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Classification of β-adrenoceptors in isolated bronchus of the pig

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1 (±)-Isoprenaline (Iso), (−)-adrenaline (Ad), (−)-noradrenaline (NA), the β_2-selective adrenoceptor agonist (±)-fenoterol (Fen) and the β_1-selective adrenoceptor agonist (±)-RO363 caused concentration-dependent relaxation of preparations of pig bronchus pre-contracted with carbachol 40 ng/ml (0.22 μM). Iso, Ad, NA and Fen caused complete relaxation of carbachol-induced tone, but RO363 caused relaxation equivalent to only 59% of the maximal response to Iso.
2 When relaxation responses to these amines were plotted as a % of their maximal effects, comparison of EC_{50} values showed that the order of potency was RO363 > Iso > NA > Fen > Ad (14.4:4.6:1:0.4:0.3).
3 pA_2 values determined for the β-adrenoceptor antagonists propranolol (non-selective) and atenolol (β_1-selective), or the partial agonist salbutamol (β_2-selective) using Iso as agonist were 8.3, 7.3 and 4.4 respectively. The pA_2 value for atenolol using RO363 as the agonist was 7.6.
4 These results indicate that porcine bronchus contains a homogeneous population of β_1-adrenoceptors.

Introduction

In the guinea-pig, β_2-adrenoceptors predominate in both central (O'Donnell, 1972; O'Donnell & Wanstall, 1979a) and peripheral airways (Siegl, Rossi & Orzechowski, 1979) while in the cat, there are β_1-adrenoceptors in central airways and β_2-adrenoceptors in the peripheral lung strip (Lulich, Mitchell & Sparrow, 1976). Recent evidence indicates that a homogeneous population of β_2-adrenoceptors also mediate catecholamine-induced relaxation of porcine lung parenchymal strip while both β_1 and β_2-adrenoceptors exist in human lung strip (Goldie, Paterson & Wale, 1982a). Thus, it was of interest to characterize the β-adrenoceptor subtype in bronchial (central) smooth muscle from the pig.

The relative potencies were determined of (±)-isoprenaline (Iso), (−)-adrenaline (Ad), (−)-noradrenaline (NA), the β_2-selective agonist (±)-fenoterol (Fen) (O'Donnell, 1970) and the highly β_1-selective catecholamine (±)-RO363 (Raper, McPherson & Iakovidis, 1978; Iakovidis, Malta, McPherson & Raper, 1980). In addition, pA_2 values were calculated for the β-adrenoceptor antagonists propranolol (non-selective) and atenolol (β_1-selective) with either Iso or RO363 as the agonist.

Methods

Bronchi (2–3 mm i.d.) were dissected from the central lobes of lung from pigs freshly slaughtered at a local abattoir. Bronchi were cut into spiral strips and suspended under 500 mg tension in Krebs Henseleit solution maintained at 37°C and aerated with 5% CO_2 in O_2. Changes in isometric tension were monitored with a Grass force-displacement transducer (FTO3C) coupled to a preamplifier and Rikadenki pen recorder (model 1328L). Preparations were left to equilibrate for 60–90 min with regular washing before any drug-induced effects were measured. Bronchial preparations developed little or no tone spontaneously so that catecholamine-induced relaxations were measured in tissues precontracted with carbachol as previously described (Goldie, Paterson & Wale, 1982b). Concentration-effect curves to catecholamines were constructed by cumulative administration and approximately 1 h was allowed to elapse between curves. Results were expressed as a % of the maximum relaxation (E_{max} = 100%) produced by each catecholamine.

In experiments to determine the relative potencies of β-agonists, two consecutive concentration-effect curves were constructed to one of Iso, Ad, NA, Fen or RO363. The EC_{50} value from the second curve

was taken as the measure of amine potency. Concentration-effect curves to two of the other amines were constructed in turn. In some experiments, after the first two control curves were established, a third curve was constructed to the same amine in the presence of normetanephrine (50 μM) to determine the effect of inhibiting extraneuronal uptake, or in the presence of phentolamine (15 μM) which inhibits both α-adrenoceptors and catecholamine uptake processes (Foster, 1967; 1968; 1969; Goldie & Paterson, 1982).

Concentration-effect curves to Iso were also established in the absence and presence of a competitive β-adrenoceptor antagonist and pA_2 values established by the method of Arunlakshana & Schild (1959). In a separate series of experiments, 4 bronchial spirals were dissected from a single pig bronchus. One preparation served as the control while the other 3 were exposed to one concentration of atenolol (0.5–20 μM) for 60 min prior to and during the construction of concentration-effect curves to RO363. Similar experiments were conducted using Iso as the agonist. Apparent atenolol-induced rightward shifts in concentration-effect curves to these relaxant amines were calculated and pA_2 values determined.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (±)-isoprenaline hydrochloride, carbamylcholine chloride, normetanephrine hydrochloride (Sigma); RO363 [(±)-1-(3, 4-dimethoxy phenyethylamino)-3- (3, 4-dihydroxyphenoxy)-2- propanol)oxalate] (synthesized at the Victorian College of Pharmacy, Melbourne, Australia); (±)-fenoterol hydrobromide (Boehringer Ingelheim); (±)-salbutamol sulphate (Allen & Hanbury); atenolol; (−)-propranolol hydrochloride (ICI); cocaine hydrochloride (M & B); imipramine hydrochloride, phentolamine mesylate (Ciba). All drug concentrations refer to the base. Drug solutions were freshly prepared using 0.9% w/v NaCl solution (saline). Solutions of sympathomimetic amines were stabilized with ascorbic acid (20 μg/ml).

Results

Effect of carbachol concentration on isoprenaline-induced bronchial relaxation.

Preparations of pig bronchus developed little or no tone spontaneously and Iso alone caused little if any decrease in resting tension. However, cumulatively added Iso caused a concentration-dependent decrease in induced tone in preparations contracted by single doses of carbachol (20–640 ng/ml; 0.11–3.5 μM). The E_{max} value for carbachol was 3.6 ± 0.2 g developed tension ($n = 18$). Iso, Ad and NA (100 μM) consistently caused complete relaxation of tone in bronchial preparations only when the concentration of carbachol was 20 ng/ml (0.11 μM; % $E_{max\ carbachol}$ ± s.e.mean = 17.1 ± 2.8%, $n = 8$) or 40 ng/ml (0.22 μM; % $E_{max\ carbachol}$ = 28.3 ± 4.0%, $n = 9$). When concentrations of carbachol higher than 80 ng/ml were used, these amines often produced less than complete relaxation of pig bronchi. In the presence of carbachol 20, 40 or 80 ng/ml, the mean Iso EC_{50} control values were 0.31 ± 0.03 μM ($n = 5$); 0.44 ± 0.02 μM ($n = 148$); and 0.68 ± 0.08 μM ($n = 5$) respectively. These values are all significantly different from one another ($P < 0.001$; non-paired t test). Unless otherwise stated, the concentration of carbachol used thereafter to pre-contract pig bronchi was 40 ng/ml (0.22 μM).

Relative potencies of β-adrenoceptor agonists

Iso, Ad, NA, Fen and RO363 caused concentration-dependent relaxation of carbachol-contracted, pig bronchus. Iso, Ad, NA and Fen caused complete relaxation in preparations pre-contracted with carbachol, 40 ng/ml (0.22 μM), while RO363 was a partial agonist, causing only 59 ± 0.03% (mean ± s.e.mean, $n = 12$) of the maximal relaxation produced by Iso.

Comparison of EC_{50} values shows that the order of potency for relaxation was RO363 > Iso > NA > Fen > Ad (14.4:4.6:1:0.4:0.3) (Table 1). The extraneuronal uptake inhibitor normetanephrine (50 μM) caused a small (2 fold) but significant increase in the potency of Iso ($P < 0.05$; paired t test) but had no significant effect on Ad or NA ($P > 0.05$). Phentolamine (15 μM) caused a small but statistically significant decrease in carbachol-induced (0.22 μM) tone of 12.3 ± 4% ($0.02 < P < 0.05$; $n = 24$) and caused significant 2–3 fold increases in the potency of all three catecholamines ($P < 0.05$). The neuronal uptake inhibitors cocaine (10 μM) and imipramine (1 μM) failed to alter significantly the potency of any of the amines tested ($P > 0.05$).

Successive concentration-effect curves to RO363 were progressively shifted in parallel to the right with no change in the maximal response; curves for the other agonists were never constructed in preparations previously exposed to RO363.

Effects of β-adrenoceptor antagonists on responses to isoprenaline or RO363 in porcine bronchus

Propranolol, atenolol, salbutamol or RO363 with isoprenaline as agonist Both the non-selective β-adrenoceptor antagonist propranolol and the β_1-selective antagonist atenolol were potent competitive inhibitors of Iso-induced bronchial relaxation having pA_2 values of 8.3 ± 0.1; (slope = 1.0) and 7.3 ± 0.1; (slope = 0.8) respectively.

Table 1 EC_{50} values and relative potency ratios for isoprenaline (Iso), adrenaline (Ad), noradrenaline (NA), fenoterol (Fen) and RO363-induced relaxation of pig bronchus

	(±)-*Iso*	(−)-*Ad*	(−)-*NA*	(±)-*Fen*	(±)-*RO363*
$EC_{50}(\mu M)$	0.44 ± 0.02 (148)	6.17 ± 0.8 (11)	2.01 ± 0.2 (25)	5.37 ± 1.0 (25)	0.14 ± 0.01 (22)
Ratio	4.6	0.3	1.00	0.4	14.4

Pig bronchi were pre-contracted with carbachol 0.22 μM. EC_{50} values are shown as mean ± s.e.mean. Numbers in parentheses indicate the number of observations.

RO363 and salbutamol had little or no relaxant effect in pig bronchi pre-contracted by a high concentration of carbachol (120 ng/ml; 0.66 μM), while Iso reduced this carbachol tone by 69 ± 2% ($n = 16$). Iso concentration-effect curves were shifted in parallel to the right in such preparations when they were pretreated with either RO363 (2–50 μM) or salbutamol (0.1–5 mM) for 60 min. The pA_2 values for RO363 and salbutamol against Iso were 5.9 ± 0.1 (slope = 1.1) and 4.4 ± 0.1 (slope = 1.0) respectively.

Atenolol with isoprenaline or RO363 as agonist RO363 was a partial agonist in pig bronchus causing β-adrenoceptor blockade. Thus accurate quantification of the β-blocking effects of atenolol with RO363 as the agonist, was not possible if a protocol was employed of successive concentration-effect curves to RO363. However, if separate bronchial preparations dissected from single bronchi were equilibrated in the absence or presence of different concentrations of atenolol and only one concentration-effect curve to RO363 constructed with each preparation, then apparent dose-ratios and thus Schild plots could be derived from the pooled results. For comparative purposes, this method was also used to calculate a pA_2 value for atenolol against Iso. When RO363 was the agonist, the pA_2 value for atenolol was 7.6 ± 0.1 (slope = 0.7; $r = 0.94$). When Iso was the agonist, the pA_2 value for atenolol was 7.2 ± 0.1 (slope = 0.8; $r = 0.86$).

Discussion

Comparison of the relaxant potencies of Iso, Ad, NA, Fen and RO363 clearly indicates that β_1-adrenoceptors predominate in bronchial smooth muscle of the pig. For example, the β_1-selective agonist RO363 (Raper *et al.*, 1978; Iakavidis *et al.*, 1980) was 3 times more potent than Iso and 38 times more potent than the β_2-selective agonist Fen (O'Donnell, 1970). Furthermore, NA which is relatively β_1-selective was 3 times more potent than Ad which is relatively β_2-selective (Lands, Arnold, McAullif, Luduena & Brown, 1967). In these experiments, the (−)-isomers of NA and Ad were used while the racemic forms of the other amines were tested. Comparison of the potencies of the (−)-isomers of NA and Iso would presumably have shown that Iso was approximately 9.2 times more potent than NA.

None of the amines tested ever caused contraction of porcine bronchus in the absence or presence of β-adrenoceptor antagonists. Thus phentolamine-induced increases in the potency of catecholamines were probably due to inhibition of uptake processes (Foster, 1967; 1968; 1969; Goldie & Paterson, 1982) rather than to inhibition of post-synaptic α-adrenoceptors. Similarly, it is unlikely that the 12.3% reduction in carbachol-induced tone produced by phentolamine (15 μM) contributed markedly to increased amine potency since Iso potency was only increased 1.3 fold when the concentration of carbachol used to pre-contract bronchi was reduced by 50%. Neuronal uptake does not appear to alter the potency of catecholamines in pig bronchus. While inhibition of extraneuronal uptake with normetanephrine (50 μM) only caused a 2 fold increase in the potency of Iso, it is possible that β-adrenoceptor blockade caused by normetanephrine partially masked sensitization to Iso (Kenakin, 1980; Goldie & Paterson, 1982).

Experiments examining the potency of β-adrenoceptor antagonists in pig bronchus support the suggestion that β_1-adrenoceptors predominate in this tissue. The pA_2 value for the β_1-selective antagonist atenolol was 7.2 when Iso was the agonist and 7.6 when the β_1-selective agonist RO363 was used, indicating the presence of a homogeneous population of β_1-adrenoceptors (O'Donnell & Wanstall, 1979a).

In guinea-pig atria where a homogeneous population of β_1-adrenoceptors also exists (O'Donnell & Wanstall, 1979a,b), the pA_2 value for atenolol against Iso was also 7.2 (Harms, 1976). In contrast, in the pig lung parenchyma strip where β_2-adrenoceptors predominate, the pA_2 value for atenolol against Iso was only 5.4 (Goldie *et al.*, 1982a). Furthermore, in the present study, salbutamol (β_2-selective) was a very weak antagonist of Iso-induced relaxation of pig bronchus ($pA_2 = 4.4$). Interestingly, the β_1-selective partial agonist RO363,

was a relatively weak competitive β_1-adrenoceptor antagonist in pig bronchus (pA_2 =5.9). This value is simlar to that obtained in carbachol contracted guinea-pig trachea (pA_2 =5.7) where RO363 was said to interact primarily with a small population of β_1-adrenoceptors (Iakovidis *et al.*, 1980).

While β_1-adrenoceptors exist in guinea-pig trachea, β_2-adrenoceptors predominate in central airways as well as in the lung parenchyma of the guinea-pig (O'Donnell & Wanstall, 1979a; Siegl *et al.*, 1979). However, in the pig there is a change from β_1-adrenoceptors in central airways to β_2-adrenoceptors in lung parenchyma strip (Goldie *et al.*, 1982a) as there is in the cat (Lulich *et al.*, 1976).

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