prejunctional β-adrenoceptors may thus have an important role in the aetiology of hypertension.

The occurrence of prejunctional β-adrenoceptors at

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sympathetic nerve endings is not universal and early work suggested that they are not present in rabbit pulmonary artery (Starke *et al.*, 1975; Endo *et al.*, 1977) or in mouse isolated atria (Farnebo & Hamberger, 1974). These studies have clouded research into prejunctional β-adrenoceptors, making it unlikely that the prejunctional β-adrenoceptor mechanism is a generalized phenomenon at sympathetic nerve endings (see Gillespie, 1980). The present studies were designed to re-investigate these two tissues and to provide a clue as to the events involved in the enhancement of noradrenaline release by β-adrenoceptor agonists.

Methods

Rabbit pulmonary artery

Rabbits were killed by cervical dislocation and the pulmonary artery dissected free and cut into half transversely. Each half was attached to a tissue hook which was passed through the lumen and connected to a 2 g weight via another hook. Each artery was placed in an organ bath containing Krebs-Henseleit solution aerated with a mixture of 5% CO_2 plus 95% O_2, and maintained at 37°C. The arteries were incubated with [^{3}H]-noradrenaline (2.9 μ Ci ml^{-1}, 0.2 μM) for 30 min and then washed repeatedly for 90 min. A priming stimulation (5 Hz for 30 s) was delivered 30 min after the commencement of the washing procedure. In some experiments ICI 63,197, phentolamine or propranolol were added to the bathing solution immediately after the priming stimulation and were present for the duration of the experiment.

To assess the effects of drugs on the stimulation-induced (S-I) outflow of radioactive compounds, the arteries were stimulated twice through a pair of platinum electrodes situated either side of the artery at a frequency of 1 Hz for 60 s with 1 ms square wave pulses at supramaximal voltage (12 V per cm). The first test stimulation was given 9 min after the 90 min washing period and a second stimulation given 30 min after the first. The effect of drugs on the S-I outflow of radioactivity was determined by adding them to the Krebs-Henseleit bathing solution 12 min before the second stimulation.

The Krebs-Henseleit bathing solution was collected after 3 min periods of contact. The spontaneous (resting) radioactive outflow 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 9 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 radioactivity in the three 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 commencement of stimulation – the fractional S-I outflow. The fractional S-I outflow of the second stimulation was expressed as a percentage of the fractional outflow for the first period.

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

Mouse atria

There were minor differences in the procedures used in experiments with mouse atria compared to the above method. Mice were killed by a blow on the head and the atria dissected free. The atria were suspended in an organ bath with Krebs-Henseleit solution containing atropine (1 μM), which was present for the duration of the experiment. The atria were incubated with [^{3}H]-noradrenaline (2.9 μCi ml^{-1}, 0.2 μM) for 20 min and then washed repeatedly for 60 min. A priming stimulation (5 Hz for 60 s) was delivered 40 min after the commencement of the washing procedure. There were two test stimulations 9 and 39 min after this washing period. The spontaneous radioactive outflow was taken as the mean radioactive content of the bathing solution during the 3 min period immediately before the test stimulation (2, 4 or 10 Hz for 60 s), and the 3 min period commencing 6 min after the onset of stimulation. The stimulation-induced component of the radioactive outflow was calculated by subtracting the spontaneous outflow from the radioactive content of the two 3 min samples collected immediately after stimulation and was then expressed as a fraction of the tissue content of radioactivity at the time of stimulation.

Statistical analysis of results

The data were analysed by the unpaired 2-tailed Student's *t* test. In some cases when the variances of the two groups were not homogeneous the Wilcoxon rank sum test was used. Where appropriate, one-way or two-way analysis of variances was performed on some groups of data. In all cases probability levels of less than 0.05 were taken to indicate statistical significance. All tests were performed using the SPSS statistical package (Nie *et al.*, 1975).

Materials

The Krebs-Henseleit solution had the following com-

position (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25.0, KH_2PO_4 1.03, D-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.07. The following drugs were used: (−)-7,8-[^{3}H]-noradrenaline (13 Ci mmol^{-1}; Amersham, U.K.); (±)-isoprenaline HCl (Sterling Pharmaceuticals, Australia); atropine sulphate (B.D.H., U.K.); phentolamine mesylate (Ciba, Australia); ICI 63, 197 (2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-s-triazolo[1,5-a]pyrimidine) and (±)-propranolol HCl were donated by ICI, U.K. and ICI, Australia.

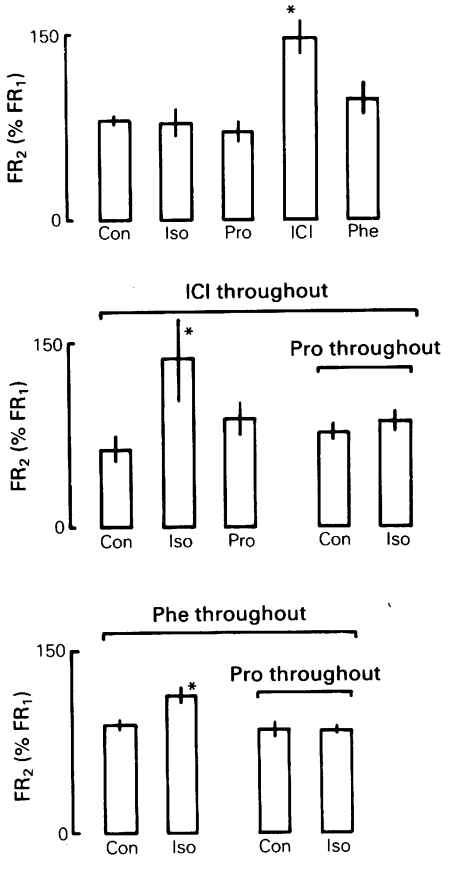

Figure 1 The effect of drugs on the fractional stimulation-induced (S-I) outflow of radioactivity from rabbit pulmonary artery incubated with [^{3}H]-noradrenaline. There were two periods of stimulation (S_1 and S_2; 1 Hz for 60 s each). Drugs (isoprenaline, Iso, 0.3 μM; propranolol, Pro, 0.1 μM; ICI 63,197, ICI, 30 μM; phentolamine, Phe, 1 μM) were present only during the second period of stimulation. In some experiments ICI 63,197 (30 μM) or a combination of ICI 63,197 (30 μM) and propranonol (0.1 μM) were present during both stimulation periods. In other experiments phentolamine (1 μM) or a combination of phentolamine (1 μM) and propranolol (0.1 μM) were present during both stimulation periods. The fractional S-I outflow in the second period of stimulation (FR_2) is expressed as a percentage of that in the first (FR_1). The vertical lines represent the s.e.mean. *Significant difference from control (Con), $P<0.05$, Student's t test. The number of experiments for each group is given in Table 1.

Results

Rabbit pulmonary artery

Rabbit pulmonary arteries were isolated and then incubated with [^{3}H]-noradrenaline. After a period of washing there were two periods of stimulation, each at a frequency of 1 Hz for 60 s, 30 min apart. The fractional S-I outflow of radioactivity in the first stimulation period was 3.6×10^{-3} (s.e.mean $= 4.4 \times 10^{-4}$, $n = 26$). Drugs were added before the second stimulation period. As can be seen from Figure 1, neither isoprenaline (0.3 μM), propranolol (0.1 μM) nor phentolamine (1 μM) had any effect on the S-I outflow of radioactivity. ICI 63,197 (30 μM) on the other hand, caused a significant increase in the stimulation-induced outflow. None of the drugs affected the spontaneous (resting) outflow of radioactivity from the tissue except for propranolol which slightly increased the outflow (Table 1).

In another series of experiments ICI 63,197 (30 μM) was present throughout. The fractional S-I outflow of radioactivity in the first stimulation period was 5.0×10^{-3} (s.e.mean $= 7.8 \times 10^{-4}$, $n = 13$). Drugs were added before the second stimulation period. As can be seen from Figure 1, isoprenaline (0.3 μM) significantly enhanced the S-I outflow of radioactivity but propranolol (0.1 μM) was without effect. Neither drug affected the spontaneous outflow of radioactivity from the tissue (Table 1). When both ICI 63,197 (30 μM) and propranolol (0.1 μM) were present throughout the fractional S-I outflow of radioactivity in the first stimulation period was 4.6×10^{-3} (s.e.mean $= 6.2 \times 10^{-4}$, $n = 8$). Isoprenaline (0.3 μM) was added before the second stimulation period and had no effect on either the S-I (Figure 1) or spontaneous outflow (Table 1) of radioactivity from the tissue, suggesting that the isoprenaline enhancement of S-I outflow was due to β-adrenoceptor activation.

Lastly, when phentolamine (1 μM) was present throughout the fractional S-I outflow of radioactivity in the first stimulation period was 4.9×10^{-3} (s.e.mean $= 5.5 \times 10^{-3}$, $n = 19$). Isoprenaline (0.3 μM) was added before the second stimulation period and significantly enhanced the S-I outflow of radioactivity (Figure 1).

When propranolol (0.1 μM) was present in addition to phentolamine (1 μM) the fractional outflow of radioactivity in the first stimulation period was 6.0×10^{-3} (s.e.mean $= 9.5 \times 10^{-4}$, $n = 8$). In this

Table 1 Effects of drugs on the spontaneous (resting) outflow of tritiated compounds in rabbit pulmonary artery

Drug	n	R_2/R_1 (%)
Control	7	84.1 ± 2.8
Iso 0.3 μM	4	77.2 ± 4.9
Pro 0.1 μM	5	93.6 ± 2.6*
ICI 30 μM	4	88.0 ± 3.9
Phe 1 μM	6	87.4 ± 3.2
ICI 30 μM throughout		
Control	4	76.2 ± 3.0
Iso 0.3 μM	4	77.3 ± 1.4
Pro 0.1 μM	5	84.6 ± 5.3
ICI 30 μM + Pro 0.1 μM throughout		
Control	4	89.6 ± 5.0
Iso 0.3 μM	4	83.4 ± 2.3
Phe 1 μM throughout		
Control	8	86.3 ± 4.2
Iso 0.3 μM	7	88.9 ± 1.8
Phe 1 μM + Pro 0.1 μM throughout		
Control	4	81.4 ± 7.5
Iso 0.3 μM	4	85.7 ± 3.9

The arteries were incubated with [^{3}H]-noradrenaline. There were two periods of stimulation 30 min apart and the spontaneous (resting) outflow of radioactivity in the second stimulation cycle (R_2) was expressed as a percentage of that in the first (R_1)). Data shown are mean ± s.e.mean. Drugs – control (no drug); isoprenaline (Iso, 0.3 μM); ICI 63,197 (ICI, 30 μM); propranolol (Pro, 0.1 μM) or phentolamine (Phe, 1 μM) were added 12 min before R_2. In some experiments ICI 63,197 (30 μM) or a combination of ICI 63,197 (30 μM) and propranolol (0.1 μM) or phentolamine (1 μM) or a combination of phentolamine (1 μM) and propranolol (0.1 μM) were present for both R_1 and R_2 measurement periods. *Represents a significant difference from control ($P<0.05$, Student's t test).

situation isoprenaline (0.3 μM), present during the second stimulation period, had no effect on the S-I outflow (Figure 1).

Mouse atria

Mouse atria were isolated and then incubated with [^{3}H]-noradrenaline. After a period of washing the intramural sympathetic nerves were field stimulated twice at frequencies of either 2, 4 or 10 Hz. The mean fractional S-I outflow of radioactivity in the first stimulation period for each frequency and drug pretreatment is given in Table 2. There were minor effects of drugs on the spontaneous resting outflow of radioactivity; these are shown in Table 3.

Table 2 Absolute fractional stimulation-induced (S-I) outflow in the first stimulation period (FR_1) in mouse atria

Pretreatment	Frequency (Hz)	FR_1 (× 100)	s.e.mean	n
–	10	5.56	0.35	26
ICI 30 μM	10	5.84	0.49	12
ICI 90 μM	10	5.59	0.68	10
Phe 1 μM	10	10.66*	0.91	10
–	4	2.26	0.25	59
–	2	0.78	0.03	63

Mouse isolated atria were incubated with [^{3}H]-noradrenaline and subjected to field stimulation. The fractional S-I outflow of radioactivity during the first stimulation period (FR_1) is shown. In some experiments atria were treated with drugs – ICI 63,197 (ICI 30 and 90 μM) or phentolamine (Phe; 1 μM) before the field stimulation. *Significantly different from 10 Hz experiments in the absence of drugs, $P<0.05$, Student's t test.

Effect of ICI 63,197 At a stimulation frequency of 4 Hz the phosphodiesterase inhibitor ICI 63,197 (30–270 μM) was added 15 min before the second stimulation period and caused a concentration-dependent enhancement in the fractional S-I outflow of radioactivity (Figure 2). However, the effect of ICI 63,197 appears to depend on the stimulation frequency. At 10 Hz, ICI 63,197 (90 μM) had no effect on the fractional S-I outflow, whereas ICI 63,197 (90 μM) significantly enhanced the fractional S-I outflow at 4 Hz and 2 Hz, the effect being significantly greater at 4 Hz ($P<0.05$, two way analysis of variance) (Figure 2).

Effect of isoprenaline At a frequency of 10 Hz, isoprenaline (0.1 μM) had no significant effect on the fractional S-I outflow of radioactivity. The fractional S-I outflow of radioactivity was not maximal since the α-adrenoceptor blocking drug phentolamine (1 μM) significantly enhanced the fractional S-I outflow (Figure 3). When phentolamine (1 μM) or ICI 63,197 (90 μM) were present throughout, isoprenaline had no significant effect on the fractional S-I outflow. However, in the presence of ICI 63,197 (30 μM), isoprenaline (0.1 μM) produced a very small but nevertheless statistically significant increase in the fractional S-I outflow (Figure 3).

At a stimulation frequency of 4 Hz, in contrast to 10 Hz, isoprenaline (0.1 μM) significantly enhanced the fractional S-I outflow of radioactivity, an effect blocked by the β-adrenoceptor blocking drug propranolol which, by itself, had no effect on the fractional S-I outflow (Figure 4).

A final series of experiments was performed at a

Table 3 Effect of drugs on the spontaneous (resting) outflow of tritiated compounds in mouse atria

Drug	n	R_2/R_1 (%)
10 Hz experiments		
Control	9	79.7 ± 3.7
Iso 0.1 μM	4	85.0 ± 5.3
ICI 30 μM	5	91.2 ± 4.8
ICI 90 μM	4	79.5 ± 2.2
Phe 1 μM	4	101.5 ± 3.9*
ICI 30 μM throughout		
Control	6	86.4 ± 2.7
Iso 0.1 μM	6	76.1 ± 5.8
ICI 90 μM throughout		
Control	6	73.4 ± 1.3
Iso 0.1 μM	4	72.4 ± 3.8
Phe 1 μM throughout		
Control	5	76.1 ± 7.3
Iso 0.1 μM	5	78.3 ± 2.2
4 Hz experiments		
Control	16	80.2 ± 3.0
Iso 0.1 μM	11	83.7 ± 3.2
Phe 1 μM	8	81.2 ± 5.1
Pro 0.8 μM + Iso 0.1 μM	6	77.3 ± 2.7
ICI 30 μM	8	88.7 ± 4.6
ICI 90 μM	4	79.3 ± 4.7
ICI 270 μM	6	85.7 ± 8.4
2 Hz experiments		
Control	12	77.6 ± 3.6
Iso 0.01 μM	7	73.6 ± 2.9
ICI 30 μM	8	73.0 ± 2.6
ICI 90 μM	9	75.9 ± 2.3
ICI 30 μM + Iso 0.01 μM	7	71.4 ± 1.8
ICI 90 μM + Iso 0.01 μM	6	74.7 ± 3.0
Phe 1 μM	7	71.4 ± 2.8
Phe 1 μM + Iso 0.01 μM	7	72.5 ± 1.8

The atria were incubated with [^{3}H]-noradrenaline. There were two periods of stimulation 30 min apart and the spontaneous (resting) outflow of radioactivity in the second stimulation cycle (R_2) was expressed as a percentage of that in the first (R_1). Drugs – control (no drug); isoprenaline (Iso, 0.1 and 0.01 μM); ICI 63,197 (ICI; 30, 90 and 270 μM); propranolol (Pro, 0.1 μM) or phentolamine (Phe, 1 μM) were added 12 min before R_2. In some experiments ICI 63,197 or phentolamine (1 μM) were present for both R_1 and R_2 measurement periods throughout. *Represents a significant difference from control ($P < 0.05$, Student's t test).

frequency of 2 Hz to elucidate further the interactions between isoprenaline, ICI 63,197 and phentolamine (Figure 5). A lower concentration of isoprenaline was used (0.01 μM) and this concentration significantly enhanced the fractional S-I outflow of radioactivity in the second stimulation period. ICI 63,197 (30 μM) had no effect on the fractional S-I outflow but when ICI 63,197 (30 μM) and isoprenaline (0.01 μM) were present together in the second stimulation period, the fractional S-I efflux of radioactivity was significantly greater than that produced by isoprenaline (0.01 μM) alone. A higher concentration of ICI 63,197 (90 μM) significantly enhanced the fractional S-I outflow but in this case the combination of ICI 63,197 (90 μM) and isoprenaline (0.01 μM) did not further enhance the fractional S-I outflow (Figure 5).

At a frequency of 2 Hz, the presence of phentolamine (1 μM) in the second period also significantly enhanced the fractional S-I outflow of radioactivity and the combination of phentolamine (1 μM) and isoprenaline (0.01 μM) enhanced the fractional S-I outflow even further (Figure 5). The difference between the fractional outflow of the combined phentolamine (1 μM) and isoprenaline (0.01 μM) experiments and that with phentolamine alone was significantly greater than the difference between that of isoprenaline (0.01 μM) and the no drug controls ($P < 0.05$, ANOVA).

Discussion

Adenylate cyclase and prejunctional β-adrenoceptors

β-Adrenoceptors are frequently linked in the literature to adenylate cyclase activity and the generation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Perkins, 1973), and it is possible that this is also true of prejunctional β-adrenoceptors. There are many studies showing that cyclic AMP derivatives and phosphodiesterase inhibitors, which prevent the breakdown of cyclic AMP (Langer, 1973; Wooten *et al.*, 1973; Cubeddu *et al.*, 1975; Celuch *et al.*, 1978; Hentrich *et al.*, 1984) and the adenylate cyclase activator forskolin (Hovevei-Sion *et al.*, 1983; Hentrich *et al.*, 1984), enhance noradrenaline release. Thus, it is possible that activation of prejunctional β-adrenoceptors could enhance noradrenaline release via production of cyclic AMP. Indeed, in the cat spleen papaverine (a phosphodiesterase inhibitor) increased the ability of isoprenaline to enhance the S-I outflow of noradrenaline as well as increasing the S-I outflow by itself (Celuch *et al.*, 1978).

In the present study the phosphodiesterase inhibitor ICI 63,197 was used. It is reported to be selective for cyclic AMP phosphodiesterase (Fredholm *et al.*, 1979) and has been shown to enhance cyclic AMP levels in the bovine sympathetic splenic nerve (Merican & Nott, 1983). ICI 63,197 enhanced the S-I outflow of radioactivity in rabbit pulmonary artery and mouse atria (as did another inhibitor, isobutylmethylxanthine in mouse atria, not shown) which had been

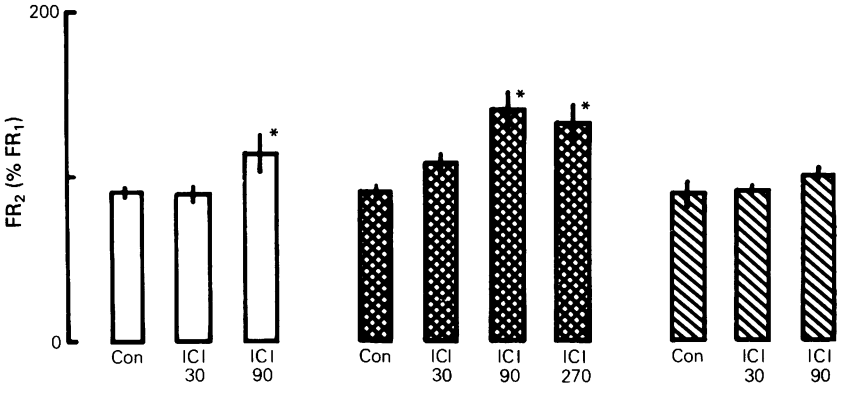

Figure 2 The effect of the phosphodiesterase inhibitor ICI 63,197 on the fractional stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]-noradrenaline. There were two periods of stimulation at 2 (open columns), 4 (cross-hatched columns) or 10 (hatched columns) Hz for 60 s each. ICI 63,197 (30, 90 or 270 μM) was present only during the second period of stimulation. The fractional S-I outflow in the second period of stimulation (Fr_2) is expressed as a percentage of that in the first (FR_1). The vertical lines represent the s.e.mean. *Significant difference from respective control ($P < 0.05$, Student's t test) after one way analysis of variance. The number of experiments for each group is given in Table 3.

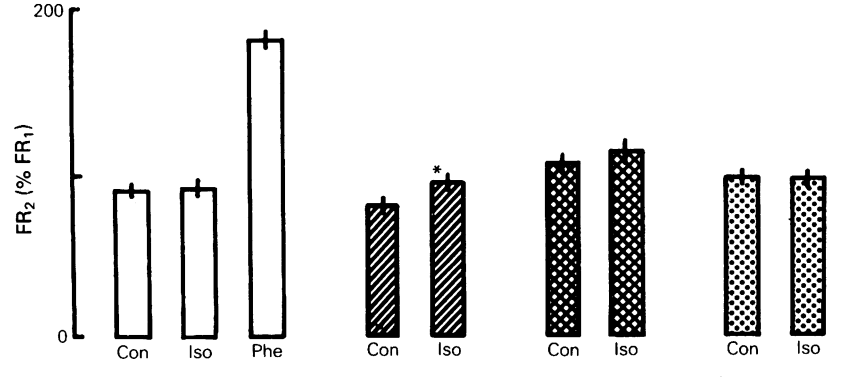

Figure 3 The effect of isoprenaline (Iso, 0.1 μM) on the fractional stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]-noradrenaline and stimulated at 10 Hz. There were two periods of stimulation at 10 Hz for 60 s each. Isoprenaline (0.1 μM) and in some experiments, phentolamine (Phe, 1 μM) were present only during the second period of stimulation. In other experiments ICI 63,197 30 μM (hatched columns) or 90 μM (cross-hatched columns) or phentolamine 1 μM (stippled columns) were present during both stimulation periods. The open columns represent the S-I outflow when no drug was present throughout. The fractional S-I outflow in the second period of stimulation (FR_2) is expressed as a percentage of that in the first (FR_1). The vertical lines represent the s.e.mean. *Significant difference from control (Con), $P < 0.05$, Student's t test. The number of experiments for each group is given in Table 3.

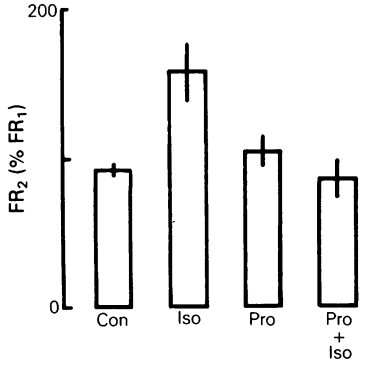

Figure 4 The effect of isoprenaline (Iso, 0.1 μM) on the fractional stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]-noradrenaline and stimulated at 4 Hz. There were two periods of stimulation (each at 4 Hz for 60 s). Drugs (isoprenaline, 0.1 μM; propranolol, Pro, 0.084 μM, or a combination of the two) were present only during the second period of stimulation. The fractional S-I outflow in the second period of stimulation (FR_2) is expressed as a percentage of that in the first (FR_1). The vertical lines represent the s.e.mean. *Significant difference from control (Con – no drug) and a combination of Pro + Iso; $P < 0.05$, Student's t test. The number of experiments for each group is given in Table 3.

incubated with [^{3}H]-noradrenaline, without altering the spontaneous outflow. In the mouse atria the effect was concentration-dependent and absent at a high stimulation frequency (10 Hz) and more pronounced during a 4 Hz stimulation than during a 2 Hz stimulation in both absolute and percentage terms. The explanation for this differential effect may be linked to the endogenous production of cyclic AMP at the various stimulation frequencies. At very low or near maximal endogenous synthesis rates of cyclic AMP, phosphodiesterase inhibition may have little effect.

Starke *et al.* (1975) and Endo *et al.* (1977) extensively studied β-adrenoceptor enhancement of noradrenaline release in the rabbit pulmonary artery at various frequencies of electrical stimulation and concluded that prejunctional β-adrenoceptors were not functional in this tissue. In the present study, in rabbit pulmonary artery incubated with [^{3}H]-noradrenaline and stimulated at 1 Hz, isoprenaline alone also failed to enhance the S-I outflow of radioactivity. However, in the presence of ICI 63,197, isoprenaline enhanced the S-I outflow, an effect blocked by propranolol. Thus the facilitatory prejunctional β-adrenoceptor mechanism is present in this tissue. This result suggests that cyclic AMP generation is an essential step in the prejunctional β-adrenoceptor facilitation of noradrenaline release and that in the rabbit pulmonary artery, a deficiency in cyclic AMP generation may explain the

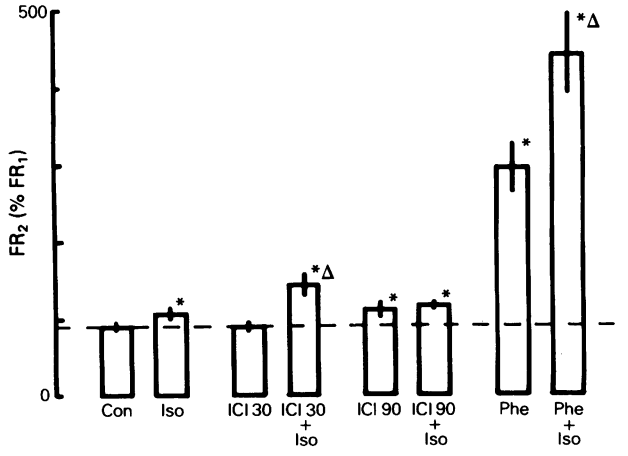

Figure 5 The effect of isoprenaline (Iso, 0.01 μM) on the fractional stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]-noradrenaline and stimulated at 2 Hz. There were two periods of stimulation (each at 2 Hz for 60 s). Drugs (isoprenaline, 0.01 μM; ICI 63,197, ICI, 30 μM or 90 μM, or phentolamine, Phe, 1 μM or combinations of these drugs) were present during the second period of stimulation. The fractional S-I outflow in the second period of stimulation (FR_2) is expressed as a percentage of that in the first (FR_1). The vertical lines represent the s.e.mean. *Significant difference from control (Con – no drug). $^\Delta$Statistically significant effect of isoprenaline compared to effect in absence of isoprenaline, $P < 0.05$, Student's t test. The difference between Phe and Phe + Iso is significantly greater than the difference between Con and Iso ($P < 0.05$, two way analysis of variance). ICI 30 + Iso is significantly greater than Iso alone ($P < 0.05$, Wilcoxon Rank Sum test). The number of experiments in each group is given in Table 3.

lack of facilitatory effect of isoprenaline alone, in this and previous studies.

In mouse isolated atria stimulated at 10 Hz, Farnebo & Hamberger (1974) failed to demonstrate a facilitatory effect of isoprenaline. In the present study, in mouse isolated atria incubated with [^{3}H]-noradrenaline and stimulated at 10 Hz, isoprenaline also failed to enhance the S-I outflow of radioactivity. In the presence of ICI 63,197, 30 μM but not 90 μM, a very slight enhancement of S-I outflow was observed with isoprenaline. The facilitatory effect of isoprenaline was, however, more substantial when the stimulation frequency was reduced to 4 Hz, an effect blocked by propranolol and thus due to β-adrenoceptor activation. It is interesting to note that the phosphodiesterase inhibitor ICI 63,197 also enhanced S-I outflow substantially at 4 Hz but not at 10 Hz, suggesting that the lack of a facilitatory effect of isoprenaline at 10 Hz may be due to the already maximal effect of endogenous cyclic AMP on release. In many tissues it has been observed that the β-adrenoceptor mediated enhancement of noradrenaline release is more pronounced at low frequencies of nerve stimulation (Langer *et al.*, 1975; Hedqvist & Moawad, 1975; Guimarães *et al.*, 1978; Ekas *et al.*, 1982), although such a frequency-dependence has been disputed (Kalsner, 1980).

Interaction between prejunctional α-adrenoceptors and prejunctional β-adrenoceptors

In a previous study in rat atria it was shown that the facilitatory effect of isoprenaline on noradrenaline release was inhibited by an α-adrenoceptor agonist (noradrenaline) and increased by the α-adrenoceptor blocker phentolamine (Majewski & Rand, 1981b). Similarly, in the rabbit ear artery isoprenaline enhanced noradrenaline release in the presence but not in the absence of phentolamine (Majewski & Rand, 1981b). Further, in guinea-pig atria noradrenaline prevented isoprenaline enhancing noradrenaline release (Kalsner, 1982). From these results it has been proposed that inhibitory prejunctional α-adrenoceptors and facilitatory prejunctional β-adrenoceptors are linked such that the activation of the former inhibits the β-adrenoceptor-mediated mechanism and that when neuronally-released noradrenaline activates inhibitory prejunctional α-adrenoceptors, prejunctional β-adrenoceptor effects are reduced (Majewski & Rand, 1981b).

In the present study in rabbit pulmonary artery incubated with [^{3}H]-noradrenaline and stimulated at 1 Hz, isoprenaline by itself did not enhance the S-I outflow of radioactivity in accord with the findings of Starke *et al.* (1975) and Endo *et al.* (1977), but in the presence of phentolamine, isoprenaline did enhance the S-I outflow, an effect blocked by propranolol. In mouse atria stimulated at 10 Hz, phentolamine did not reveal a facilitatory effect of isoprenaline, suggesting that at this high frequency other factors need to be considered. Nevertheless, in other experiments in mouse atria conducted at the lower frequency of 2 Hz and with a low concentration of isoprenaline (0.01 μM), it was demonstrated that in the presence of phentolamine, the facilitatory effect of isoprenaline was markedly enhanced.

One explanation for these results is that isoprenaline may activate inhibitory prejunctional α-adrenoceptors and this may mask its effects on prejunctional β-adrenoceptors. However, this is unlikely. Firstly, the concentration of isoprenaline is extremely low (e.g., 0.01 μM in mouse atria). Secondly, when isoprenaline facilitated release in rabbit pulmonary artery in the presence of ICI 63,197, or in mouse atria stimulated at a frequency of 4 Hz, and when these effects were blocked by propranolol, no inhibitory effect on noradrenaline release was revealed. This suggests that isoprenaline did not activate inhibitory prejunctional α-adrenoceptors.

Another explanation for these results is the interaction proposed by Majewski & Rand (1981b) between inhibitory prejunctional α-adrenoceptors and facilitatory prejunctional β-adrenoceptors outlined above. There is one discrepancy, however, in the rabbit pulmonary artery stimulated at 1 Hz, phentolamine did not enhance noradrenaline release. This suggests that under these conditions neuronally-released noradrenaline did not activate prejunctional α-adrenoceptors, but phentolamine nevertheless revealed a facilitatory effect of isoprenaline.

One possibility to explain this finding is that phentolamine is in fact a phosphodiesterase inhibitor and there is contrary biochemical evidence on this point (compare findings of McNeill *et al.*, 1972 with those of Stock & Thomas, 1975). However, if phentolamine were acting as a phosphodiesterase inhibitor, like ICI 63,197, it should enhance the S-I outflow in the rabbit pulmonary artery, but it did not. An alternative explanation is required and we propose two prejunctional α-adrenoceptors: one independent of adenylate cyclase and one linked to adenylate cyclase. Usual α-adrenoceptor inhibition of noradrenaline release appears to be independent of adenylate cyclase since the facilitatory effect of phentolamine and the inhibitory effect of clonidine on noradrenaline release are unaltered by phosphodiesterase inhibition by either ICI 63,197 or isobutylmethylxanthine in mouse atria (Johnston & Majewski, unpublished observations). Similar results for phentolamine have been observed in cat spleen (Cubeddu *et al.*, 1974) and rat atria (Majewski, unpublished observations). However, there may be another prejunctional α-adrenoceptor which is linked to adenylate cyclase. The linking of both α- and β-adrenoceptors to adenylate cyclase has been proposed in other systems (see

Schultz & Jakobs, 1981) and they inhibit and facilitate adenylate cyclase activity, respectively. If this is also true of adenylate cyclase on nerve endings, α-adrenoceptor mediated inhibition of noradrenaline release by neuronally-released noradrenaline may only occur appreciably through this alternative adenylate cyclase linked α-adrenoceptor system when the adenylate cyclase is activated by β-adrenoceptor agonists. In this case phentolamine may reveal a facilitatory effect of isoprenaline without enhancing noradrenaline release by itself.

Effect of propranolol

Adler-Graschinsky & Langer (1975) initially proposed that neuronally-released noradrenaline activates prejunctional β-adrenoceptors, thus explaining a decrease in noradrenaline release produced by propranolol alone. However, there are many studies showing that β-adrenoceptor blocking drugs alone have no inhibitory effect on noradrenaline release (see Majewski, 1983). Adler-Graschinsky & Carrara (1982) have suggested that neuronal uptake blockade prevents the inhibition of noradrenaline release by β-adrenoceptor blocking drugs and that many of the negative findings with β-adrenoceptor blocking drugs may be explained by concomitant use of these agents. However, in the present study neuronal uptake blocking drugs were not used, yet no decrease in the S-I outflow by propranolol was observed in either rabbit pulmonary artery or mouse atria in the presence or absence of phentolamine or ICI 63,197, suggesting that neuronally-released noradrenaline does not activate prejunctional β-adrenoceptors in these tissues.

Conclusion

The results in rabbit pulmonary artery and mouse atria demonstrate the operation of prejunctional β-adrenoceptors in these tissues and remove the important 'exceptions to the rule' about the prejunctional β-adrenoceptor concept (see Gillespie, 1980; Majewski, 1983). The ability of phentolamine and the phosphodiesterase inhibitor ICI 63,197 to reveal or increase the facilitatory effect of isoprenaline in both tissues may be linked to cyclic AMP and we propose that cyclic AMP generation is an essential step in prejunctional β-adrenoceptor-mediated facilitation of noradrenaline release. It is possible that the effect of phentolamine may be related to α-adrenoceptor inhibition of adenylate cyclase. This latter suggestion, although consistent with the findings, requires further investigation.

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