Br. J. Pharmac. (1984), **81**, 611–615

Classification of β-adrenoceptors in human isolated bronchus

Roy G. Goldie, James W. Paterson, Domenico Spina & Janet L. Wale

Department of Pharmacology, University of Western Australia, Nedlands, Perth, 6009, Australia

1 (±)-Isoprenaline (Iso), (−)-adrenaline (Ad), (−)-noradrenaline (NA), (±)-phenylephrine (Phe) and the β_2-selective adrenoceptor agonist (±)-fenoterol (Fen) caused a concentration-dependent relaxation of human isolated bronchial preparations. Iso, Ad and NA caused complete relaxation of both spontaneous and carbachol-induced bronchial tone. Fen, which was only tested in preparations where tone was induced with carbachol, also caused complete relaxation. However, Phe was a partial agonist in all preparations tested.

2 When relaxation responses to these amines were calculated as a % of their maximal effects, comparison of EC_{50} values showed that the order of potency was Iso > Ad = Fen > NA > Phe (92:27:25:1:0.2) in preparations with carbachol-induced tone and Iso > Ad > NA > Phe (112:38:1:0.3) in preparations with spontaneous tone.

3 pA_2 values determined for the β-adrenoceptor antagonists propranolol (non-selective), atenolol (β-selective) and ICI-118, 551 (β_2-selective), using Iso as an agonist were, 9.3, 5.3 and 9.1 respectively.

4 These results indicate that β_2-adrenoceptors mediate relaxation of human isolated bronchus to sympathomimetic amines in preparations obtained 4–14 h post-mortem from non-diseased lung. α-Adrenoceptors were apparently sparse or absent in this tissue.

Introduction

Sympathomimetic bronchodilators continue to play a major role in the treatment of asthma (Svedmyr, 1977; Paterson *et al.*, 1979). In asthma, the major site of airways obstruction is in the central airways. Isolated central airways preparations from several laboratory animal species have been used as models in the evaluation of β-adrenoceptor agonists as bronchodilators. While these different central airways preparations have been of great value in the screening of potential bronchodilator amines, many of them do not accurately reflect human central airways with respect to the β-adrenoceptor subtypes that they contain. Evidence from studies in conscious man strongly suggests a predominance of β_2-adrenoceptors in central airways (Larsson & Svedmyr, 1977; Madsen *et al.*, 1979). However, β_1-adrenoceptors predominate in the trachea of the cat (Lulich, *et al.*, 1976; O'Donnell & Wanstall, 1983), rabbit (Bristow *et al.*, 1970; Toda *et al.*, 1978) and pig (Goldie *et al.*, 1983) while β_2-adrenoceptors predominate in guinea-pig trachea (O'Donnell 1972; O'Donnell & Wanstall, 1979). Harms (1976) has shown that β_2-adrenoceptors predominate in isolated rings of human primary bronchi obtained from surgically excised lung samples. We have previously shown that preparations from smaller bronchi obtained 4–14 h post-mortem from non-diseased lung, respond to a range of relaxant and spasmogenic agents (Goldie *et al.*, 1982). The present investigation sought to determine the predominant β-adrenoceptor subtype in this tissue, with a view to assessing the value of the preparation as in *in vitro* model of human central airways.

Methods

Bronchi (2–3 mm i.d.) were removed from 44 separate macroscopically normal specimens of human lung (11 female, 34 male) obtained 4–14 h post-mortem. The subjects were aged 9–55 years. Excised bronchi were dissected free of all peripheral lung tissue, including visible blood vessels and cut into spirals. Preparations were suspended under 500 mg tension in Krebs-Henseleit solution aerated with 5% CO_2 plus 95% O_2 at 37°C. The composition of

Krebs-Henseleit solution (mM) was NaCl 117.6, KCl 5.4, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.57, D-glucose 11.1 and $CaCl_2$ 2.5. Isometric changes in tension were measured with a Grass force-displacement transducer (FTO3C) coupled to a preamplifier and a Rikadenki pen recorder (Model 1328L). Preparations were left to equilibrate for 2 h with washing at 30 min intervals, before any drug induced effects were measured.

Firstly, experiments were conducted to determine the possible effect of bronchial α-adrenoceptors (mediating contraction) on the relaxant potency of sympathomimetic amines which stimulate both α- and β-adrenoceptors. The effects of cumulative concentrations of Phe (α-adrenoceptor selective) were assessed in human isolated bronchi, in the absence and presence of the β-adrenoceptor antagonist propranolol (0.5 μM). In most cases, human isolated bronchi developed tone spontaneously and thus the relaxant effects of sympathomimetic amines could be measured following their cumulative addition to the organ bath. However, in some preparations, tone had to be induced with carbachol before the relaxant effects of agonists could be measured. When bronchial tone did not develop spontaneously, a complete cumulative concentration-effect curve to carbachol was constructed. After washing and resting the preparation for 1 h, tone was re-established using a concentration of carbachol which induced 50% of the maximum response to this spasmogen. Relaxation responses to cumulative concentrations of β-adrenoceptor agonists were then superimposed. Results were expressed as a % of the maximum relaxation produced by each sympathomimetic amine. In experiments to determine the relative potencies of relaxant amines, a cumulative concentration-effect curve was constructed to each of (±)-isoprenaline (Iso), (−)-adrenaline (Ad), (−)-noradrenaline (NA), (±)-phenylephine (Phe) or (±)-fenoterol (Fen) and the EC_{50} value taken as a control measure of amine potency. Concentration-effect curves to two of the other amines were then constructed in turn at intervals of approximately 90 min.

Concentration-effect curves to Iso were also established in the absence and presence of the competitive β-adrenoceptor antagonists propranolol (non-selective), atenolol (β_1-selective) or ICI-118,551 (β_2-selective) in bronchi pre-contracted with carbachol. Preparations were exposed to one concentration of one of these antagonists for 1 h before another cumulative concentration-effect curve to Iso was produced. In some preparations, this procedure was repeated once using a higher concentration of the same antagonist. The EC_{50} values for Iso in the presence and absence of antagonist and the ratio of these values was then determined. Schild plots ($\log_{10}$ concentration ratio − 1 versus $\log_{10}$ molar antagonist concentration) were constructed and pA_2 values were determined by regression analysis of individual data points. Consecutive Iso concentration-effect curves were also constructed in preparations not exposed to β-adrenoceptor antagonists to monitor possible spontaneous changes in agonist potency.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (±)-isoprenaline hydrochloride, (±)-phenylephrine hydrochloride, carbamylcholine chloride (Sigma); fenoterol hydrobromide (Boehringer Ingelheim); propranolol hydrochloride, atenolol, ICI-118,551 (erythro-DL-1-(7-methylindom-4-yloxy)-3-(isopropylaminobutan-2-ol) hydrochloride) (ICI). 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^{-1}).

Results

In the absence of spontaneously developed or carbachol-induced tone, cumulative concentrations of the relatively α-adrenoceptor-selective agonist Phe (0.8–800 μM) failed to cause either contraction or relaxation in isolated human bronchial preparations. In preparations with spontaneous tone, Phe caused concentration-dependent relaxation (Table 1), but was a partial agonist producing only 48.9 ± 9.7% (mean ± s.e.mean, 8 preparations from 3 lung specimens) of the maximal response to Iso. Carbachol-induced tone was reduced by Phe in 10 of 11 preparations tested (4 lung specimens) by only 46.5 ± 10.4% and was unaltered in the remaining preparation. In the presence of the β-adrenoceptor antagonist propranolol (0.5 μM), Phe caused weak concentration-dependent increases in tone in 3 of the 9 preparations tested. Maximal Phe-induced contractions were equivalent to 8, 17 and 29% of the respective carbachol response maxima. Thus α-adrenoceptor-mediated bronchial contraction was not considered to be of any importance when measuring the β-adrenoceptor potency of agonists such as NA and Ad which stimulate both α- and β-adrenoceptors.

The relative relaxant potencies of β-adrenoceptor agonists in isolated human bronchus are summarized in Table 1. In preparations with spontaneous tone and in carbachol-contracted bronchi, the relative potencies were Iso > Ad > NA > Phe. The highly selective β_2-agonist Fen (O'Donnell, 1970), which was only tested against carbachol-induced tone, was about 25 times more potent than the relatively β_1-selective agonist NA, but was approximately equipotent with the relatively β_2-selective agonist Ad. Iso was only 3–4 times more potent than Fen and Ad, but was about 92 times more potent than NA. Similarly, in bronchi that gained tone spontaneously, Iso

Table 1 EC_{50} values and relative relaxant potency ratios for sympathomimetic amines in human isolated bronchus

	(±)-Iso	*(−)-Ad*	*(±)-Fen*	*(−)-NA*	*(±)-Phe*
Carbachol-induced tone					
EC_{50}(μM)	0.021 ± 0.003	0.076 ± 0.012	0.082 ± 0.029	2.02 ± 0.27	9.86 ± 1.80
n	16 (10)	6 (5)	8 (4)	6 (5)	10 (4)
Ratio	91.8	26.6	24.6	1.0	0.2
Spontaneous tone					
EC_{50}(μM)	0.025 ± 0.009	0.074 ± 0.020		2.81 ± 0.82	10.04 ± 1.90
n	10 (9)	6 (6)		4 (4)	8 (3)
Ratio	112.4	38.0		1.0	0.3

EC_{50} values are given as mean ± s.e.mean and *n* indicates the number of bronchial preparations used. Numbers in parentheses indicate the number of separate lung specimens from which bronchi were obtained.

was only about 3 times more potent than Ad but was about 112 times more potent than NA (Table 1). Except for Phe, each of the relaxant amines caused complete relaxation of tone in all of the preparations tested. Phe was nearly 5 times less potent than NA in carbachol-contracted bronchi and 3.6 times less potent than NA in preparations with spontaneous tone. The absolute potencies of particular sympathomimetic amines were similar in bronchi with spontaneously developed or carbachol-induced tone.

Figure 1 shows the Schild plots for three competitive β-adrenoceptor antagonists with Iso as the agonist, in carbachol-contracted human bronchi. Propranolol (non-selective), and ICI-118,551 (β_2-selective) were both potent inhibitors of Iso-induced relaxation in human isolated bronchi. Conversely, atenolol (β_1-selective) was a weak inhibitor of Iso-induced relaxation (Table 2).

Table 2 pA_2 values for propranolol (non-selective), ICI-118,551 (β_2-selective) and atenolol (β_1-selective) against isoprenaline-induced relaxations in carbachol contracted human isolated bronchus

	pA_2	*slope*	n
Propranolol	9.3 ± 0.4	0.9 ± 0.2	14 (7)
ICI-118,551	9.1 ± 0.2	1.1 ± 0.1	22 (5)
Atenolol	5.3 ± 0.4	1.2 ± 0.4	14 (6)

pA_2 values are given as mean ± s.e.mean and *n* indicates the number of individual measurements of antagonist-induced rightward shifts in concentration-effect curves. Numbers in parentheses indicate the number of different lung specimens from which bronchi were dissected.

Discussion

The present study has clearly demonstrated the predominance of β_2-adrenoceptors in human isolated bronchial spirals dissected from non-diseased lung obtained 4–14 h post-mortem. Comparison of the potencies of several sympathomimetic amines in carbachol-contracted bronchi, showed that the β_2-selective amines Fen and Ad were about 25 times and 27 times more potent, respectively than the relatively

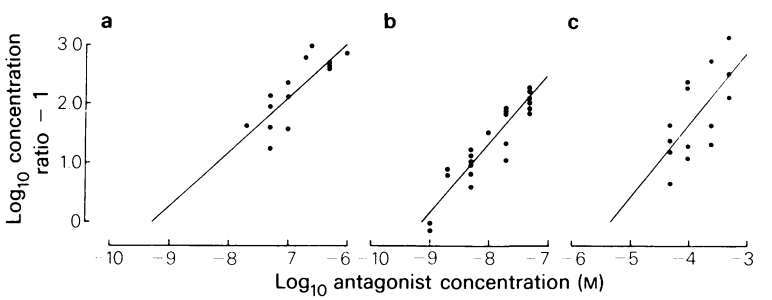

Figure 1 Schild plots for the β-adrenoceptor antagonists (a) propranolol, (b) ICI-118,551 and (c) atenolol with isoprenaline (Iso) as the agonist, in human isolated bronchus.

β_1-selective catecholamine NA. Furthermore, Fen and Ad were only 3–4 times less potent than Iso which was the most potent amine tested. In these experiments, the (−)-isomers of NA and Ad were used while the racemic mixtures of the other agonists were tested. Presumably, (−)-Iso would have been about 180 times more potent than NA. Although Fen was not tested in human bronchi that developed tone spontaneously, the potencies and relative potencies of the other sympathomimetics tested were similar to those observed in carbachol-contracted bronchi. In effect, preparations with either spontaneous or carbachol-induced tone behaved identically with respect to β-adrenoceptor-mediated relaxations. However, the levels of tone developed spontaneously varied considerably. Thus, carbachol pre-contracted bronchi were easier to use in that reproducible levels of tone could be induced within given preparations.

In accord with the findings of Harms (1976), comparison of the pA_2 values for β-adrenoceptor antagonists suggests that a relatively pure population of β_2-adrenoceptors exist in human isolated bronchus. β_2-Adrenoceptors predominate in the guinea-pig trachea, although a small population of functional β_1-adrenoceptors also exists (O'Donnell & Wanstall, 1979). The pA_2 value for ICI-118,551 (β_2-selective antagonist) in the guinea-pig trachea was 8.69, using Fen as the agonist (O'Donell & Wanstall, 1980). In the present study in human bronchus, this antagonist was even more potent. Furthermore, the β_1-selective antagonist atenolol was 6,300 times less potent than ICI-118,551 in human bronchi. In contrast, atenolol was 30–70 times more potent against Iso, in pig bronchi (Goldie *et al.*, 1983) and in guinea-pig atria (Harms, 1976; O'Donnell & Wanstall, 1979), where β_1-adrenoceptors predominate, than in human bronchi (this study).

In agreement with the findings of Mathé *et al.*, (1971) and of Simonsson *et al.*, (1972), α-adrenoceptors mediating contraction of human isolated bronchi were revealed in the present study. However, as in these other studies, contractions were only observed following blockade of bronchial β-adrenoceptors with propranolol. Even then, Phe only caused weak contractions in 3 of the 9 preparations tested. Thus it would seem that in non-diseased human isolated bronchi, α-adrenoceptors are of no functional significance. The present study indicates that human isolated bronchial preparations, obtained 4–14 h post-mortem may provide a valuable model for the assessment of the pharmacological reactivity of human central airways smooth muscle.

R.G.G. is a Senior Research Officer funded by the National Health and Medical Research Council of Australia. This work was also supported by a Post Doctoral research fellowship to J.L.W. from the University of Western Australia. The authors wish to thank Boehringer Ingelheim for their gift of fenoterol hydrobromide.

References

BRISTOW, M., SHERROD, T.R. & GREEN, R.D. (1970). Analysis of β-receptor drug interactions in isolated rabbit atrium, aorta, stomach and trachea. *J. Pharmac. exp. Ther.*, **171,** 52–61.

GOLDIE, R.G., PATERSON, J.W. & WALE, J.L. (1982). Pharmacological responses of human and porcine lung parenchyma, bronchus and pulmonary artery. *Br. J. Pharmac.*, **76,** 515–521.

GOLDIE, R.G., PATERSON, J.W. & WALE, J.L. (1983). Classification of β-adrenoceptors in porcine isolated bronchus. *Br. J. Pharmac.*, **79,** 177–180.

HARMS, H.H. (1976). Isoproterenol antagonism of cardioselective beta adrenergic receptor blocking agents: A comparative study of human and guinea-pig cardiac and bronchial beta-adrenergic receptors. *J. Pharmac. exp. Ther.*, **199,** 329–335.

LARSSON, S. & SVEDMYR, N. (1977). Bronchodilating effect and side effects of $beta_2$ adrenoceptor stimulants by different modes of administration (tablets, metered aerosol and combinations thereof). A study with salbutamol in asthmatics. *Am. Rev. Resp., Dis.*, **116,** 861–869.

LULICH, K.M., MITCHELL, H.W. & SPARROW, M.P. (1976). The cat lung strip as an *in vitro* preparation of peripherals airways: A comparison of β-adrenoceptor agonists, autacoids, and anaphylactic challenge on the lung strip and trachea. *Br. J. Pharmac.*, **58,** 71–79.

MADSEN, B.W., TANDON, M.K. & PATERSON, J.W. (1979). Cross-over study of the efficacy of four β_2-sympathomimetic bronchodilator aerosols. *Br. J. clin. Pharmac.*, **8,** 75–82.

MATHÉ, A.A., ASTRÖM, A. & PERSSON, N.-A. (1971). Some bronchoconstricting and bronchodilating responses of human isolated bronchi: Evidence for the existence of α-adrenoceptors. *J. Pharm. Pharmac.*, **23,** 905–910.

O'DONNELL, S.R. (1970). A selective β-adrenoceptor stimulant (Th 1165a) related to orciprenaline. *Eur. J. Pharmac.*, **12,** 35–43.

O'DONNELL, S.R. (1972). An examination of some β-adrenoceptor stimulants for selectivity using the isolated trachea and atria of the guinea-pig. *Eur. J. Pharmac.*, **19,** 371–379.

O'DONNELL, S.R. & WANSTALL, J.C. (1979). The importance of choice of agonist in studies designed to predict β_1:β_2 adrenoceptor selectivity of antagonists from pA_2 values on guinea-pig trachea and atria. *Naunyn-Schmiedebergs Arch. Pharmac.*, **308,** 183–190.

O'DONNELL, S.R. & WANSTALL, J.C. (1980). Evidence that ICI 118,551 is a potent, highly $beta_2$-selective ad-

renoceptor antagonist and can be used to characterize beta-adrenoceptor populations in tissues. *Life Sci.*, **27,** 671–677.

O'DONNELL, S.R. & WANSTALL, J.C. (1983). Relaxation of cat trachea by β-adrenoceptor agonists can be mediated by both β_1 and β_2-adrenoceptors and potentiated by inhibitors of extraneuronal uptake. *Br. J. Pharmac.*, **78,** 417–424.

PATERSON, J.W., WOOLCOCK, A.J. & SHENFIELD, G.M. (1979). Bronchodilator drugs. *Am. Rev. Resp. Dis.*, **120,** 1149–1188.

SIMONSSON, B.G., SVEDMYR, N., SKOOGH, B.E., ANDERSSON, R. & BERGH, N.P. (1972). *In vivo* and *in vitro* studies on alpha-receptors in human airways. Potentiation with bacterial endotoxin. *Scand. J. resp. Dis.*, **53,** 227–236.

SVEDMYR, N. (1977). Treatment with β-adrenostimulants. *Scand. J. resp. Dis., Suppl.*, **101,** 59–68.

TODA, N., HAYASHI, S., HATANO, Y., OKUNISHI, H. & MIYAZAKI, M. (1978). Selectivity and steric effects of metoprolol isomers on isolated rabbit atria, arteries and tracheal muscles. *J. Pharmac. exp. Ther.*, **207,** 311–319.

(Received July 18, 1983.
Revised October 21, 1983.)