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ADRENALINE ACTIVATION OF PREJUNCTIONAL β-ADRENOCEPTORS IN GUINEA-PIG ATRIA

H. MAJEWSKI, M.W. McCULLOCH, M.J. RAND & D.F. STORY

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

1 Adrenaline in a concentration of 1.0 μM depressed the stimulation-induced efflux of tritium from the guinea-pig atria incubated with [^{3}H]-noradrenaline, whereas adrenaline in a concentration of 0.5 nM significantly enhanced the stimulation-induced efflux of tritium. This enhancement was blocked by metoprolol (0.1 μM) and thus appears to be mediated by β-adrenoceptors.

2 In guinea-pig atria incubated with unlabelled adrenaline and then with [^{3}H]-noradrenaline, both catecholamines were released by field stimulation. In such atria metoprolol, practolol, oxprenolol or propranolol decreased the stimulation-induced efflux of tritium. These effects did not occur if the atria were incubated with unlabelled noradrenaline and then with [^{3}H]-noradrenaline, suggesting that neuronally released adrenaline activates prejunctional β-adrenoceptors.

3 The effect of oxprenolol in decreasing the release of tritium from guinea-pig atria, incubated with unlabelled adrenaline and then with [^{3}H]-noradrenaline was greater in the presence of phentolamine. This may reflect the α-adrenoceptor blocking activity of oxprenolol.

Introduction

At sites of noradrenergic transmission there exists a facilitatory mechanism which involves prejunctional β-adrenoceptors and which may play a physiological role in sympathetic transmitter release. Enhancement of transmitter noradrenaline release by β-adrenoceptor agonists has been shown in many tissues (see review by Starke, 1977). β-Adrenoceptor blocking drugs reduce the release of transmitter noradrenaline by nerve stimulation in guinea-pig atria (Adler-Grashinsky & Langer, 1975), in the phenoxybenzamine-pretreated perfused calf muscle of the cat (Dahlöf, Åblad, Borg, Ek & Waldeck, 1975), in the heart of the anaesthetized dog (Yamaguchi, de Champlain & Nadeau, 1977), and in the phenoxybenzamine-treated rat portal vein (Dahlöf, Ljung & Åblad, 1978). β-Adrenoceptor blocking drugs may reduce transmitter release by antagonizing the action of transmitter noradrenaline on prejunctional β-adrenoceptors (Adler-Grashinsky & Langer, 1975). However, some findings are at variance with this proposition: for example, β-adrenoceptor blocking drugs do not reduce transmitter noradrenaline release in human omental arteries and veins (Stjärne & Brundin, 1975), guinea-pig atria (Rand, Law, Story & McCulloch, 1976) and human oviduct (Hedqvist & Moawad, 1975), even though the existence of β-adrenoceptor-mediated facilitation of transmitter release by β-adrenoceptor agonists has been demonstrated in all of these tissues. The ineffectiveness of β-adrenoceptor-blocking drugs in reducing transmitter release in these tissues suggests that, under the conditions of the experiments, transmitter noradrenaline does not activate prejunctional β-adrenoceptors to facilitate its own release.

Low concentrations of adrenaline facilitate the release of transmitter noradrenaline in human omental blood vessels (Stjärne & Brundin, 1975) and in the rat portal vein (Westfall, Peach & Tittermary, 1979). These authors suggested that prejunctional β-adrenoceptors, rather than being activated by transmitter noradrenaline, were more likely to be activated by circulating adrenaline. This system would thus constitute a hormonal mechanism for the modulation of the noradrenergic transmission process. Moreover, adrenaline in the circulation may also be taken up, actively, by sympathetic nerve endings (Andén, 1964) and adrenaline, once incorporated into the nerve terminals, may be released as a co-transmitter with noradrenaline. Under these circumstances, neuronally-released adrenaline may activate prejunctional β-adrenoceptors and constitute a positive feedback system which facilitates transmitter release. The feasibility of this hypothesis was tested in guinea-pig atria. Reports of preliminary findings in this study have been published previously (Majewski, McCulloch, Rand & Story, 1979; Rand, Majewski, McCulloch & Story, 1979).

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Methods

Preparation of guinea-pig atria

Guinea-pigs of either sex (300 to 500 g) were killed by cervical dislocation, exsanguinated and the hearts rapidly removed. The atria were dissected free and set up in an organ bath containing 2.5 ml of Krebs–Henseleit solution. The solutions in the organ bath and in the reservoir supplying the organ bath were gassed with a mixture of 5% CO_2 in O_2 and maintained at 37°C. Platinum electrodes were placed on either side of the atria for electrical stimulation of the intramural sympathetic nerves. Electrical stimulation was by monophasic square wave pulses of 1 ms duration; the field gradient was about 12 V/cm which was supramaximal for increasing the efflux of radioactivity in atria which had been incubated in [^{3}H]-catecholamines.

Incubation with [^{3}H]-noradrenaline

After 30 min of equilibration the atria were incubated with [^{3}H]-(−)-noradrenaline (4 μCi/ml; 0.4 μM) for 20 min and then washed repeatedly for 60 min with catecholamine-free Krebs–Henseleit solution to remove loosely bound tritiated compounds. Cocaine (100 μM) was added 15 min before the end of the washing procedure and then remained present for the duration of the experiment. The cocaine was used to prevent the displacement of [^{3}H]-noradrenaline from the transmitter storage vesicles by the adrenaline which was used subsequently.

Combined incubation with adrenaline and noradrenaline

These experiments were designed to incorporate both adrenaline and noradrenaline into the transmitter stores of the sympathetic nerves of guinea-pig atria and to investigate the effects of drugs on [^{3}H]-noradrenaline release.

[^{3}H]-adrenaline labelling Guinea-pig atria were dissected and equilibrated as previously described. The intramural sympathetic nerves of the atria were then stimulated at a frequency of 1 Hz for 45 min in order to produce some depletion of endogenous transmitter stores. The atria were then preincubated with [^{3}H]-(±)-adrenaline (0.9 μCi/ml; 3 μM), which was prepared by diluting [^{3}H]-(±)-adrenaline with (−)-adrenaline, for 60 min and washed with drug-free solution for 30 min to remove loosely bound adrenaline. Then the atria were incubated with unlabelled noradrenaline (0.4 μM) for 10 min and washed in drug-free solution for 60 min to remove loosely bound catecholamines.

[^{3}H]-noradrenaline labelling Guinea-pig atria were dissected, equilibrated and stimulated as described above. The atria were then preincubated in either unlabelled adrenaline (3 μM) or noradrenaline (3 μM), after which they were washed for 30 min, and incubated in [^{3}H]-(−)-noradrenaline (4 μCi/ml; 0.4 μM) for 10 min followed by washing in drug-free Krebs–Henseleit solution for 60 min.

Estimation of the atrial content of [^{3}H]-noradrenaline and [^{3}H]-adrenaline

To determine the proportions of [^{3}H]-noradrenaline and [^{3}H]-adrenaline retained in the preparations as such, after the incubation and washing procedures described in the previous sections, guinea-pig atria were dissected, equilibrated and then incubated in one of three ways: (i) with [^{3}H]-noradrenline alone; (ii) with [^{3}H]-adrenaline followed by unlabelled noradrenaline or (iii) with unlabelled adrenaline followed by [^{3}H]-noradrenaline.

These incubations were all carried out as outlined in the preceding sections. The atria were taken for estimation of the respective tritiated catecholamines 15 min after the 60 min period of washing in each case. The atria were homogenized in 2 ml of ice cold perchloric acid (0.4 M) containing disodium edetate (2.7 mM). To facilitate chromatographic separation of the catecholamines and metabolites, 30 μl of a carrier solution of noradrenaline, adrenaline and their metabolites was added before homogenization. The aliquot of carrier solution contained the following substances, each in an amount of 30 μg: (−)-noradrenaline hydrochloride, (−)-adrenaline bitartrate, metanephrine hydrochloride, normetanephrine hydrochloride, 3,4-dihydroxymandelic acid, bis(3-methoxy-4-hydroxy-phenylglycol) piperazine, 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxymandelic acid. Then the homogenate was centrifuged at 1200 *g* for 15 min at 4°C. A 1.0 ml aliquot of the supernatant was taken for estimation of radioactivity, and a further 0.1 ml sample of the supernatant was applied 7.5 cm from the bottom of a sheet of Whatman No. 3 paper (57 cm × 2 cm) and dried under a stream of nitrogen at room temperature. The sheets were chromatographed as described by Majewski & Story (1977) in the following solvent: *n*-butanol: ethyl acetate: glacial acetic acid: sulphur dioxide solution (7% w/v): formic acid (90% w/v): HCl (10 M) in the ratios of 200:170:90:140:5:10, respectively. Ascending chromatograms were run at 4°C, until the solvent had moved about 30 cm from the origin (which took 18 to 24 h). The papers were then allowed to dry at room temperature and divided into 3 mm horizontal strips between the origin and the solvent front. The strips were placed in liquid scintillation counting vials to which 0.2 ml of HCl (6 M) was added, followed by 1

ml of distilled water. After 30 min, 10 ml of scintillation fluid was added and the vials were shaken vigorously and left to stand for 1 h at room temperature. Radioactivity was measured in a Packard 3380 Tri-Carb Liquid Scintillation Counter. The locations of norarenaline and adrenaline on the chromatograms were determined by comparing the R_F values of the peaks of radioactivity with those of authentic [^{3}H]-noradrenaline and [^{3}H]-adrenaline, respectively.

Stimulation procedure and measurement of tritium efflux

In all experiments in which stimulation-induced efflux was measured, the intramural nerves of the atria were stimulated at a frequency of 5 Hz for 30 s. The first period of stimulation was given 15 min after the 60 min period of washing; a second period of stimulation was given 35 min after the first. The effects of drugs on the stimulation-induced tritium effluxes were determined by adding them to the Krebs–Henseleit solution bathing the atria 28 min before the second period of stimulation.

The Krebs–Henseleit solution bathing the atria was collected after 3 min periods of contact with the atria for determination of the efflux of tritium from the tissue, three consecutive collections being made, starting 6 min before each stimulation was applied. Radioactivity was measured by liquid-scintillation counting. The resting tritium efflux was taken as the mean tritium content of the sample of bathing solution collected during the two 3 min periods immediately preceding the period of stimulation. The stimulation-induced component of the tritium efflux was calculated by subtracting the resting efflux from the tritium content of the samples collected in the 3 min period in which stimulation was applied. In each experiment the stimulation-induced efflux for the second period of stimulation was calculated as a percentage of the corresponding efflux for the first period (% S_2/S_1). The results were then expressed as a ratio of the values obtained in control experiments in the absence of drugs.

Statistical analysis of results

Except where otherwise indicated, the data were analysed by one-way analysis of variance followed by the unpaired 2-tailed Student's t test. Probability levels of less than 0.05 were taken to indicate statistical significance.

Estimation of radioactivity in collections of tissue bathing solution

The amount of radioactivity present in the collections of Krebs–Henseleit solution bathing the atria was estimated by placing 1 ml aliquots in vials containing 0.1 ml HCl (6 M) and 10 ml of scintillation solution. The radioactivity (counts/min) was measured in a Packard 3380 scintillation counter and expressed as disintegrations per min after correction for counting efficiency. Counting efficiency as determined by automatic external standardization (A.E.S.) ranged from 20 to 25%.

Materials

The Krebs–Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, $MgSO_4$ 0.45, KH_2PO_4 1.03, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.07.

The scintillation solution had the following composition: 5.5 g of 2,5-diphenyloxazole (PPO), 0.1 g of 1,3-*bis*-2-(5-phenyloxazolyl) benzene (POPOP) and 333 ml of Triton X-100 made up to 1 litre in toluene.

The following drugs were used: cocaine hydrochloride (MacFarlan Smith); (−)-adrenaline bitartrate (Calbiochem); propranolol hydrochloride (ICI); practolol (ICI); atenolol (ICI); pindolol (Sandoz); metoprolol tartrate (Ciba) and oxprenolol hydrochloride (Ciba).

The following compounds were obtained from Sigma, U.S.A.: (−)-noradrenaline hydrochloride, (±)-normetanephrine hydrochloride; metanephrine hydrochloride, 3,4-dihydroxyphenylglycol, 3,4-dihydroxymandelic acid, (±)-3-methoxy-4-hydroxymandelic acid, *bis*-(3-methoxy-4-hydroxyphenylglycol) piperazine.

All drugs were dissolved in distilled water and then diluted with Krebs–Henseleit solution to the required concentrations except pindolol and practolol which were first dissolved in 0.1 ml of HCl (1.0 M) and then diluted.

The following radiolabelled compounds were obtained from the Radiochemical Centre, Amersham, U.K.: (−)-[7-^{3}H]-noradrenaline hydrochloride (15 Ci/mmol) and (±)-[7-^{3}H]-adrenaline bitartrate (7.25 Ci/mmol).

Results

The effect of adrenaline on the stimulation-induced efflux of [^{3}H]-noradrenaline

The effect of adrenaline on the stimulation-induced efflux of tritium in the second period of stimulation from guinea-pig atrial preparations which had been incubated with [^{3}H]-noradrenaline was investigated in the presence of cocaine (100 μM). After atria had been incubated with [^{3}H]-noradrenaline and washed for 60 min, the mean amount of radioactivity retained

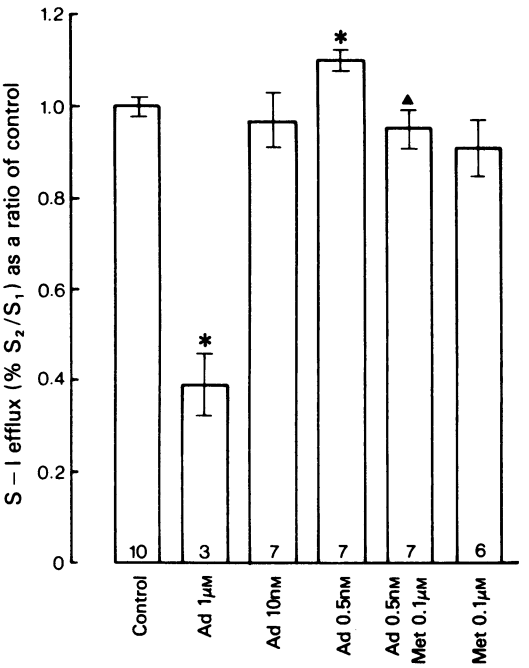

Figure 1 Effect of adrenaline (Ad) on the stimulation-induced (S–I) efflux of tritium from guinea-pig atria incubated with [^{3}H]-noradrenaline. Cocaine (100 μM) was present continuously after incubation. There were two periods of stimulation each at a frequency of 5 Hz for 30 s. Drugs Ad and metoprolol (Met) were present during the second period of stimulation where indicated. The S–I efflux of tritium during the second period of stimulation is expressed as a percentage of that in the first. All results are expressed as a ratio of the control value. The vertical bars represent s.e. mean and the numbers at the base of the histograms represent the number of experiments performed. The asterisks indicate a statistically significant difference from control ($P < 0.05$). The filled triangle indicates a statistically significant difference in the effect from that of adrenaline (0.5 nM) ($P < 0.05$). Note that the effect of the combination of metoprolol (0.1 μM) and adrenaline (0.5 nM) was significantly different from that of adrenaline (0.5 nM) alone ($P < 0.05$) but not significantly different from control.

by the atria was 6.04×10^6 d/min (s.e. mean = 0.34×10^6, $n = 4$). Chromatographic analysis showed that 93.2% of the radioactivity (s.e. mean = 2.5, $n = 4$) was present as [^{3}H]-noradrenaline. The atria were subjected to two periods of field stimulation (5 Hz for 30 s), 35 min apart. In these experiments the mean total amount of radioactivity from 10 atrial preparations released by the first period of stimulation was 15,895 d/min (s.e. mean = 2144). The stimulation-induced tritium efflux for the second period of stimulation was 69.9% (s.e. mean = 1.4, $n = 10$) of that for the first. The effects of adrenaline on the stimulation-induced efflux of tritium were concentration-dependent. At a concentration of 0.5 nM, adrenaline significantly enhanced the stimulation-induced tritium efflux, at 10 nM, it was without effect, whereas at 1.0 μM, it markedly reduced the efflux. The enhancement of stimulation-induced tritium efflux produced by 0.5 nM adrenaline was prevented by metoprolol (0.1 μM). These results are shown in Figure 1.

Simultaneous release of both adrenaline and noradrenaline

To determine if there was simultaneous stimulation-induced release of adrenaline and noradrenaline from guinea-pig preparations which had been incubated first with adrenaline and then with noradrenaline, two series of experiments were performed.

In one series of experiments, atria were first incubated with non-radioactive adrenaline (3 μM) and then they were incubated with [^{3}H]-noradrenaline (0.4 μM) as described in Methods. After incubation and washing, the mean atrial content of radioactivity was 4.26×10^6 d/min (s.e. mean = 0.29×10^6, $n = 4$). Chromatographic analysis showed that 92.5% (s.e. mean = mean 1.8, $n = 4$) of this was present as [^{3}H]-noradrenaline. After an identical regime of incubation and washing, atria were subjected to two periods of field stimulation (5 Hz for 30 s), 35 min apart. In these experiments the mean amount of radioactivity released from 17 preparations by the first period of stimulation was 8549 d/min (s.e. mean = 733). The stimulation-induced tritium efflux for the second period of stimulation was 65.9% (s.e. mean = 3.2, $n = 17$) of that for the first.

In the second series of experiments atria were preincubated with [^{3}H]-adrenaline (3 μM) and then incubated with non-radioactive noradrenaline (0.4 μM). After atria had been incubated in this way and then washed for 75 min, the mean content of radioactivity was 6.30×10^6 d/min (s.e. mean = 0.91×10^6, $n = 4$). Chromatographic analysis showed that 90.3% (s.e. mean = 1.5, $n = 4$) of this was present as [^{3}H]-adrenaline. The mean total amount of radioactivity released from 3 such preparations by a first period of stimulation was 7240 d/min (s.e. mean = 1740). The stimulation-induced tritium efflux for the second period of stimulation was 65.2% (s.e. mean = 6.1, $n = 3$) of that for the first period. [^{3}H]-adrenaline is therefore released by nerve stimulation in a similar manner to [^{3}H]-noradrenaline.

Effects of β-adrenoceptor blocking drugs

The effects of some β-adrenoceptor blocking drugs on the stimulation-induced efflux of tritium from atria

which had been preincubated with non-radioactive adrenaline and then incubated with [^{3}H]-noradrenaline were compared with their effects in atria which had been preincubated with non-radioactive noradrenaline and then incubated with [^{3}H]-noradrenaline. This was to determine whether prejunctional β-adrenoceptors were activated by neuronally released adrenaline or noradrenaline.

Preincubation with noradrenaline In atria which had been preincubated with non-radioactive noradrenaline (3 μM) and then incubated with [^{3}H]-noradrenaline (0.4 μM), neither metoprolol (0.1 μM), practolol (0.1 μM) nor propranolol (0.001 and 0.01 μM) had any effect on the stimulation-induced tritium efflux (Table 1).

Preincubation with adrenaline In contrast, when the atria were preincubated with adrenaline (3 μM) before being incubated with [^{3}H]-noradrenaline (0.4 μM), metoprolol (0.1 μM), practolol (0.1 μM) and propranolol (0.001 and 0.01 μM) reduced significantly the stimulation-induced tritium efflux (Table 1). However, atenolol (0.1 and 0.01 μM) and pindolol (1.0 μM) had no significant inhibitory effect on stimulation-induced tritium efflux and pindolol (0.1 μM) significantly enhanced stimulation-induced tritium efflux. These results are summarized in Table 1.

Effect of oxprenolol on the stimulation-induced tritium efflux

The effects of oxprenolol on the stimulation-induced efflux of tritium from atria preincubated with non-radioactive adrenaline and then incubated with [^{3}H]-noradrenaline were compared with its effects on atria preincubated with non-radioactive noradrenaline and then incubated with [^{3}H]-noradrenaline.

Noradrenaline preincubation In atria which had been preincubated with non-radioactive noradrenaline (3 μM) and then incubated with [^{3}H]-noradrenaline (0.4 μM), oxprenolol in concentrations of 0.001 and 0.01 μM significantly enhanced the stimulation-induced efflux of tritium, whereas oxprenolol in higher concentrations (0.1 and 1.0 μM) had no effect (Figure 2b). The effects of oxprenolol were further investigated in the presence of phentolamine in order to preclude any effects of oxprenolol on prejunctional α-adrenoceptors. Phentolamine (1.0 μM) present during the second period of stimulation significantly enhanced the stimulation-induced efflux of tritium in atria which had been incubated with non-radioactive noradrenaline and then with [^{3}H]-noradrenaline, the mean % S_2/S_1 being increased from 68.2% (s.e. mean = 3.8, n = 5) in the absence of phentolamine to 194.7% (s.e. mean = 7.1, n = 4) in the presence of phentolamine ($P < 0.05$). The effect of a combination of phentolamine (1.0 μM) with oxprenolol (either 0.001, 0.01 or 0.1 μM) was not significantly different from the effect of phentolamine alone (Figure 3b).

Adrenaline preincubation In atria preincubated with non-radioactive adrenaline (3 μM) and then incubated with [^{3}H]-noradrenaline (0.4 μM), oxprenolol (in concentrations of 0.001, 0.01 and 0.1 μM) had no effect on the stimulation-induced efflux of tritium. In the higher concentration of 1.0 μM oxprenolol significantly de-

Table 1 Effect of β-adrenoceptor blocking drugs on the stimulation-induced (S–I) efflux of tritium from guinea-pig atria

	Preincubated in noradrenaline (3 μM) then [^{3}H]-noradrenaline			*Preincubated in noradrenaline (3 μM) then [^{3}H]-noradrenaline*		
	Mean	s.e. mean	n	Mean	s.e. mean	n
Control	(=) 1.000	0.049	17	(=) 1.000	0.073	7
Metoprolol (0.1 μM)	0.502*	0.046	5	1.263	0.128	6
Practolol (0.1 μM)	0.698*	0.041	5	1.115	0.083	4
Propranolol (0.001 μM)	0.498*	0.038	4	1.220	0.062	4
Propranolol (0.01 μM)	0.566*	0.110	4	0.947	0.092	5
Pindolol (1.0 μM)	1.153	0.093	4	—	—	—
Pindolol (0.1 μM)	1.169*	0.084	5	—	—	—
Atenolol (0.1 μM)	0.884	0.074	6	—	—	—
Atenolol (0.01 μM)	0.951	1.024	5	—	—	—

There were two periods of stimulation each at 5 Hz for 30 s. The stimulation-induced efflux of tritium during the second period of stimulation was expressed as a percentage of that in the first. Drugs were present during the second period. All results were expressed as a ratio of control experiments.
The asterisks indicate a significant difference from control ($P < 0.05$).

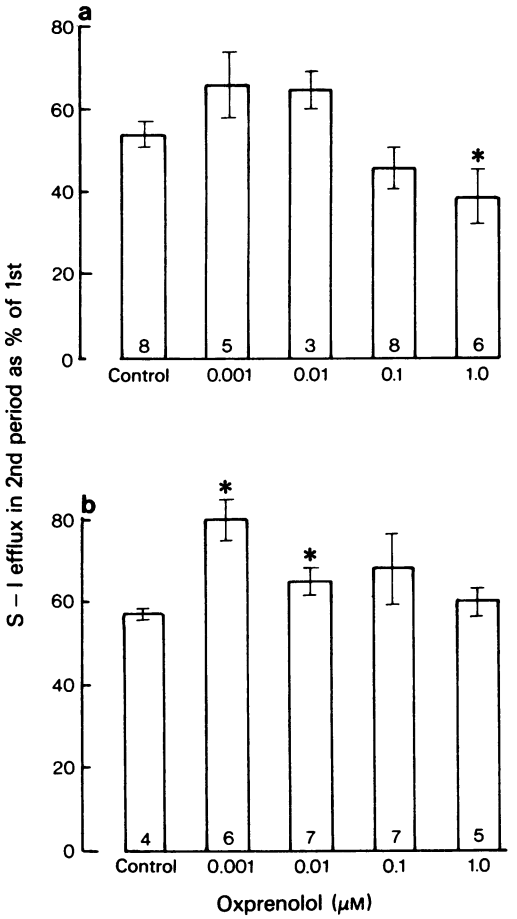

Figure 2 The effect of oxprenolol on the stimulation-induced (S–I) efflux of tritium from guinea-pig atria preincubated with either adrenaline (3 μM) (a), or noradrenaline (3 μM) (b) then incubated with [^{3}H]-noradrenaline (0.4 μM). There were two periods of stimulation each at 5 Hz for 30 s. The S–I efflux of tritium in the second period of stimulation is expressed as a percentage of that in the first. Oxprenolol was present during the second period of stimulation. The vertical bars represent s.e. means and the numbers at the base of the histograms refer to the number of experiments performed. The asterisks indicate a statistically significant difference from control ($P < 0.05$).

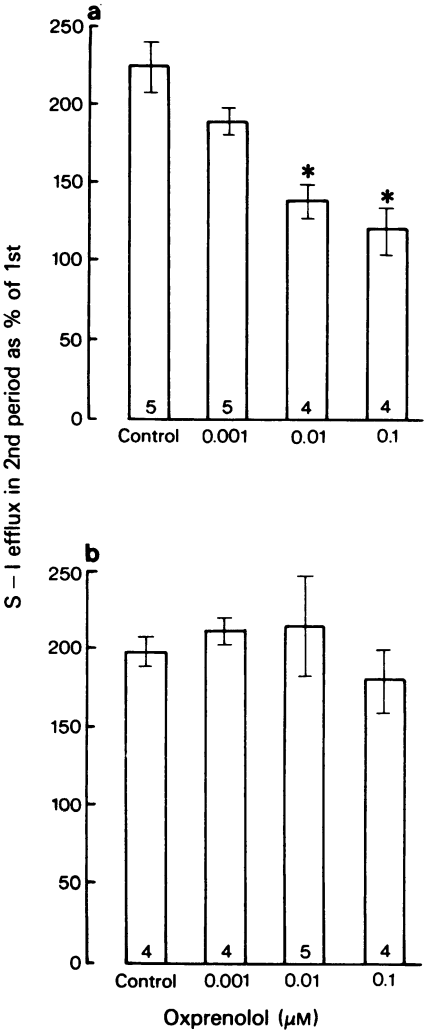

Figure 3 The effect of oxprenolol in the presence of phentolamine (1 μM) on the stimulation-induced (S–I) efflux of tritium from guinea-pig atria preincubated with either adrenaline (3 μM) (a) or noradrenaline (3 μM) (b) then incubated with [^{3}H]-noradrenaline (0.4 μM). There were two periods of stimulation each at a frequency of 5 Hz for 30 s. The S–I efflux of tritium in the second period of stimulation was expressed as a percentage of that in the first. Oxprenolol and phentolamine (1.0 μM) were present during the second period of stimulation. The vertical bars represent s.e. mean and the numbers at the base of the histograms refer to the number of experiments performed. The asterisks indicate a statistically significant difference from control ($P < 0.05$) when only phentolamine was present.

creased the stimulation-induced efflux of tritium (Figure 2a). The effects of oxprenolol were investigated further in the presence of phentolamine in order to preclude any effects of oxprenolol or prejunctional α-adrenoceptors. Phentolamine (1.0 μM) present during the second period of stimulation, significantly enhanced the stimulation-induced efflux of tritium from atria which had been preincubated with adrenaline and then incubated with [^{3}H]-noradrenaline ($P < 0.05$), the mean % S_2/S_1 being increased from 54.5% (s.e. mean = 3.3, $n = 8$) in the absence of phentolamine to 225.6% (s.e. mean = 16.1, $n = 5$) in the

presence of phentolamine. A combination of oxprenolol (0.01 μM or 0.1 μM) with phentolamine (1.0 μM) resulted in an enhancement of the stimulation-induced tritium efflux which was in each case less than that produced by phentolamine (1.0 μM) alone (Figure 3a).

Discussion

When guinea-pig atria were incubated with (i) [^{3}H]-noradrenaline, (ii) non-radioactive adrenaline followed by [^{3}H]-noradrenaline or (iii) with [^{3}H]-adrenaline followed by non-radioactive noradrenaline, more than 90% of the radioactivity retained in the atria in each case was in the form of the unmetabolized tritiated catecholamine present during incubation. It has been established that the metabolism of transmitter noradrenaline released from isolated tissues occurs subsequent to its release from storage vesicles; thus for the calculation of the actual output of transmitter, it is important to include the metabolites and not to rely on the determination of noradrenaline alone (Langer, 1970). Therefore, in this study, the release of tritium from the tissue was taken as an index of actual transmitter release since this measurement includes the respective catecholamine and its tritiated metabolites formed after the release of the catecholamine from the storage vesicles.

The effect of adrenaline on the stimulation-induced efflux of tritium from guinea-pig atria that had been incubated with [^{3}H]-noradrenaline depended on its concentration. In low concentrations (0.5 nM), adrenaline enhanced stimulation-induced tritium efflux, whereas in a higher concentration (1.0 μM), adrenaline reduced stimulation-induced tritium efflux; in an intermediate concentration (10 nM) adrenaline was without effect on release, probably indicating a balance between the inhibitory and facilitatory effects of adrenaline. These findings are similar to those of Stjärne & Brundin (1975) for human omental blood vessels. The marked inhibitory effect produced by adrenaline (1.0 μM) is probably due to activation of inhibitory prejunctional α-adrenoceptors as has been shown in other tissues (see review by Starke, 1977). The small facilitatory effect produced by the low concentration of adrenaline (0.5 nM) was abolished by the β-adrenoceptor-blocking drug, metoprolol (0.1 μM), indicating the involvement of β-adrenoceptors. The concentrations of adrenaline which facilitated transmitter release both in the present study (0.5 nM) and in that of Stjärne & Brundin (1975) (1.6 to 8.0 nM) are within the range of those found in human plasma as a result of enhanced adrenomedullary secretion (Vendsalu, 1960). Thus it seems possible that prejunctional β-adrenoceptors at noradrenergic nerve terminals may be activated *in vivo* by hormonal levels of adrenaline as suggested by Stjärne & Brundin (1975).

Transmitter noradrenaline does not appear to activate prejunctional β-adrenoceptor sites in guinea-pig atria as evidenced by the inability of metoprolol (0.1 μM) alone to decrease the stimulation-induced efflux of tritium. This agrees with the findings of Rand *et al.* (1976) who found that propranolol (0.1 μM) did not inhibit transmitter noradrenaline release in guinea-pig atria. A similar finding has also been obtained for human omental arteries and veins by Stjärne & Brundin (1975), and by Hedqvist & Moawad (1975) for human oviduct. In the preceding three reports, β-adrenoceptor-blocking drugs by themselves did not inhibit transmitter noradrenaline release, even though facilitation of transmitter release by β-adrenoceptor agonists had been demonstrated in these tissues. These results suggest that at least in some tissues, transmitter noradrenaline does not normally facilitate its own release by acting on prejunctional β-adrenoceptors. Noradrenaline is a much weaker β_2-adrenoceptor agonist than is adrenaline (Lands, Arnold, McAuliff, Luduena & Brown, 1967); thus if the β-adrenoceptors which mediate the facilitation of transmitter release are of the β_2-subtype, noradrenaline would be expected to be much less effective than adrenaline. This has in fact, been demonstrated in the phenoxybenzamine-treated rat portal vein, where noradrenaline (10 μM) enhanced the stimulation-induced release of transmitter noradrenaline; however, adrenaline produced the same degree of enhancement in a concentration of only 0.05 μM (Dahlöf *et al.*, 1978).

The possibility exists that the facilitatory effect of adrenaline on transmitter noradrenaline release is mediated through different β-adrenoceptor subtypes in different tissues and animals. The relatively selective β_2-adrenoceptor agonists, terbutaline and salbutamol, enhance transmitter release in human omental arteries and veins, whereas the β_1-adrenoceptor agonist, H 110/38, does not (Stjärne & Brundin, 1976). Similar results were obtained with the rat portal vein in which stimulation-induced release of noradrenaline was enhanced by isoprenaline and terbutaline but not by the β_1-adrenoceptor agonist, dobutamine, whilst the facilitatory effects of isoprenaline and terbutaline were antagonized by the relatively selective β_2-adrenoceptor blocking drug, butoxamine, but not by practolol, a relatively selective β_1-adrenoceptor blocking drug (Westfall *et al.*, 1979). Dahlöf *et al.* (1975) suggested that the facilitatory receptors were of the β_1-subtype on the basis of the finding that metoprolol, a relatively selective β_1-adrenoceptor blocking drug reduced transmitter noradrenaline release in the blood vessels of the cat hind limb.

Circulating adrenaline can be incorporated into the transmitter stores of sympathetic nerves (Andén, 1964). It is possible that adrenaline taken up from the

circulation and accumulated into nerves is released by nerve stimulation along with transmitter noradrenaline. Thus, in addition to the relatively low levels of circulating adrenaline, neuronally-released adrenaline may also contribute to the activation of prejunctional β-adrenoceptors.

To investigate the hypothesis that adrenaline, once incorporated into sympathetic nerves, can be released by nerve stimulation and activate prejunctional β-adrenoceptors, another series of experiments was designed to incorporate both adrenaline and noradrenaline into sympathetic transmitter stores of guinea-pig atria; the atria were first incubated with adrenaline (3 μM) followed by noradrenaline (0.4 μM). In preparations preincubated with tritiated adrenaline there was a release of radioactivity in response to field stimulation, that is, adrenaline was released together with noradrenaline. In preparations preincubated with non-radioactive adrenaline and then incubated with [^{3}H]-noradrenaline, several β-adrenoceptor blocking drugs: metoprolol (0.1 μM), oxprenolol (1.0 μM), practolol (0.1 μM) and propranolol (0.001 and 0.01 μM), significantly reduced the stimulation-induced efflux of tritium. However, in guinea-pig atria in which the adrenaline preincubation was replaced with non-radioactive noradrenaline preincubation (but retaining the subsequent incubation with [^{3}H]-noradrenaline), metoprolol (0.1 μM), practolol (0.1 μM) and propranolol (0.001 μM and 0.01 μM) did not alter the stimulation-induced efflux of tritium. Taken together, these results suggest that in guinea-pig atria the facilitatory mechanism mediated by prejunctional β-adrenoceptors is not activated by transmitter noradrenaline but when the transmitter stores contain adrenaline as well as noradrenaline, the facilitatory mechanism is activated by neuronally released adrenaline.

Pindolol in a concentration of 0.1 μM (but not 1.0 μM) enhanced the stimulation-induced efflux of tritium from atria which were preincubated with adrenaline and then with [^{3}H]-noradrenaline. The enhancement may be due to activation of prejunctional β-adrenoceptors since pindolol has been shown to possess intrinsic sympathomimetic (β-adrenoceptor agonistic) activity (Clark, 1976). Atenolol (0.01 and 0.1 μM) had no statistically significant effect on the stimulation-induced efflux of tritium from guinea-pig atria incubated with adrenaline then [^{3}H]-noradrenaline. Further concentrations of atenolol need to be tested to determine fully its effects on transmitter noradrenaline release.

In atria preincubated with adrenaline and then incubated with [^{3}H]-noradrenaline, oxprenolol in a concentration of 1.0 μM decreased the stimulation-induced efflux of tritium; however, lower concentrations of oxprenolol had no effect. In atria that were preincubated with non-radioactive noradrenaline and then incubated with [^{3}H]-noradrenaline, oxprenolol (0.1 μM and 1.0 μM) had no effect on the stimulation-induced efflux of tritium, whereas lower concentrations (0.01 μM and 0.001 μM) enhanced the stimulation-induced efflux of tritium. Thus the presence of neuronally released adrenaline appears necessary for oxprenolol to decrease the stimulation-induced efflux of tritium. The increases in stimulation-induced efflux of tritium observed in the presence of oxprenolol (0.001 μM and 0.01 μM), in preparations that were preincubated with noradrenaline and then incubated with [^{3}H]-noradrenaline, may be due to the blockade of prejunctional α-adrenoceptors, since oxprenolol has been shown to possess α-adrenoceptor blocking activity (Law, Rand & Story, 1978; Rosello, Guinot & Jane, 1978). Phentolamine is more potent in blocking α-adrenoceptors than is oxprenolol (Law, 1979); hence in the presence of phentolamine it is likely that the α-adrenoceptor blocking action of oxprenolol may be masked by that of phentolamine. In guinea-pig atria that were preincubated with either adrenaline or noradrenaline and then incubated with [^{3}H]-noradrenaline, phentolamine (1.0 μM) by itself enhanced the stimulation-induced efflux of tritium by blockade of transmitter noradrenaline activation of the prejunctional α-adrenoceptor-mediated inhibitory feedback mechanism. When atria were preincubated with non-radioactive noradrenaline and then incubated with [^{3}H]-noradrenaline, oxprenolol (0.01 μM and 0.001 μM) in the presence of phentolamine (1.0 μM) had no effect on the stimulation-induced efflux of tritium, whereas these concentrations of oxprenolol enhanced the stimulation-induced efflux of tritium in the absence of phentolamine. These findings support the suggestion that the enhancement of [^{3}H]-noradrenaline release produced by these concentrations of oxprenolol may be due to prejunctional α-adrenoceptor blockade.

In guinea-pig atria preincubated with adrenaline and then incubated with [^{3}H]-noradrenaline, oxprenolol (0.01 μM and 0.1 μM) had no effect on the stimulation-induced efflux of tritium. However, in the presence of phentolamine (1.0 μM), the same concentrations of oxprenolol decreased the stimulation-induced efflux of tritium; this may be due to occupation of prejunctional α-adrenoceptors by phentolamine, thereby occluding the α-adrenoceptor blocking action of oxprenolol and therefore unmasking the blocking action of oxprenolol on prejunctional β-adrenoceptors. This effect was not apparent if the transmitter stores contained only noradrenaline and thus further supports the contention that neuronally released adrenaline but not noradrenaline activates the facilitatory prejunctional β-adrenoceptors in this preparation.

That neurally released adrenaline but not noradrenaline appears to mediate a positive feedback

loop on noradrenergic transmission suggests that the β-adrenoceptors involved are of the β_2-subtype. However, the relative efficacies of the β-adrenoceptor drugs used in reducing release do not appear to bear any relationship to their reported selectivity for β_1- or β_2-adrenoceptors and suggests that under the conditions of the present study they are non-selective.

In essential hypertension in man, increased levels of circulating adrenaline have been observed (de Champlain, Farley, Cousineau & van Ameringen, 1974; Franco-Morselli, Elghozi, Joly, di Guilio & Meyer, 1977) and may, through enhancing transmitter release from sympathetic nerves, be important in the aetiology and maintenance of high blood pressure. Drugs which block β-adrenoceptors prevent this action of adrenaline *in vitro* and it has been shown that they decrease sympathetic transmitter release in man (de Champlain, Cousineau, van Ameringen & Marc Aurèle, 1977). Blockade of adrenaline-mediated facilitation of sympathetic transmitter release may explain at least in part, the antihypertensive activity of these agents.

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