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Identification of presynaptic β_2-adrenoceptors on the sympathetic nerve fibres of the human pulmonary artery

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1 Strips of human pulmonary arteries from patients undergoing surgery for lung tumour were incubated with [^{3}H]-noradrenaline. Subsequently, they were superfused with physiological salt solution containing cocaine and corticosterone. Tritium overflow from the strips was stimulated by transmural electrical impulses (2 Hz). The electrically evoked overflow of tritium consisted of 91% unmetabolized [^{3}H]-noradrenaline, and this percentage was not altered by isoprenaline.

2 Adrenaline (in the presence of rauwolscine), isoprenaline and the preferential β_2-adrenoceptor agonist, procaterol, concentration-dependently increased the electrically evoked tritium overflow. Prenalterol, a β-adrenoceptor agonist with moderate preference for β_1-adrenoceptors, was considerably less active than the previously mentioned agonists; noradrenaline (in the presence of rauwolscine) was ineffective.

3 The concentration-response curve of procaterol was shifted to the right by the preferential β_2-adrenoceptor antagonist ICI 118-551 but was not affected by the β_1-selective antagonist, atenolol. Propranolol, but not atenolol, produced a shift to the right of the concentration-response curve of isoprenaline.

4 It is concluded that the sympathetic nerve fibres of the human pulmonary artery are endowed with facilitatory presynaptic β_2-adrenoceptors.

Introduction

Facilitatory presynaptic β-adrenoceptors on noradrenergic nerve fibres were first detected in guinea-pig atria (Adler-Graschinsky & Langer, 1975) and in human omental blood vessels (Stjärne & Brundin, 1975) and oviduct (Hedqvist & Moawad, 1975). This finding has been confirmed in several other tissues of various species including man (for reviews, see Langer, 1977; 1980; Starke 1977; 1981; Majewski 1983). Generally, it was suggested that presynaptic β-adrenoceptors belong to the β_2-subtype (e.g., Stjärne & Brundin, 1976; Dahlöf *et al.*, 1980; Dahlöf, 1981; Misu *et al.*, 1983), but many of the studies available were based only on a limited number of β-adrenoceptor agonists and antagonists; furthermore, it has not yet been demonstrated that β-adrenoceptor antagonists are acting competitively at these receptors. In some models (perfused hind leg of the cat and perfused gracilis muscle of the dog) data compatible with the presence of β_1-adrenoceptors on sympathetic nerve fibres were obtained (Dahlöf *et al.*, 1975; Dahlöf, 1981).

Species differences concerning the presence of presynaptic β-adrenoceptors also have to be considered; e.g., such receptors are present in the guinea-pig pulmonary artery (Misu *et al.*, 1981; 1983), but are not found in the rabbit pulmonary artery (Endo *et al.*, 1977). The aims of the present study were (1) to investigate whether facilitatory presynaptic β-adrenoceptors are detectable on the sympathetic nerve fibres of the human pulmonary artery, and (2) if present, to determine the subtype (β_1 or β_2) to which these receptors belong. Some of these results were reported at the 5th International Congress on Neuroeffector Mechanisms, Paris 1984 (Göthert *et al.*, 1984a).

Methods

Segments of human pulmonary arteries were obtained

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from male or female patients aged 35–70 years undergoing surgery for lung tumour. The patients did not suffer from pulmonary or systemic hypertension, and they were not treated with α- or β-adrenoceptor agonists or antagonists or with drugs influencing the storage or release of noradrenaline. After premedication with pethidine, promethazine and atropine, anaesthesia was induced with hexobarbitone, flunitrazepam or etomidate and maintained with enflurane, halothane or fentanyl (plus diazepam or enflurane). During maintenance of anaesthesia, the patients were breathing mixtures of N_2O and O_2. Suxamethonium and pancuronium were administered for neuromuscular blockade. The specimens of arteries used for the experiments were prepared immediately after pneumonectomy from lobular or segment arteries in macroscopically tumour-free areas of the lung.

The segments of human pulmonary arteries were cut spirally into strips (about 3 × 20 mm). Subsequently, the strips were incubated for 60 min in 1.5 ml physiological salt solution (37°C; composition see below) containing (−)-[ring-2,5,6-^{3}H]-noradrenaline 0.4 μmol l^{-1} (specific activity 43.9–53.5 Ci $mmol^{-1}$). The strips were mounted vertically in an organ bath (tension adjusted to 2 g) between two parallel platinum electrodes and superfused with [^{3}H]-noradrenaline-free physiological salt solution of 37°C at a rate of 2 ml min^{-1}. The composition of the solution was as follows (mmol l^{-1}): NaCl 118, NaH_2PO_4 1.2, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, glucose 11, ascorbic acid 0.3, Na_2EDTA 0.03 (aerated with 95% O_2 and 5% CO_2). Throughout superfusion this solution contained cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. The superfusate was collected continuously in 3 or 6 min fractions. Five 3 min periods of transmural electrical stimulation (2 Hz, rectangular pulses of 150 mA and 0.3 ms) were applied to each strip after 93 (S_1), 117 (S_2), 141 (S_3), 165 (S_4) and 189 min (S_5) of superfusion. At the end of superfusion, each strip was solubilized with Soluene, and the radioactivity in the superfusate samples and arteries was determined by liquid scintillation counting.

In some experiments, [^{3}H]-noradrenaline and its ^{3}H-metabolites contained in total tritium efflux from pulmonary arteries preincubated with (−)-[ring 2,5,6-^{3}H]-noradrenaline (53.5 Ci $mmol^{-1}$) were separated by combined use of alumina and Dowex 50W × 4 columns (as described by Graefe *et al.*, 1973). The recoveries of [^{3}H]-noradrenaline ([^{3}H]-NA) and each of its ^{3}H-metabolites ([^{3}H]-3,4-dihydroxyphenylglycol, [^{3}H]-DOPEG; [^{3}H]-3,4-dihydroxymandelic acid, [^{3}H]-DOMA; [^{3}H]-normetanephrine, [^{3}H]-NMN; ^{3}H-*O*-methylated deaminated metabolites, [^{3}H]-OMDA, i.e. sum of [^{3}H]-3-methoxy-4-hydroxyphenylglycol and [^{3}H]-3-methoxy-4-hydroxymandelic acid) was at least 77%, and corrections were made for recovery in each fraction.

Tritium efflux was calculated as the fraction of tritium present in the strip at the onset of the respective collection period. Basal ^{3}H efflux was expressed as the ratio of the fractional rate during the collection period immediately before S_3 (t_3, from 138–140 min), S_4 (t_4, from 162–164 min) or S_5 (t_5, from 186–188 min) to that immediately before S_2 (t_2; from 114–116 min).

Stimulation-evoked overflow of tritium or, when relevant, of [^{3}H]-noradrenaline and ^{3}H-metabolites was calculated by subtraction of the basal efflux (assumed to decrease linearly from the collection period before to that 12–15 min after stimulation) from the total efflux during the 12 min subsequent to the onset of stimulation. Evoked ^{3}H overflow was given as a fraction of tissue tritium at the onset of stimulation, and the ratios of the overflow evoked by S_3, S_4 or S_5 to that evoked by S_2 were determined.

Mean ± s.e.mean of the number of experiments (n) indicated are given throughout the paper. Student's t test was used for comparison of the mean values. Apparent pA_2 values of antagonists were calculated according to the following formula given by Furchgott (1972):

$$pA_2 = \log([E']/[E] - 1) - \log[B]$$

[E'] and [E] are those concentrations which caused half-maximum effects in the presence and absence of the antagonist, respectively. [B] is the concentration of the antagonist.

Drugs

The following drugs were used: (−)-[ring-2,5,6-^{3}H]-noradrenaline (New England Nuclear, Dreieich, FRG); cocaine hydrochloride (Merck, Darmstadt, FRG); corticosterone (Sigma, St. Louis, MO, USA); (−)-noradrenaline base, (−)-adrenaline base (Hoechst, Frankfurt, FRG); (−)-isoprenaline sulphate (Boehringer, Ingelheim, FRG); (±)-procaterol hydrochloride (Otsuka, Osaka, Japan); (±)-prenalterol hydrochloride (Hässle, Göteborg, Sweden); (±)-propranolol hydrochloride, (±)-atenolol, (±)-ICI 118-551 (erythro-DL-l(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; ICI, Plankstadt, FRG); rauwolscine hydrochloride (Roth, Karlsruhe, FRG).

Results

Basal and stimulation-evoked outflow of tritium under control conditions

In control experiments, the basal tritium efflux from superfused strips of the human pulmonary artery preincubated with [^{3}H]-noradrenaline slightly de-

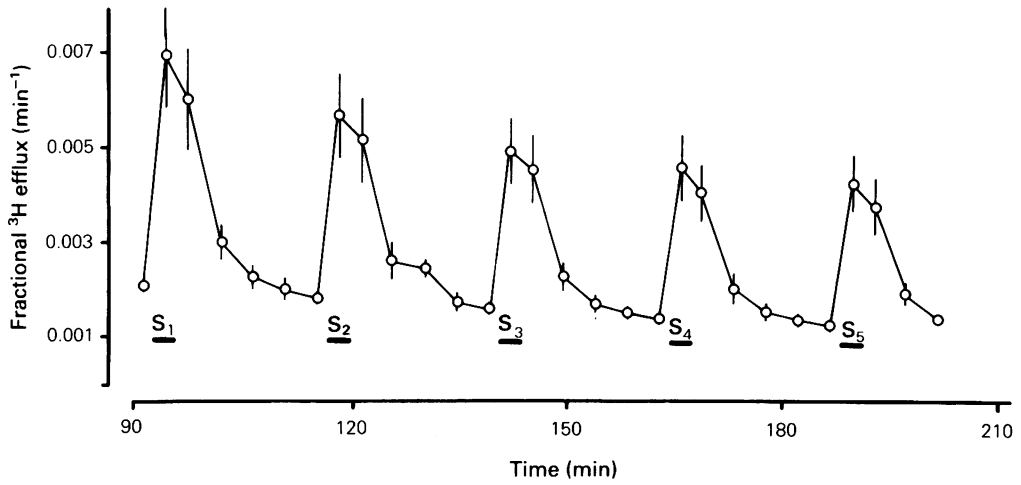

Figure 1 Tritium efflux from strips of the human pulmonary artery preincubated with [^{3}H]-noradrenaline and superfused with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Collection of the superfusates in 3 or 6 min samples. Five periods of transmural electrical stimulation (2 Hz; S_1-S_5). Abscissa scale: time after onset of superfusion. Ordinate scale: efflux of tritium per min, expressed as fraction of tissue tritium at the onset of the respective collection period. Mean of 6 experiments; vertical lines show s.e.means.

creased with time (Figure 1, Table 1). The outflow of tritium was reproduceably increased by transmural electrical stimulation (Figure 1, Table 1). It should be noted that in all experiments the superfusion fluid contained cocaine and corticosterone throughout.

Effects of β-adrenoceptor agonists

The basal efflux of tritium, measured in the 3-min period immediately before S_3, S_4 or S_5 (i.e. after 6–9 min of exposure to the drugs) was not affected by isoprenaline, procaterol, prenalterol, adrenaline or noradrenaline at the concentrations investigated (the latter two drugs also in the presence of rauwolscine 10 μmol l^{-1} throughout superfusion; all results not shown).

Isoprenaline, procaterol and prenalterol increased the electrically evoked tritium overflow in a concentra-

Table 1 Control values for basal tritium efflux and stimulation-evoked tritium overflow from strips of the human pulmonary artery[a]

Basal ^{3}H efflux[b,c,d]				
t_2		t_3/t_2	t_4/t_2	t_5/t_2
nCi	*Fractional rate* (min^{-1})[e]			
2.72 ± 0.46	0.0019 ± 0.0001	0.89 ± 0.01	0.80 ± 0.01	0.74 ± 0.02
Electrically evoked ^{3}H overflow[c,d]				
S_2		S_3/S_2	S_4/S_2	S_5/S_2
nCi	*% of tissue tritium*			
12.54 ± 4.04	2.50 ± 0.54	0.89 ± 0.02	0.81 ± 0.02	0.78 ± 0.04

[a] Strips preincubated with [^{3}H]-noradrenaline were superfused with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}.
[b] t_2-t_5 represent the 3 min periods of superfusate sampling immediately before the respective stimulation periods S_2-S_5.
[c] Basal efflux during t_3, t_4, t_5 and overflow evoked by S_3, S_4, S_5 are given as fractions of the respective t_2 and S_2 values.
[d] Mean ± s.e.mean of 6 experiments.
[e] Efflux of tritium per min, expressed as fraction of tissue tritium.

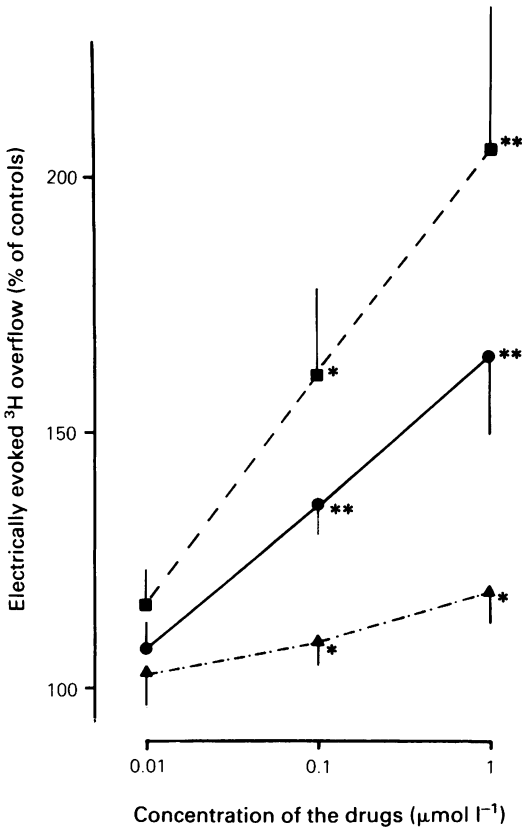

Figure 2 Effects of β-adrenoceptor agonists on the electrically evoked tritium overflow from strips of the human pulmonary artery preincubated with [^{3}H]-noradrenaline and superfused with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Five periods of transmural electrical stimulation (S_1-S_5. see Figure 1). The ratios of the ^{3}H overflow evoked by S_3, S_4 and S_5 to that evoked by S_2 are given. All ratios are expressed as percentages of the ratios in the control experiments. Each concentration of the agonists (isoprenaline ●, procaterol ■, prenalterol ▲) was present 9 min before and during the stimulation period S_3, S_4 or S_5 (see Figure 1). Mean of 5–6 experiments; vertical lines show s.e.means. $*P < 0.05$; $**P < 0.005$ (compared to the corresponding controls).

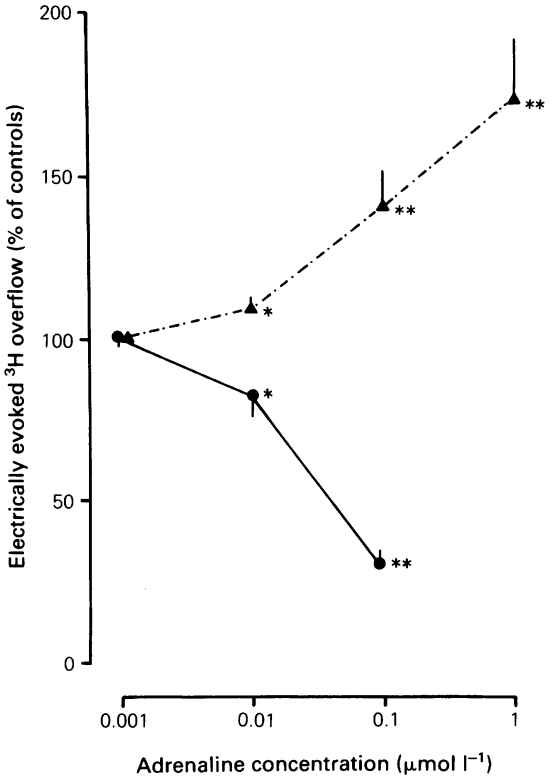

Figure 3 Effects of adrenaline on the electrically evoked tritium overflow from strips of the human pulmonary artery preincubated with [^{3}H]-noradrenaline and superfused with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Five periods of transmural electrical stimulation (S_1-S_5, see Figure 1). The ratios of the ^{3}H overflow evoked by S_3, S_4 or S_5 to that evoked by S_2 are given. All ratios are expressed as percentages of the ratios in the respective adrenaline-free control experiments. Each concentration of adrenaline was present 9 min before and during the stimulation period S_3, S_4 or S_5 (see Figure 1). Experiments were carried out either in the absence (●) or in the presence of rauwolscine 10 μmol l^{-1} (▲) throughout superfusion. Mean of 4–6 experiments; vertical lines show s.e.means. $*P < 0.05$; $**P < 0.005$ (compared to the corresponding controls).

tion-dependent manner (Figure 2). However, the facilitatory effect of prenalterol was clearly less pronounced than that of isoprenaline or procaterol: at the highest concentration applied, namely 1 μmol l^{-1}, prenalterol increased the evoked overflow by about 20%, whereas isoprenaline and procaterol produced increases of about 70 and 100%, respectively (Figure 2). Preliminary experiments with constant concentrations of the agonists, present from 9 min before S_3 until 12 min after S_5, revealed a constant degree of facilitation (i.e. no time-dependent alteration; results not shown). In contrast to the compounds mentioned so far, adrenaline caused a concentration-dependent inhibition of the electrically evoked overflow of tritium, but in the presence of rauwolscine 10 μmol l^{-1}, this effect was reversed to a facilitation (by about 70% at 1 μmol l^{-1} adrenaline; Figure 3). Under the latter condition, i.e. blockade of α-adrenoceptors, unlabelled noradrenaline did not alter the evoked overflow; when unlabelled noradrenaline 0.01, 0.1 or

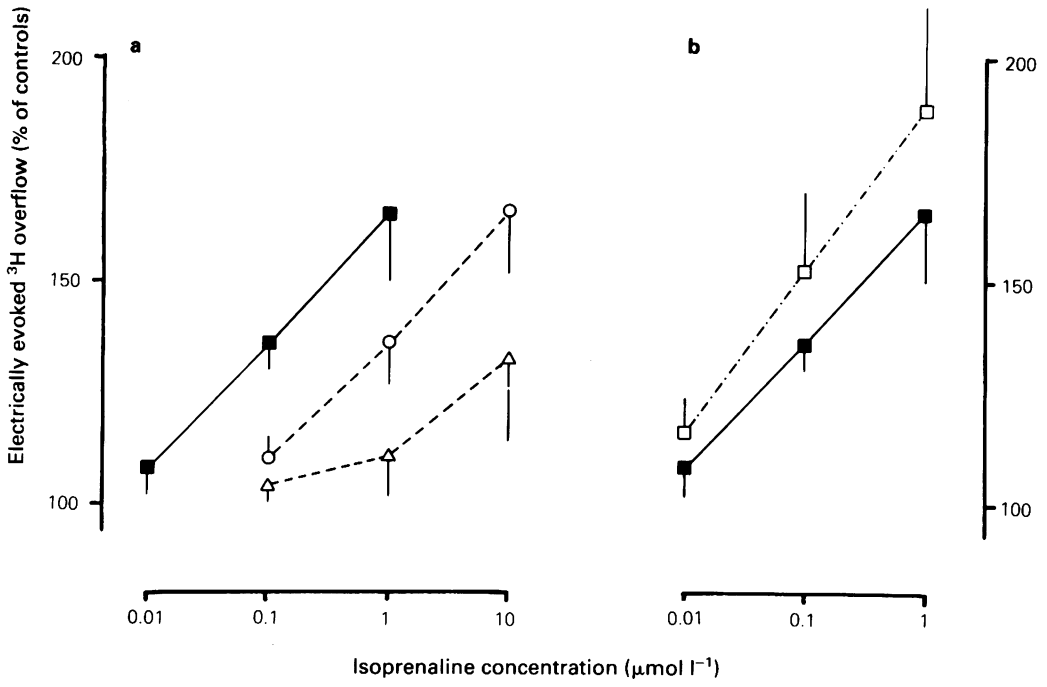

Figure 4 Effect of isoprenaline on the electrically evoked tritium overflow from strips of the human pulmonary artery and interaction with propranolol (a) or atenolol (b). Preincubation of the strips with [^{3}H]-noradrenaline and superfusion with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Five periods of transmural electrical stimulation (S_1-S_5, see Figure 1). The ratios of the ^{3}H overflow evoked by S_3, S_4 and S_5 to that evoked by S_2 are given. All ratios are expressed as percentages of the ratios in the respective isoprenaline-free control experiments. Each concentration of isoprenaline was present 9 min before and during the stimulation period S_3, S_4 or S_5 (see Figure 1). Effect of isoprenaline without propranolol or atenolol (■); effect of isoprenaline in the presence of propranolol 0.1 (○) or 1 μmol l^{-1} (△) or of atenolol 10 μmol l^{-1} (□). The antagonists were present from 3 min before S_1 until the end of the experiments. Mean of 5 or 6 experiments; vertical lines show s.e.means.

1 μmol l^{-1} was present in the superfusion fluid 9 min before and during stimulation ($n = 4$), the evoked overflow was 102 ± 3, 93 ± 2 and 96 ± 1% of controls, respectively (no significant difference from 6 controls).

Effects of β-adrenoceptor antagonists

The same time schedule as in the experiments with agonists was applied in a series of experiments with propranolol, i.e. the drug was added to the superfusion fluid at concentrations of 0.01, 0.1 and 1 μmol l^{-1} 9 min before and during S_3, S_4 and S_5 respectively ($n = 4$–5). Propranolol 0.01 and 0.1 did not alter the basal tritium efflux, and at 1 μmol l^{-1} it caused a negligible increase by 8 ± 1% ($P < 0.05$; results not shown). The electrically evoked tritium overflow was not affected by the β-adrenoceptor antagonist: in the presence of propranolol 0.01, 0.1 and 1 μmol l^{-1}, the stimulated overflow was 104 ± 3, 109 ± 6 and 101 ± 14% of controls, respectively (no significant difference from 6 controls). Propranolol (0.1 and 1 μmol l^{-1}) produced a concentration-dependent shift to the right of the concentration-response curve of isoprenaline for its increasing effect on the electrically evoked ^{3}H overflow (Figure 4a). The mean apparent pA_2 value of propranolol against isoprenaline (determined at the level of its EC_{50} values) was 7.96. In the presence of atenolol 10 μmol l^{-1} no shift to the right (but rather a negligible shift to the left) of the concentration-response curve of isoprenaline was obtained (Figure 4b).

The concentration-response curve of procaterol for its facilitatory effect on the electrically evoked ^{3}H overflow was also not affected by atenolol 10 μmol l^{-1}, but it was shifted to the right by ICI 118-551 0.1 μmol l^{-1} (Figure 5). The apparent pA_2 value of ICI 118-551 against procaterol (determined at the level of its EC_{50} value) was 7.99.

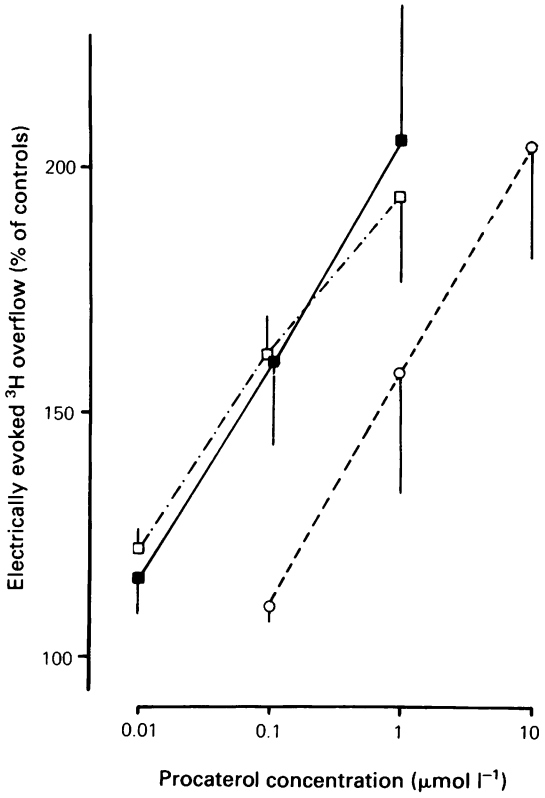

Figure 5 Effect of procaterol on the electrically evoked tritium overflow from strips of the human pulmonary artery and interaction with ICI 118-551 and atenolol. Preincubation of the strips with [^{3}H]-noradrenaline and superfusion with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Five periods of transmural electrical stimulation (S_1-S_5, see Figure 1). The ratios of the ^{3}H overflow evoked by S_3, S_4 and S_5 to that evoked by S_2 are given. All ratios are expressed as percentages of the ratios in the respective procaterol-free control experiments. Each concentration of procaterol was present 9 min before and during the stimulation period S_3, S_4 or S_5 (see Figure 1). Effect of procaterol without ICI 118-551 and atenolol (■); effect of procaterol in the presence of ICI 118-551 0.1 μmol l^{-1} (○) or atenolol 10 μmol l^{-1} (□). The antagonists were present from 3 min before S_1 until the end of the experiments. Mean of 5 or 6 experiments; vertical lines show s.e.means.

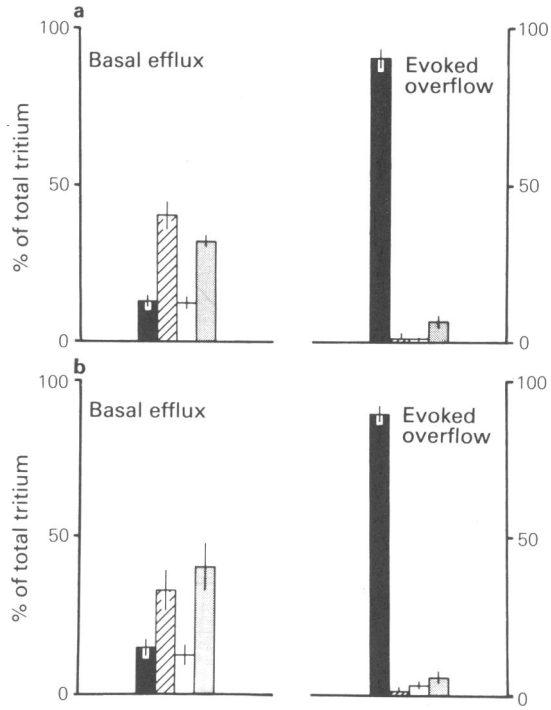

Figure 6 Percentages of [^{3}H]-noradrenaline and ^{3}H-metabolites contained in basal and electrically evoked efflux of tritium from strips of the human pulmonary artery. Preincubation of the strips with [^{3}H]-noradrenaline and superfusion with [^{3}H]-noradrenaline-free solution containing cocaine 30 μmol l^{-1} and corticosterone 40 μmol l^{-1}. Three periods of transmural electrical stimulation (S_1-S_3, see Figure 1). Basal efflux: percentages in the 3 min sample immediately before S_3. Evoked overflow: percentages contained in the ^{3}H overflow evoked by S_3 (above basal efflux). In (a), control experiments without isoprenaline. (b) Experiments with isoprenaline 1 μmol l^{-1} (present 9 min before and during S_3). Mean of 4 experiments; vertical lines show s.e.means. Solid columns, [^{3}H]-NA; hatched columns, [^{3}H]-DOPEG; open columns, [^{3}H]-DOMA; stippled columns [^{3}H]-OMDA; for explanation of abbreviations see Methods. Only negligible percentages of basal and stimulation evoked tritium efflux were accounted for by [^{3}H]-NMN (range: 0.1 ± 0.6 to 1.8 ± 1.0%).

Separation of [^{3}H]-noradrenaline from its ^{3}H-metabolites

Under control conditions, only a minor part of the basal efflux of tritium from the human pulmonary artery was accounted for by unmetabolized [^{3}H]-noradrenaline (Figure 6); mainly [^{3}H]-DOPEG and [^{3}H]-OMDA were found. In contrast, the electrically evoked ^{3}H overflow consisted mainly of [^{3}H]-noradrenaline and only to a minor part of ^{3}H-metabolites (Figure 6). Neither the percentage composition of the basal efflux of tritium nor that of the stimulation evoked tritium overflow was altered by isoprenaline 1 μmol l^{-1} (Figure 6).

Discussion

Only one attempt has been made previously to identify presynaptic receptors in the human pulmonary artery: Freeman *et al.* (1981) showed that in agreement with data obtained in rabbits (Endo *et al.*, 1977), presynaptic 5-hydroxytryptamine receptors do not exist in this blood vessel. In view of the failure to identify presynaptic β-adrenoceptors in rabbit pulmonary arteries (Endo *et al.*, 1977) but, on the other hand, their presence in the corresponding vessels from guinea-pigs (Misu *et al.*, 1981; 1983), it was of particular interest to investigate whether these receptors are present in human pulmonary arteries.

Preliminary studies in such arteries preincubated with [^{3}H]-noradrenaline revealed that the electrically evoked tritium overflow was abolished by tetrodotoxin or omission of calcium ions from the superfusion fluid (unpublished results). Since in the present experiments neuronal and extraneuronal uptake were blocked, the evoked overflow consisted almost exclusively of [^{3}H]-noradrenaline; however, even in the presence of cocaine and corticosterone, basal efflux of tritium was accounted for mainly by deaminated ^{3}H-metabolites. These findings are in agreement with the pattern of ^{3}H-substances found under similar conditions in the superfusates of other tissues with a noradrenergic nerve supply (Langer *et al.*, 1976; Endo *et al.*, 1977; Göthert, 1984; Göthert *et al.*, 1984b). According to the findings discussed so far, it is evident that the electrically evoked tritium overflow reflects action potential-induced noradrenaline release from the sympathetic nerve fibres.

Isoprenaline which is known to activate both β_1- and β_2-adrenoceptors increased the stimulation-evoked tritium overflow, and it did not alter the pattern of [^{3}H]-noradrenaline and ^{3}H-metabolites. Adrenaline, administered under identical conditions, inhibited the evoked release. This effect was due to an activation of presynaptic α_2-adrenoceptors, since after blockade of these autoreceptors by rauwolscine, adrenaline caused an increase in noradrenaline release, suggesting a β-adrenoceptor-mediated facilitation. In other human blood vessels, like omental, digital and metatarsal arteries and veins (Stjärne & Brundin, 1975; Stevens *et al.*, 1982; Moulds & Stevens, 1983), the facilitatory effect of adrenaline was already apparent in the absence of α_2-adrenoceptor antagonists, suggesting a different proportion of presynaptic α- and β-adrenoceptors in different tissues. In agreement with findings in guinea-pig atria (Rand *et al.*, 1976; Majewski *et al.*, 1980), rat portal vein (Westfall *et al.*, 1979), dog saphenous vein (Guimarães *et al.*, 1978), human digital arteries and metatarsal veins (Moulds & Stevens, 1983), propranolol given alone did not affect the stimulated noradrenaline release in the human pulmonary artery. However, propranolol produced a concentration-dependent shift to the right of the concentration-response curve of isoprenaline. In conclusion, the ability of isoprenaline and adrenaline (the latter in the presence of rauwolscine) to increase noradrenaline release and the ability of propranolol to antagonize competitively the effect of isoprenaline indicate that the sympathetic nerve fibres of the human pulmonary artery are endowed with facilitatory presynaptic β-adrenoceptors. In this respect, the human pulmonary artery resembles that of the guinea-pig (Misu *et al.*, 1981; 1983), but differs from that of the rabbit (Endo *et al.*, 1977).

Another aim of the present study was to characterize the subtype to which these receptors belong. For this purpose, agonists and antagonists preferentially acting on either β_1- or β_2-adrenoceptors were applied. The selective β_2-adrenoceptor agonist, procaterol (Brodde *et al.*, 1983), caused a pronounced increase in evoked noradrenaline release. Prenalterol, an agonist with moderate preference for β_1-adrenoceptors (Carlsson *et al.*, 1977; Johansson & Waldeck, 1980; Brodde *et al.*, 1983; Cook *et al.*, 1984), produced only a slight facilitation of release which may be explained by its weak stimulating effect on β_2-adrenoceptors. Apart from its ability to activate α-adrenoceptors, noradrenaline stimulates β-adrenoceptors and exhibits a preference for the β_1-subtype (Lands *et al.*, 1967; Furchgott, 1967; Daly & Levy, 1979). In the presence of rauwolscine, noradrenaline did not affect the evoked noradrenaline release. In this respect, noradrenaline was basically different from the unselective agonist, adrenaline, which under identical conditions facilitated release (see above). The selective β_2-adrenoceptor antagonist, ICI 118-551 (Nahorski *et al.*, 1979; Cook *et al.*, 1984), caused a clear-cut shift to the right of the concentration-response curve of procaterol for its facilitatory effect on evoked noradrenaline release. In contrast, the preferential β_1-adrenoceptor antagonist, atenolol (Barrett *et al.*, 1973; Nahorski *et al.*, 1979; Vincent *et al.*, 1982; Cook *et al.*, 1984), even at a very high concentration, did not produce a shift to the right of the concentration-response curve of procaterol or of the unselective agonist isoprenaline. All results obtained with the preferential β-adrenoceptor agonists and antagonists are compatible with the conclusion that the facilitatory presynaptic β-adrenoceptors in the human pulmonary artery belong to the β_2-subtype. The same conclusion was drawn from experiments in human omental arteries and veins (Stjärne & Brundin, 1976). However, results obtained in other blood vessels were equivocal. Thus, Stevens *et al.* (1982) found that in human digital arteries both the β_2-selective agonist, salbutamol, and the β_1-preferential agonist, dobutamine, facilitated [^{3}H]-noradrenaline release; they supposed dobutamine to be a non-selective agonist at the presynaptic β-adrenoceptors of this

blood vessel. In the guinea-pig pulmonary artery, Misu *et al.* (1983), found that salbutamol produced a facilitation, but that metoprolol, which is known to exhibit a preference for β_1-adrenoceptors, was active as an antagonist; the authors suggested that in this preparation metoprolol may act as a non-selective antagonist.

The present study is the first investigation of presynaptic β-adrenoceptors in which shifts to the right by β-adrenoceptor antagonists of concentration-response curves of agonists are shown, indicating competitive antagonism. It is clearly demonstrated that facilitatory presynaptic β_2-adrenoceptors are located on the sympathetic nerve fibres of the human pulmonary artery.

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