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β-Adrenoceptor reserve in human lung: a comparison between airway smooth muscle and mast cells

Lee K. Chong, Peter T. Peachell *

Section of Molecular Pharmacology and Pharmacogenetics, University of Sheffield, The Royal Hallamshire Hospital (Floor L), Glossop Road, Sheffield S10 2JF, UK

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Abstract

The effects of several β-adrenoceptor agonists on the relaxation of precontracted human bronchial rings and the inhibition of IgE-mediated histamine release from human lung mast cells (HLMC) were studied. For the relaxation of bronchial rings, isoprenaline, fenoterol and terbutaline were full agonists whereas salbutamol was a full agonist in some (two out of six) experiments and a partial agonist in the remainder. For the inhibition of histamine release, relative to isoprenaline, neither fenoterol, terbutaline nor salbutamol was a full agonist. Studies with the irreversible β-adrenoceptor antagonist, bromoacetylalprenolol menthane, indicated that there was a larger β-adrenoceptor reserve for the relaxation of precontracted bronchial rings than for the inhibition of histamine release from HLMC. Further studies indicated that the isoprenaline inhibition of histamine release was considerably more susceptible to desensitizing treatments than the isoprenaline relaxation of bronchial rings. Collectively, these data suggest that a larger β-adrenoceptor reserve exists for the relaxation of smooth muscle than the inhibition of histamine release from HLMC and that differences in receptor reserve may contribute to the relative susceptibilities of the two systems to desensitization.

Keywords: β-Adrenoceptor; Desensitization; Mast cell; Receptor reserve

1. Introduction

Bronchodilator β_2-adrenoceptor agonists continue to be important in the therapeutic management of asthma. The primary action of this class of drug is to relax airway smooth muscle although additional therapeutically desirable effects may include the stabilization of mast cell responses (Tattersfield, 1992; Barnes, 1995).

A potential limitation with the continued use of β_2-adrenoceptor agonists in asthma therapy is the development of tolerance although it is unclear how important this may be at conventional therapeutic doses (Svedmyr, 1990). However, clinical studies indicate that tolerance to the mast cell-stabilizing properties of β_2-adrenoceptor agonists occurs more readily than tolerance to bronchodilation (O'Connor et al., 1992; Cockcroft et al., 1993) findings which are supported by in vitro studies in the guinea pig (Van der Heijden et al., 1984).

At the molecular level, tolerance may reflect receptor desensitization (Hausdorff et al., 1990). A large number of in vitro studies has indicated that exposure of β_2-adrenoceptors to agonists induces receptor desensitization (Conolly and Greenacre, 1976; Davis and Conolly, 1980; Galant et al., 1980; Avner and Jenne, 1981; Hui et al., 1982; Hasegawa and Townley, 1983; Van der Heijden et al., 1984). However, dependent on the system studied, very variable levels of desensitization are possible (Hasegawa and Townley, 1983). Our own studies (Chong et al., 1995, 1997; Drury et al., 1998) in human lung mast cells (HLMC) and those of others (Van der Heijden et al., 1984) in guinea pig mast cells indicate that desensitization may be readily induced in these systems. Although studies with human bronchial rings show that the relaxation of smooth muscle by β-adrenoceptor agonists is attenuated following pretreatment with agonists, high concentrations of agonists are necessary to demonstrate appreciable levels of desensitization (Davis and Conolly, 1980; Avner and Jenne, 1981; Hauck et al., 1997). Alternative studies with guinea pig airway smooth muscle indicate that β-adrenoceptor-mediated relaxation is relatively resistant to desensitization

* Corresponding author. Tel.: +44-114-271-2063; fax: +44-114-272-0275; E-mail: p.t.peachell@sheffield.ac.uk

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(Herepath and Broadley, 1992). One possible explanation for this relative resistance to desensitization is that a large receptor reserve exists for the relaxation of airway smooth muscle.

The aim of the present study was to determine, employing functional responses, whether a larger β-adrenoceptor reserve exists for the relaxation of airway smooth muscle than for the inhibition of histamine release.

2. Materials and methods

2.1. Buffers

Phosphate buffered saline (PBS) contained (mM): NaCl 137, $Na_2HPO_4 \cdot 12H_2O$ 8, KCl 2.7, KH_2PO_4 1.5. PBS-BSA was PBS which additionally contained: $CaCl_2 \cdot 2H_2O$ 1 mM, $MgCl_2 \cdot 6H_2O$ 1 mM, glucose 5.6 mM, bovine serum albumin (BSA) 1 mg/ml, DNase 15 μg/ml. PBS-HSA was PBS additionally supplemented with: $CaCl_2 \cdot 2H_2O$ 1 mM, $MgCl_2 \cdot 6H_2O$ 1 mM, glucose 5.6 mM, human serum albumin (HSA) 30 μg/ml. The pH of all PBS buffers was titrated to 7.3.

Krebs' buffer contained (mM): NaCl 118, $NaHCO_3$, 25, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 0.6, KH_2PO_4 1.2, glucose 11.1, $CaCl_2 \cdot 2H_2O$ 1.3.

2.2. Preparation of compounds

Salbutamol and terbutaline were prepared daily in buffer as 10^{-2} M solutions. Fenoterol was prepared daily (10^{-1} M) in dimethyl sulphoxide (DMSO). Bromacetylalprenolol menthane (BAAM) was prepared (10^{-2} M) in DMSO and stored frozen in appropriate aliquots. Isoprenaline (10^{-2} M) was dissolved in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl), an anti-oxidant, and this stock solution was made weekly and stored at 4°C.

2.3. Lung tissue

Macroscopically normal lung tissue from resections was used in this study. Most of the patients were undergoing surgery for carcinoma. The male to female split was 70% to 30% and 90% of the patients were white caucasians.

2.4. Preparation of bronchial rings

Bronchi (≤ 3 mm diameter) were dissected free from parenchymal tissue and bronchial rings prepared. The rings were mounted under a resting tension of 1 g in 10 ml organ baths attached to force transducers for isometric tension. The rings were allowed to equilibrate in aspirated (O_2 95%/CO_2 5%) Kreb's buffer with several washes over 40 min before challenge with anti-IgE (1:1000) in order to induce contraction. Preliminary experiments indicated that this concentration of anti-IgE induces between 70% and 95% of the maximal contractile response obtained with an optimal concentration (1:300) of anti-IgE. After contraction had plateaued (15–20 min), isoprenaline or alternative agonists were added cumulatively to the bath. In experiments with BAAM, following the 40 min equilbration period, rings were incubated for 25 min with the antagonist and then the rings were washed six times over 40 min before challenge with anti-IgE. In desensitization experiments, rings were incubated (24 h) with or without isoprenaline (1 μM) in RPMI 1640 buffer supplemented with penicillin/streptomycin (10 μg/ml) and gentamicin (50 μg/ml). For the first 23 h of the incubation, rings were placed in a CO_2 incubator and for the final hour of the incubation, the rings were mounted in the organ baths in the original RPMI buffer used during the 23 h in the CO_2 incubator. Following this period, the rings were washed three times in Kreb's buffer over 20 min before challenge with anti-IgE (1:1000). Relaxations were expressed relative to the maximal response observed with 10 μM isoprenaline. Aminophylline (1 mM) was added to all rings at the end of each experiment. In all experiments, the addition of aminophylline did not cause any increases in relaxation of the rings over that seen with 10 μM isoprenaline.

2.5. Isolation of HLMC

Mast cells were isolated from human lung tissue by a modification of the method described by Ali and Pearce (1985). Lung tissue was stripped of its pleura and chopped vigorously for 15 min with scissors in a small volume of PBS buffer. The chopped tissue was washed over a nylon mesh (100 μm pore size; Cadisch and Sons, London, UK) with 0.5–1 l of PBS buffer to remove lung macrophages. The tissue was reconstituted in PBS-BSA (10 ml per gram of tissue) containing collagenase Ia (350 Units per milliliter of PBS-BSA) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37°C. The supernatant (containing some HLMC) was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of PBS-BSA buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with PBS-BSA (300–600 ml). The pooled filtrates were sedimented ($120 \times g$, RT, 8 min), the supernatant discarded and the pellets reconstituted in PBS-BSA (100 ml). The pellet was washed a further two times. On occasion, the mast cell isolation procedure described above was performed in the absence of enzymes (collagenase and DNase). HLMC were visualized by microscopy using an alcian blue stain (Gilbert and Ornstein, 1975). Of the total cells, 3–13% were mast cells. Enzymatic and physical disruption of lung tissue generated $1–6 \times 10^5$ HLMC per gram of tissue whereas physical disruption alone generated $0.1–0.9 \times 10^5$ HLMC per gram of tissue. HLMC prepared

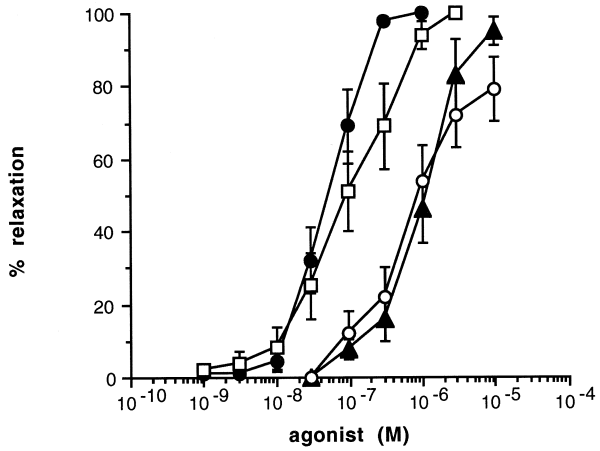

Fig. 1. Relaxation of airway smooth muscle. Human bronchial rings were precontracted with anti-IgE (1:1000) and once the contraction had plateaued, the effects of cumulative additions of β-adrenoceptor agonists on the contraction determined. The effects of isoprenaline (closed circles), fenoterol (open squares), terbutaline (closed triangles) and salbutamol (open circles) on the contraction were assessed. The control contraction was 1.2 ± 0.2 g. Results are expressed as a percentage of the maximal relaxation observed with 10 μM isoprenaline. Values are means ± S.E.M, $n = 6$.

Table 1
E_{max} and pD_2 values for β-adrenoceptor agonists for the inhibition of histamine release and the relaxation of contraction
E_{max} values are %relaxation (bronchial rings) or %inhibition (HLMC). Data have been generated from Figs. 1 and 2 and further details may be found in the legends to those figures.

Agonist	Bronchial rings		HLMC	
	E_{max}	pD_2	E_{max}	pD_2
Isoprenaline	102 ± 2	7.3 ± 0.1	61 ± 8	8.3 ± 0.3
Fenoterol	109 ± 5	6.9 ± 0.1	53 ± 12	7.3 ± 0.6
Salbutamol	86 ± 4	6.2 ± 0.1	38 ± 1	7.0 ± 0.1
Terbutaline	110 ± 8	5.9 ± 0.1	47 ± 1	6.4 ± 0.7

by these methods were used in mediator release experiments.

2.6. Mediator release from HLMC

Histamine release from HLMC was initiated immunologically with anti-IgE (1:1000). We have previously reported that this concentration of anti-IgE induces between 63% and 95% of the maximal response obtained with an optimal releasing concentration of anti-IgE (Chong et al., 1997). Secretion was allowed to proceed for 25 min at 37°C after which time the cells were pelleted by centrifugation ($400 \times g$, RT, 3 min). Histamine released into the supernatant was determined by a modification (Ennis, 1991) of the automated fluorometric method of Siraganian (1974). When isoprenaline or other agonists were employed, cells were incubated with agonist for 10 min at 37°C before the addition of stimulus and then samples were processed as indicated above. Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in buffer alone served as a measure of spontaneous histamine release ($< 6\%$). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release.

In experiments with BAAM, cells (0.5×10^6 HLMC in 5 ml) were incubated with the antagonist for 25 min. After this time, the cells were washed in a large volume (50 ml) of PBS-HSA followed by centrifugation ($120 \times g$, RT, 10 min). The pellet was then resuspended in PBS-HSA and allowed to stand for 5 min before centrifugation. This step was then repeated before the cells were used in histamine release experiments.

In experiments in which long-term (24 h) incubations were performed, RPMI 1640 buffer supplemented with penicillin/streptomycin (10 μg/ml) and gentamicin (50 μg/ml) was employed. Cells were incubated at a density of 0.1×10^6 HLMC per milliliter in 12 well plates with, usually, 0.5×10^6 HLMC per condition with or without isoprenaline (1 μM). After completion of the incubations, the cells were washed three times with PBS-HSA before use in mediator release experiments. Incubations of HLMC with isoprenaline had no effect on either the total number of HLMC recovered, the total histamine content or the spontaneous histamine release compared to HLMC incubated in buffer. The spontaneous histamine release did not change with time with values of 5 ± 1 and $6 \pm 1\%$ at times 0 and 24 h, respectively. The percentage recovery of HLMC following a 24 h incubation was $94 \pm 4\%$.

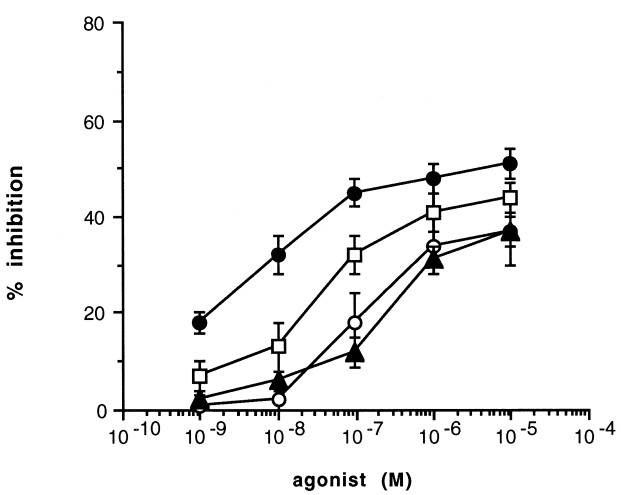

Fig. 2. Inhibition of histamine release. HLMC were incubated for 10 min with or without a β-adrenoceptor agonist before challenge with anti-IgE (1:1000) for a further 25 min. The effects of isoprenaline (closed circles), fenoterol (open squares), terbutaline (closed triangles) and salbutamol (open circles) on histamine release were assessed. Results are expressed as the percent inhibition of the control histamine release which was $17 \pm 1\%$. Values are means ± S.E.M, $n = 6$.

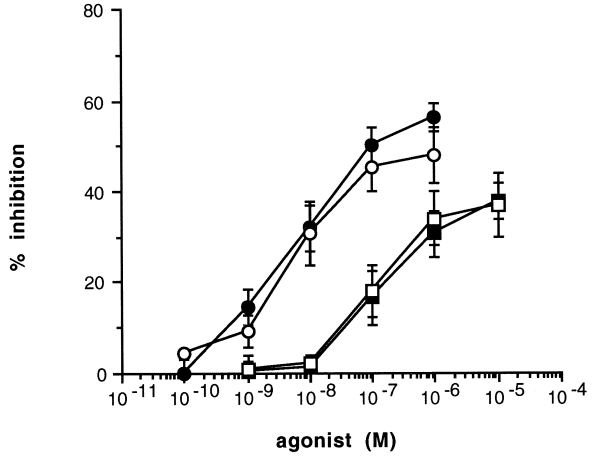

Fig. 3. Effects of β-adrenoceptor agonists on either enzymatically or physically dispersed mast cells. Lung tissue was dispersed in parallel either enzymatically (open symbols) or physically (closed symbols) and HLMC derived from either of these treatments were incubated with either isoprenaline (circles) or salbutamol (squares) for 10 min before challenge with anti-IgE (1:1000) for a further 25 min. Results are expressed as the percent inhibition of the control histamine releases which were 21 ± 3% and 26 ± 5% for physically and enzymatically dispersed HLMC, respectively. Values are means ± S.E.M., $n = 5$.

2.7. Calculations of receptor occupancy

The fraction of receptors unoccupied, q, following treatments with BAAM was calculated according to methods described by Furchgott (1966). The reciprocal of the concentration of agonist against the reciprocal of the concentration of agonist required to elicit an identical response following BAAM treatment was plotted from which q (slope = $1/q$) was calculated.

Table 2
Effect of BAAM on E_{max} and pD_2 values for isoprenaline
E_{max} values are %relaxation (bronchial rings) or %inhibition (HLMC). Data have been generated from Fig. 4 and further details may be found in the legend to that figure.

BAAM (nM)	Bronchial rings		HLMC	
	E_{max}	pD_2	E_{max}	pD_2
–	110 ± 7	7.6 ± 0.1	72 ± 2	8.7 ± 0.1
1	104 ± 4	7.9 ± 0.1	65 ± 2	8.0 ± 0.2
10	107 ± 3	7.4 ± 0.1	47 ± 5	7.6 ± 0.2
100	108 ± 4	6.8 ± 0.1	43 ± 2	6.8 ± 0.1

2.8. Materials

The following were purchased from the sources indicated: aminophylline, anti-human IgE, BSA, collagenase, DNase, DMSO, fenoterol, HSA, (−)isoprenaline, salbutamol, terbutaline (all Sigma, Poole, UK); RPMI 1640, gentamicin, penicillin/streptomycin (Gibco BRL, Dundee UK); BAAM (Research Biochemicals, Natick, MA, USA).

2.9. Statistics

Maximal responses (E_{max}) and potencies (EC_{50}) were calculated by a non-linear regression technique using SPSS (version 6.0). The statistical significance of drug-related effects was analyzed by comparing control and treated

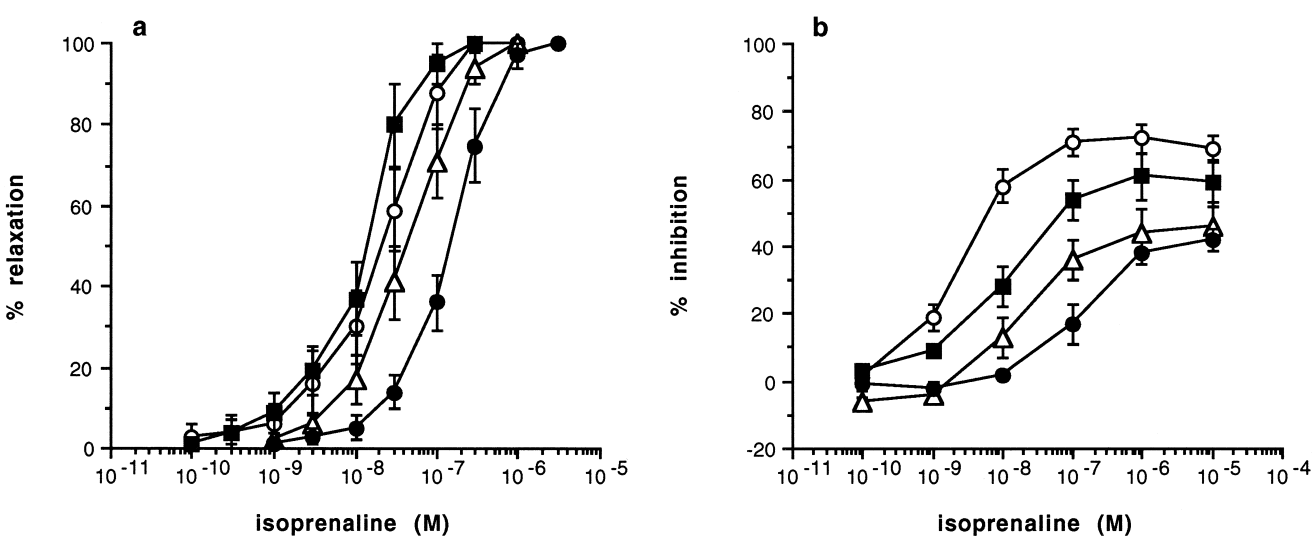

Fig. 4. Effects of BAAM on isoprenaline-induced relaxation of smooth muscle (a) and inhibition of histamine release (b). Bronchial rings and HLMC derived from the same lung were incubated without (open circles) or with 1 (closed squares), 10 (open triangles) or 100 (closed circles) nM BAAM for 25 min followed by extensive washings. The effects of these BAAM treatments on (a) the isoprenaline relaxation of smooth muscle and (b) the isoprenaline inhibition of histamine release were determined. (a) Results are expressed as a percentage of the maximal relaxation observed with 10 μM isoprenaline. The control contractions were 1.6 ± 0.3 g (control), 1.5 ± 0.2 g (1 nM BAAM), 1.2 ± 0.1 g (10 nM BAAM) and 1.4 ± 0.3 g (100 nM BAAM). (b) Results are expressed as the percent inhibition of the control histamine releases which were 29 ± 5% (control), 28 ± 5% (1 nM BAAM), 29 ± 5% (10 nM BAAM) and 25 ± 3% (100 nM BAAM). Statistically significant ($P < 0.05$) shifts in the control dose–response curve were observed at all concentrations of BAAM in HLMC and following treatment of bronchial rings with 100 nM BAAM. Values are means ± S.E.M., $n = 4$.

Table 3
Fraction of adrenoceptors unoccupied (q) following treatment with BAAM
The fraction of receptors unoccupied, q, following treatment of either HLMC or airway smooth muscle with a given concentration of BAAM was calculated according to the method of Furchgott (1966) utilising data from Fig. 4. Further details may be found in the legend to that figure.

BAAM (nM)	q	
	HLMC	Smooth muscle
1	0.20	–
10	0.07	0.53
100	0.01	0.11

cells using repeated measures two-way ANOVA with respect to concentration and treatments. Long-term treatment with isoprenaline or treatments with BAAM had no effect on the levels of control histamine release from HLMC or contractions of bronchial rings. In all experiments, the transformed data (i.e., %inhibition or relaxation values) were subjected to statistical analyses. Values were considered significant at the $P < 0.05$ level.

3. Results

The effects of isoprenaline, fenoterol, terbutaline and salbutamol on relaxation of bronchial rings precontracted with anti-IgE (1:1000) were investigated (Fig. 1). All four agonists relaxed precontracted tissue in a dose-dependent manner and with the following rank order of potency, isoprenaline > fenoterol > salbutamol > terbutaline (see Table 1 for pD_2 values). Isoprenaline, fenoterol and terbutaline were full agonists relative to isoprenaline whereas salbutamol was a full agonist in two, and a partial agonist in four out of six experiments (see Table 1 for E_{max} values).

The same agonists were studied for effects on the inhibition of IgE-mediated histamine release from HLMC (Fig. 2). All four agonists inhibited histamine release in a dose-dependent manner and with the following rank order of potency, isoprenaline > fenoterol > salbutamol > terbutaline (see Table 1 for pD_2 values). Relative to isoprenaline, neither fenoterol, terbutaline nor salbutamol was a full agonist (see Table 1 for E_{max} values).

Isolation of mast cells from human lung involves enzymatic dispersion of the tissue and the possibility existed that the enzymes (collagenase and DNase) may have affected β-adrenoceptor-mediated responses in HLMC. To test this, mast cells were isolated from lung tissue that had been either physically disrupted or physically and enzymatically dispersed and the effects of isoprenaline (and salbutamol) on histamine release determined (Fig. 3). Mast cells generated by either treatment responded equieffectively to isoprenaline (and salbutamol).

The effects of the irreversible β-adrenoceptor antagonist, BAAM, on the relaxation of smooth muscle precontracted with anti-IgE was assessed (Fig. 4a). Bronchial rings were incubated (25 min) either in buffer or with BAAM (1–100 nM) followed by extensive washing. Rings were then challenged with anti-IgE (1:1000) and the effectiveness of isoprenaline to relax the rings was determined. A low (1 nM) concentration of BAAM caused a modest leftward shift in the dose–response curve for the relaxation by isoprenaline of the contraction whereas higher (10 and

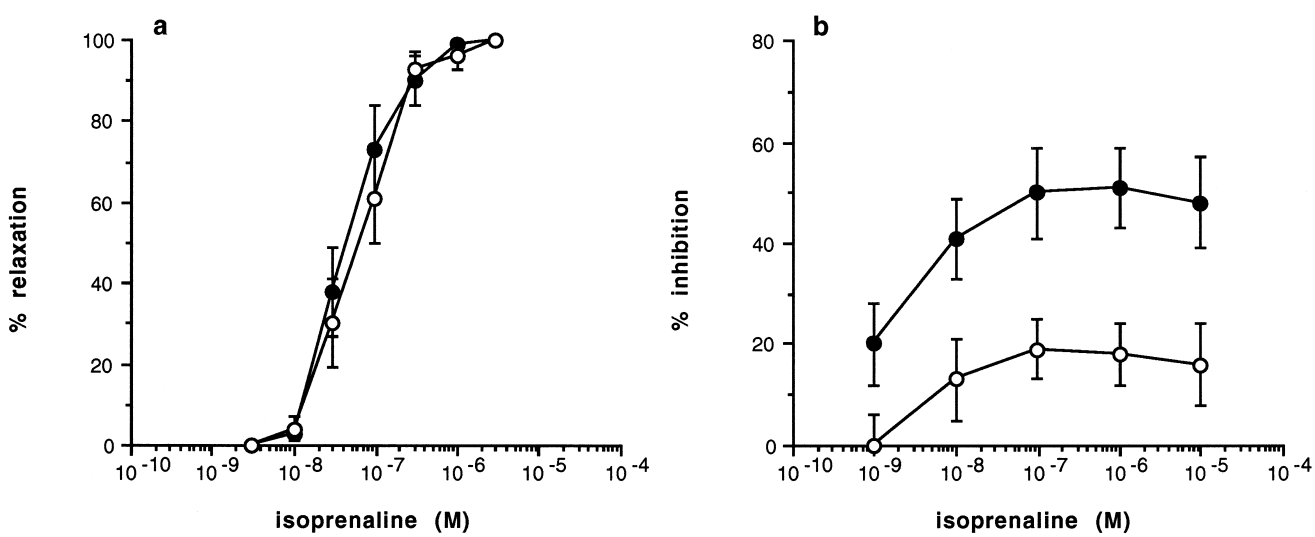

Fig. 5. Susceptibility of β-adrenoceptor-mediated responses to desensitization. Bronchial rings and HLMC derived from the same lung were incubated either with (desensitizing treatment; open circles) or without (control; closed circles) isoprenaline (1 μM) for 24 h. After this time the rings and cells were washed and the subsequent effectiveness of isoprenaline (a) to relax contracted rings or (b) to inhibit histamine release determined. (a) Results are expressed as a percentage of the maximal relaxation observed with 10 μM isoprenaline. The control contractions were 0.9 ± 0.1 g (control) and 1.0 ± 0.1 g (desensitizing treatment). (b) Results are expressed as the percent inhibition of the control histamine releases which were 39 ± 6% (control) and 31 ± 6% (desensitizing treatment). The desensitizing treatment caused a statistically significant ($P < 0.05$) reduction in the effectiveness of isoprenaline to inhibit histamine release but had no significant effect ($P > 0.05$) on the isoprenaline relaxation of precontracted rings. Values are means ± S.E.M., $n = 5$.

100 nM) concentrations of BAAM caused concentration-dependent rightward shifts in the dose–response curve (see Table 2 for pD_2 values). Maximal relaxations were unaffected by increasing concentrations of BAAM (see Table 2 for E_{max} values).

The effects of BAAM on the isoprenaline inhibition of histamine release from HLMC were determined in parallel (Fig. 4b). Concentrations of BAAM of 1, 10 and 100 nM caused concentration-dependent rightward shifts for the isoprenaline inhibiton of histamine release (see Table 2 for pD_2 values). Moreover, there was a concentration-dependent reduction in the maximal inhibitory response obtained with isoprenaline with increasing concentrations of BAAM (see Table 2 for E_{max} values). Employing the method of Furchgott (1966), the proportions of receptors unoccupied following BAAM treatments in both systems were calculated (Table 3).

In experiments to establish the susceptibility of smooth muscle β-adrenoceptors to desensitization, bronchial rings were incubated for 24 h with or without isoprenaline (1 μM) followed by extensive washing and then challenged with anti-IgE (1:1000) to contract the tissue. The relaxant effects of isoprenaline were determined following these treatments (Fig. 5a). Desensitizing conditions caused a very slight rightward shift in the dose–response curve for isoprenaline although this effect was not statistically significant ($P > 0.05$). In contrast, desensitizing conditions (24 h with 1 μM isoprenaline) attenuated the isoprenaline inhibition of histamine release from HLMC to a statistically significant ($P < 0.05$) degree (Fig. 5b).

4. Discussion

In the therapeutic management of asthma, bronchodilator β-adrenoceptor agonists act primarily to relax airway smooth muscle although effects on inflammatory cells such as the mast cell may also be important (Tattersfield, 1992; Barnes, 1995). In the present study, we have compared the responses of smooth muscle and mast cells to a variety of β-adrenoceptor agonists. In both systems, the rank order of potency of isoprenaline > fenoterol > salbutamol > terbutaline held for both the relaxation of smooth muscle and the inhibition of histamine release from HLMC. However, whereas fenoterol, terbutaline and, occasionally, salbutamol were full agonists when compared to isoprenaline for the relaxation of smooth muscle, neither fenoterol, terbutaline nor salbutamol was a full agonist for the inhibition of histamine release. These findings suggest that there is a larger receptor reserve available for the relaxation of smooth muscle as compared with the inhibition of histamine release. This contention stems from the widely-recognized observation that partial agonists in some systems may act as full agonists in alternative systems if a larger receptor reserve exists (Kenakin, 1984; Hoyer and Boddeke, 1993; MacEwan et al., 1995).

A concern existed that our methods of cell isolation, involving the use of enzymes to disperse lung tissue, may have in some manner modified β-adrenoceptor-mediated responses in mast cells. However, comparison of the responses of mast cells that had been isolated by either physically or physically and enzymatically disrupting lung tissue indicated that mast cells generated by either method responded equieffectively to agonists suggesting that enzymatic dispersion of lung tissue does not affect β-adrenoceptor-mediated responses in mast cells.

The irreversible β-adrenoceptor antagonist, BAAM, has been employed quite widely in order to establish whether a β-adrenoceptor reserve exists in a given system (Mahan and Insel, 1986; Minneman and Mowry, 1986; Undem et al., 1988; MacEwan et al., 1995). In the present study, for a given concentration of BAAM, there was a considerably larger relative shift in the dose–response curve for the isoprenaline inhibition of histamine release than the relaxation of smooth muscle. Because the extent of inactivation of adrenoceptors by BAAM is both concentration and time-dependent, it is not surprising that, for a fixed exposure time at a given concentration of BAAM, effects were less pronounced in intact tissue than in isolated cells as diffusional differences of the drug to receptor sites in the two systems would be anticipated. Interestingly, when a similar proportion of receptors were inactivated in either system, the maximal response for smooth muscle relaxation was unaffected whereas that for the inhibition of histamine release was depressed. These data suggest that a larger β-adrenoceptor reserve exists for the relaxation of smooth muscle than the inhibition of histamine release. The factors that are responsible for this difference in receptor reserve are unknown although differences in receptor density and the efficiency of receptor-effector coupling mechanisms could contribute to the variable receptor reserves observed in the two systems (Kenakin, 1984).

Previous studies of our own have demonstrated that receptor reserve influences desensitization (Drury et al., 1998). Because airway smooth muscle would appear to possess a larger β-adrenoceptor reserve compared to mast cells, it might be predicted that the relaxation of smooth muscle by isoprenaline would be relatively resistant to desensitization compared to the isoprenaline inhibition of histamine release from mast cells. Support for this prediction was obtained in the present study because conditions which were found to cause extensive desensitization of the isoprenaline inhibition of mast cell responses were relatively ineffective at inducing desensitization to the isoprenaline relaxation of smooth muscle. Similar studies in the guinea pig have shown that β-adrenoceptor-mediated responses of bronchial mast cells are more susceptible to desensitization than β-adrenoceptor-mediated relaxation of smooth muscle (Van der Heijden et al., 1984). These studies would appear to support the findings of several clinical studies which suggest that tolerance to the mast cell-stabilizing properties of β-adrenoceptor agonists oc-

curs without affecting the bronchodilator properties of these drugs (O'Connor et al., 1992; Cockcroft et al., 1993).

The present study has involved a comparative assessment of β-adrenoceptor reserve in smooth muscle and mast cells. However, it should be noted that comparisons have been made between two very different biological responses, relaxation and secretion, and in which completely different assay conditions have been employed. Moreover, comparisons have been made between a tissue system (bronchial rings) and an isolated cell system (HLMC) in which differences in a number of factors, such as drug access, may exist. These considerations indicate that it would have been desirable to have performed the mediator release component of the study on mast cells in intact bronchial tissue. However, this would have required very large quantities of bronchial tissue which would have been available only on a very infrequent basis.

In summary, the present study has shown that full agonists in smooth muscle act as partial agonists in mast cells and that inactivation of a similar proportion of receptors in either system reduces the maximal response to isoprenaline for the inhibition of histamine release but not the relaxation of smooth muscle. Collectively, these data suggest that a larger β-adrenoceptor reserve exists for the relaxation of smooth muscle than for the inhibition of histamine secretion. Additionally, our data suggest that differences in receptor reserve could influence the relative susceptibilities of these two systems to desensitization. However, it should be stressed that desensitization is a complex process that may be influenced by a variety of factors (Hausdorff et al., 1990). For example, the suggestion has been made that the high levels of β-adrenoceptor mRNA that have been detected in airway smooth muscle provide the potential for rapid rates of β-adrenoceptor synthesis which, in turn, could lead to a relative resistance to desensitization (Hamid et al., 1991). Moreover, a recent study has shown that the expression of mRNA for G-protein receptor kinases (GRKs) is higher in a mast cell line (HMC-1) than in airway smooth muscle cells (McGraw and Liggett, 1997). Because GRKs are thought to induce desensitization by phosphorylating the receptor, differences in the complement of GRKs between airway smooth muscle and HLMC could serve as an alternative explanation for the relative susceptibilities of the two systems to desensitization.

To conclude, the present study has shown that a larger β-adrenoceptor reserve exists for the relaxation of smooth muscle than for the inhibition of mast cell secretion. This difference in receptor reserve may influence the sensitivity of the two systems to β-adrenoceptor agonists and the susceptibility of the two systems to desensitization.

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