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Influence of receptor reserve on β-adrenoceptor-mediated responses in human lung mast cells

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1 The effects of the β-adrenoceptor agonists isoprenaline and salbutamol on IgE-mediated histamine release from human lung mast cells (HLMC) were evaluated. Both agonists (10^{-10}–10^{-5} M) inhibited histamine release in a dose-dependent manner and isoprenaline (pD_2, 8.3±0.1, mean±s.e.mean) was more potent than salbutamol (7.3±0.1). Moreover, the mean data indicated that salbutamol was a partial agonist when compared with isoprenaline. However, there was a large degree of inter-experimental variability because, in 11 of 32 experiments, salbutamol was a full agonist and, in 21 of 32 experiments, a partial agonist relative to isoprenaline. These data suggest that different HLMC preparations possess variable receptor reserves.

2 The effect of the irreversible β-adrenoceptor antagonist, bromoacetylalprenolol menthane (BAAM), on the inhibition of IgE-mediated histamine release by both isoprenaline and prostaglandin E_2 (PGE_2) was assessed. Whereas BAAM (100 nM) antagonized the isoprenaline inhibition of histamine release from activated HLMC, BAAM had no effect on the PGE_2 inhibition. Pretreatment of HLMC with the β_2-selective competitive antagonist, ICI 118551 (100 nM), protected against the loss in responsiveness to isoprenaline following treatment with BAAM.

3 Concentrations of 1, 10 and 100 nM of BAAM caused dose-dependent rightward shifts in the dose-response curve for the isoprenaline inhibition of histamine release. Furthermore, there was a dose-dependent reduction in the maximal inhibitory response obtained with isoprenaline following treatments with increasing concentrations of BAAM. Although the rightward shifts in the isoprenaline dose-response curves, with a given concentration of BAAM, were similar in all experiments, there was some variability in the depression of the maximal response in individual experiments. Thus, in 6 of 16 experiments, BAAM (1 nM) did not depress the maximal response to isoprenaline, whereas in 10 of 16 experiments there was a depression (7 to 49% reduction) in the maximal response. These data suggest that different HLMC preparations possess variable receptor reserves.

4 Isoprenaline was more potent as an inhibitor in those HLMC preparations in which there was a larger receptor reserve (i.e. preparations in which the maximal inhibitory response to isoprenaline was unaffected by pretreatment with 1 nM BAAM).

5 The influence of receptor reserve on the inhibition by salbutamol of histamine release from HLMC was evaluated. There was a good correlation ($r=0.77$) between receptor reserve and the maximal response (relative to isoprenaline) obtained with salbutamol. Thus, HLMC preparations with larger receptor reserves were more responsive to salbutamol.

6 Receptor reserve influenced the desensitization of β-adrenoceptor-mediated responses in HLMC. Cells were incubated (24 h) with isoprenaline (1 μM), washed and then the ability of a second isoprenaline (10^{-10}–10^{-5} M) exposure to inhibit histamine release was assessed. The pretreatment caused a reduction in the isoprenaline inhibition of histamine release although the extent of desensitization was highly variable, ranging from essentially negligible levels in some preparations to substantial reductions (93% desensitization) in the ability of isoprenaline to inhibit histamine release. There was a reasonable correlation ($r=0.59$) between receptor reserve and desensitization. Preparations that possessed a larger receptor reserve were more resistant to desensitization.

7 Collectively, these data suggest that a receptor reserve exists for the β-adrenoceptor-mediated inhibition of histamine release from HLMC but that the size of this reserve varies between HLMC preparations. Moreover, the size of this receptor reserve may influence the sensitivity of HLMC to β-adrenoceptor agonists and the susceptibility of individual HLMC preparations to desensitization.

Keywords: β-adrenoceptors; desensitization; mast cells; receptor reserve

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 airways smooth muscle (Tattersfield, 1992; Barnes, 1995). However, it is possible that β_2-adrenoceptor agonists may also act in asthma to stabilize mast cells and thereby prevent the generation of mediators that could cause bronchospasm and inflammation. Certainly, a large number of *in vitro* studies has shown that isoprenaline and alternative β_2-adrenoceptor agonists are effective inhibitors of the stimulated release of mediators from human lung mast cells (HLMC) (Assem & Schild, 1969; Orange *et al*., 1971; Butchers *et al*., 1980; Church & Hiroi, 1987; Peachell *et al*., 1988; Undem *et al*., 1988).

Previous studies have shown that the response of HLMC to isoprenaline is highly variable (Undem *et al*., 1988; Chong *et al*., 1997). Moreover, the susceptibility to desensitization of β_2-adrenoceptor-mediated response in HLMC is also very

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variable with some HLMC preparations totally resistant to desensitization and others highly sensitive (Chong *et al*., 1995; 1997). Although the reasons for this large inter-preparation variability in responses to isoprenaline and sensitivity to desensitization have not been established, one possible explanation may be that different HLMC preparations possess variable receptor reserves.

The concept of receptor reserve has been appreciated for some time and describes the situation in which only a proportion of the receptors needs to be occupied for a full agonist to induce a maximal response (Kenakin, 1984). Experimental strategies that have been employed to study receptor reserve have included an evaluation of the responses to agonists following inactivation of a proportion of the receptors by treatment with an irreversible antagonist (Mahan & Insel, 1986; Minneman & Mowry, 1986; Undem *et al*., 1988; Hoyer & Boddeke, 1993; MacEwan *et al*., 1995). The use of partial agonists is another potentially useful approach to study receptor reserve because a partial agonist could convert into a full agonist if a larger receptor reserve exists (Hoyer & Boddeke, 1993; MacEwan *et al*., 1995). By employing approaches of this type, we provide evidence that receptor reserve may influence the variability in responses of HLMC to β-adrenoceptor agonists and the susceptibility of β-adrenoceptor-mediated responses to desensitization.

Methods

Isolation of HLMC

Mast cells were isolated from human lung tissue by a modification of the method described by Ali and Pearce (1985). Macroscopically normal tissue from lung resections of patients with carcinoma 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 U.K.) with 0.5–1 l of −PBS buffer to remove lung macrophages. The tissue was reconstituted in PBS (10 ml g^{-1} of tissue) containing collagenase la (350 u ml^{-1} PBS) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37°C for 90 min. 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 buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with PBS (300–600 ml). The pooled filtrates were sedimented ($120 \times g$, RT, 8 min), the supernatant discarded and the pellets reconstituted in PBS (100 ml). The pellet was washed a further two times. HLMC were visualized by microscopy using an alcian blue stain (Gilbert & Ornstein, 1975). Of the total cells, 3–13% were mast cells and disruption of lung tissue generated 2 to 9×10^5 HLMC g^{-1} tissue.

Mediator release from HLMC

Histamine release experiments were performed in +PBS buffer. Histamine release was initiated immunologically with anti-IgE (1:1000). 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 inhibitors were employed, cells were incubated with an inhibitor for 10 min at 37°C before the addition of stimulus and then samples were processed as indicated above. In studies with ICI 118551, cells were incubated with the antagonist for 5 min and then together with isoprenaline for a further 10 min before challenge with anti-IgE. 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. When ICI 118551 was used, cells were pretreated for 10 min with the reversible antagonist before the addition of BAAM for 25 min. After this time, the cells were washed in a large volume (50 ml) of +PBS followed by centrifugation ($120 \times g$, RT, 10 min). The pellet was then resuspended in 50 ml of +PBS and allowed to stand for 5 min before centrifugation. This step was then repeated before the cells were resuspended in +PBS for histamine release experiments.

In experiments in which long-term (24 h) incubations were performed, RPMI 1640 buffer supplemented with penicillin/streptomycin (10 μg ml^{-1}) and gentamicin (50 μg ml^{-1}) was used. Cells were incubated at a density of 0.1×10^6 HLMC ml^{-1} 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 and reconstituted in +PBS for 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±1 at times 0 and 24 h, respectively. The percentage recovery of HLMC following 24 h incubations was 94±4% (mean±s.e.mean).

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) and the agonist dissociation constant, K_D (K_D = slope-1/intercept), were calculated.

Buffers

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

Preparation of inhibitors

Salbutamol was prepared daily in buffer as a 10^{-2} M solution. ICI 118551 was prepared as a 10^{-2} M solution in distilled water and stored at 4°C. BAAM was prepared (10^{-2} M) in dimethyl sulphoxide (DMSO) and stored frozen in appropriate aliquots. Prostaglandin E_2 (PGE_2) was prepared as a 10^{-1} M

solution in ethanol and stored at −20°C. Isoprenaline (10^{-2} M) was dissolved in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl) and this stock solution was made weekly and stored at 4°C.

Materials

The following were purchased from the sources indicated; anti-human IgE, BSA, collagenase, DNase, DMSO, HSA, (−)-isoprenaline, prostaglandin E_2 (PGE_2), salbutamol, (all Sigma, Poole, U.K.); RPMI 1640, gentamicin, penicillin/streptomycin (Gibco BRL, Dundee U.K.); BAAM (bromoacetylalprenolol menthane; Research Biochemicals Inc., Natick, MA, U.S.A.); ICI 118551 (erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol; Cambridge Research Biochemicals, Northwich, U.K.).

Statistics

The statistical significance of drug-related effects was analysed by comparing control and treated cells using two-way ANOVA with Bonferroni correction (Bland & Altman, 1995) with respect to concentration and treatments. Maximal responses (E_{max}) and potencies (EC_{50}) were calculated by a non-linear regression technique using SPSS (version 6.0). In order to compare changes in pD_2 values and changes in maximal responses following treatments, *t* tests were performed with Bonferroni correction when there were multiple comparisons. Correlation coefficients were determined by use of linear regression analyses. Values were considered significant at the $P<0.05$ level.

Results

The effects of isoprenaline and salbutamol on the release of histamine from HLMC challenged with anti-IgE (1 : 1000) were determined (Figure 1). Both agonists inhibited histamine release in a dose-dependent manner and isoprenaline (pD_2; 8.3 ± 0.1, mean ± s.e.mean) was more potent than salbutamol (7.3 ± 0.1). The mean data show that salbutamol, relative to isoprenaline, was a partial agonist. However, inspection of the individual data for each of the experiments reveals that salbutamol, when compared with isoprenaline, was a full agonist in some experiments (11 out of 32 experiments) and a partial agonist (33 to 91% of the maximal response obtained with isoprenaline) in the remainder. The wide spectrum of responses to salbutamol suggests that different HLMC preparations possess variable receptor reserves.

The irreversible antagonist, BAAM, has been used widely to investigate receptor reserve and, in one study, the effects of BAAM in HLMC has been studied (Undem *et al*., 1988). In the present study, we have attempted to establish the selectivity of action of BAAM. HLMC were incubated with or without BAAM (100 nM) for 25 min, washed and then incubated for

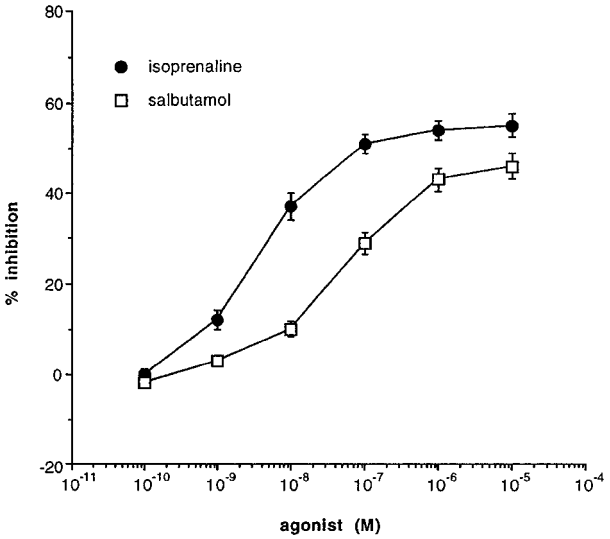

Figure 1 Effect of β-adrenoceptor agonists on histamine release from HLMC. HLMC were incubated with either isoprenaline or salbutamol for 10 min before challenge with anti-IgE (1 : 1000) for a further 25 min. Results are expressed as the % inhibition of the control histamine release which was 28 ± 2%. Values are means and vertical lines show s.e.mean, $n=32$.

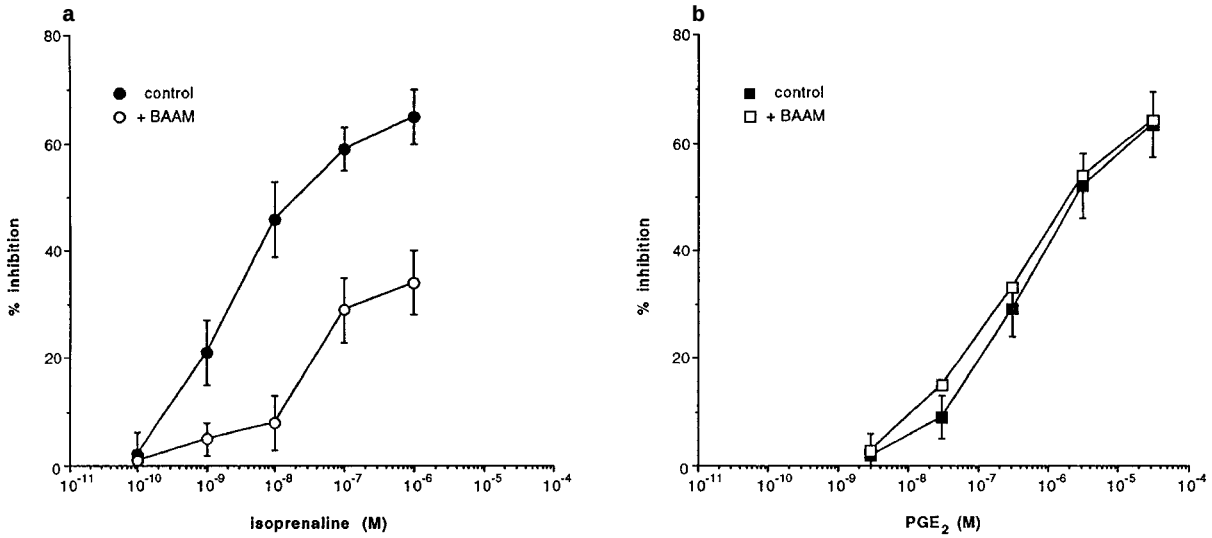

Figure 2 Effect of BAAM on the isoprenaline and PGE_2-mediated inhibition of histamine release. HLMC were incubated with or without BAAM (100 nM) for 25 min after which the cells were washed extensively and then incubated for 10 min with either isoprenaline (a) or PGE_2 (b) before challenge with anti-IgE (1 : 1000) for 25 min. Results are expressed as the % inhibition of the control histamine releases which were 24 ± 5 and 25 ± 5% in the absence and presence of BAAM, respectively. Values are means and vertical lines show s.e.mean, $n=5$.

10 min with increasing concentrations of either isopranaline ($10^{-10}-10^{-5}$ M) or an alternative receptor-mediated activator of adenylate cyclase, PGE_2 ($3\times10^{-9}-3\times10^{-5}$ M), before challenge with anti-IgE. The data indicate that the isoprenaline inhibition of histamine release was antagonized by pretreatment with BAAM (Figure 2a). There was a rightward shift in the isoprenaline dose-response curve following BAAM treatment with pD_2 values of 8.4 ± 0.2 and 7.6 ± 0.2 in the absence and presence, respectively, of BAAM. This difference in pD_2 values was statistically significant ($P<0.005$). Moreover, there was a statistically significant ($P<0.0005$) reduction (mean ± s.e.mean, $49\pm8\%$ reduction; range 30 to 77%) in the maximal response observed with isoprenaline following BAAM treatment. In contrast, BAAM treatment had no significant ($P>0.05$) effect on either the potency (pD_2 values of 6.5 ± 0.2 and 6.6 ± 0.1 in the absence and presence, respectively, of BAAM) or efficacy of PGE_2 (Figure 2b).

Experiments were performed with the competitive β_2-selective antagonist, ICI 118551, to establish whether pretreatment with ICI 118551 would protect against a loss in responsiveness to isoprenaline following treatment with BAAM. Preliminary studies indicated that ICI 118551 (100 nM) caused a 100 fold rightward shift in the isoprenaline dose-response curve for the inhibition of histamine release (data not shown, $n=5$). In subsequent experiments, HLMC were incubated for 10 min with or without ICI 118551 (100 nM) and then together with or without BAAM (100 nM) for a further 25 min. The cells were then washed extensively and the ability of isoprenaline ($10^{-10}-10^{-5}$ M) to inhibit histamine release was determined following these treatments

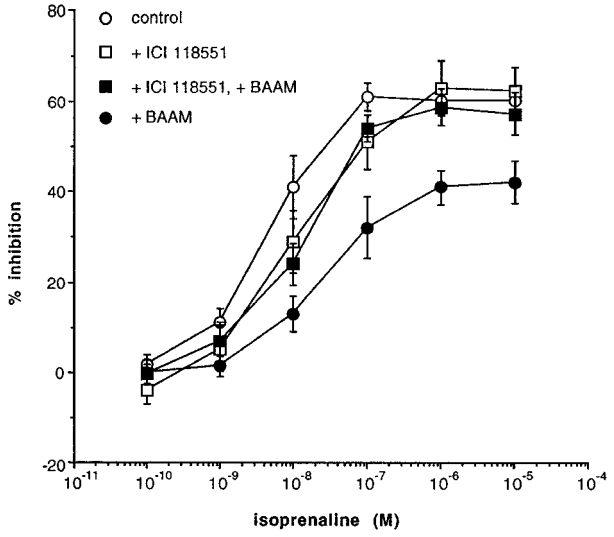

Figure 3 Protection by ICI 118551 of BAAM effects. HLMC were incubated with or without ICI 118551 for 10 min and then together with or without BAAM for an additonal 25 min. After this time the cells were washed extensively and incubated for 10 min with increasing concentrations of isoprenaline before challenge with anti-IgE (1 : 1000). Results are expressed as the % inhibition of the control histamine releases which were, (control) $21\pm3\%$, (BAAM treatment) $22\pm3\%$, (ICI 118551 treatment) $22\pm3\%$, (ICI 118551 and BAAM treatments) $20\pm2\%$. Treatment with BAAM (solid circles) caused a statistically significant ($P<0.005$) shift in the dose-response curve for isoprenaline (open circles). Treatments with ICI 118551 (open squares, solid squares) had no significant ($P>0.05$) effect on the ability of isoprenaline to inhibit histamine release compared to control (open circles). However, the presence of ICI 118551 protected (solid squares) against the reduction in the effectiveness of isoprenaline to inhibit histamine release following BAAM treatment (solid circles) to a significant degreee ($P<0.005$). Values are means and vertical lines show s.e.mean, $n=5$.

(Figure 3). BAAM treatment caused a statistically significant ($P<0.01$) change in the pD_2 values (8.4 ± 0.2 and 7.5 ± 0.2 in the absence and presence of BAAM, respectively) and a reduction ($30\pm6\%$) in the maximal response that was also statistically significant ($P<0.0005$). ICI 118551 protected against these BAAM-mediated effects by restoring the maximal response and increasing the potency of isoprenaline. Although the pD_2 value (7.8 ± 0.2) for isoprenaline following the ICI 118551 plus BAAM treatment was different from the control pD_2 value (8.4 ± 0.2), it was no different from that obtained after ICI 118551 treatment alone (7.9 ± 0.2).

The effect of increasing concentrations of BAAM on the isoprenaline inhibition of histamine release from HLMC was investigated (Figure 4). Increasing concentrations of BAAM caused dose-dependent rightward shifts for the isoprenaline inhibition of histamine release. Concentrations of BAAM of 1, 10 and 100 nM altered, to a statistically significant degree ($P<0.05$ at least), the pD_2 value for the isoprenaline inhibition from 8.6 ± 0.1 to 7.9 ± 0.1, 7.6 ± 0.1 and 7.1 ± 0.1, respectively. Moreover, concentrations of BAAM of 1, 10 and 100 nM caused statistically significant ($P<0.05$, at least) reductions in the maximal inhibitory response obtained with isoprenaline of $21\pm5\%$ (range, 0 to 37%), $32\pm7\%$ (range, 0 to 62%) and $46\pm6\%$ (range, 12 to 69%), respectively. From these data and employing the method described by Furchgott (1966), the proportion of receptors unoccupied following treatments with 1, 10 and 100 nM was 0.25, 0.1 and 0.02, respectively. The K_D value for isoprenaline was estimated as 260 nM using data following treatment of HLMC with 100 nM BAAM. In all 9 experiments in this series, there was a depression in the maximal response following this BAAM (100 nM) treatment which would be a requirement for a reliable estimation of the K_D.

Although the mean data in Figure 4 indicate that BAAM effectively antagonized the response of HLMC to isoprenaline, there was a large degree of inter-experimental variation. For a given concentration of BAAM, the rightward shift in the dose-

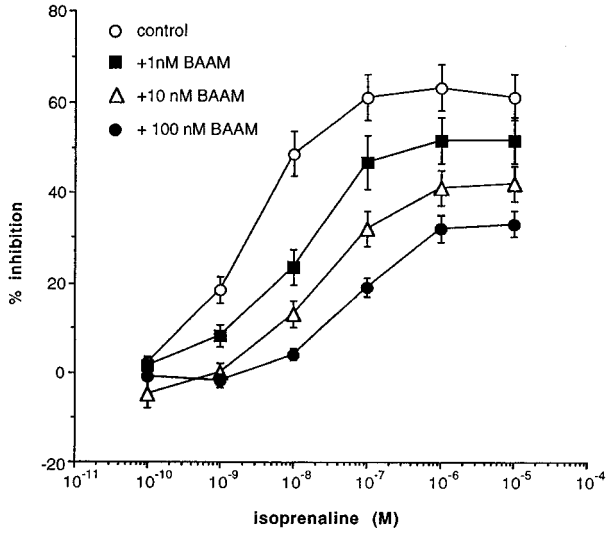

Figure 4 Effect of BAAM treatments on the isoprenaline inhibition of histamine release. HLMC were incubated for 25 min either in buffer or with increasing concentrations of 1, 10 or 100 nM BAAM. After this time, the cells were washed extensively and then incubated for 10 min with increasing concentrations of isoprenaline before challenge with anti-IgE (1 : 1000). Values are expressed as the % inhibition of the control histamine release which ranged from $23\pm2\%$ to $26\pm4\%$. Values are means and vertical lines show s.e.mean, $n=9$.

response curve to isoprenaline was similar between experiments. However, there was considerable variability in the depression of the maximal response. For example, in 6 of 16 experiments (Figure 5a), BAAM (1 nM) had no effect on the maximal response to isoprenaline whereas in the remaining experiments (Figure 5b, BAAM reduced the maximal response by $32\pm4\%$ (range, 7 to 49%). In those experiments in which the maximal response was unaffected by BAAM, isoprenaline was more potent than in those experiments in which BAAM depressed the maximal response (pD_2, values of 8.7 ± 0.1 and 8.3 ± 0.1, respectively). The difference in pD_2 values between the two sets was statistically significant ($P<0.05$).

The effect of BAAM treatment on the effectiveness of salbutamol to inhibit histamine release from HLMC was also investigated (Figure 6). HLMC were incubated (25 min) with or without BAAM (10 nM), washed and then incubated (10 min) with either isoprenaline ($10^{-10}-10^{-5}$ M) or salbutamol ($10^{-10}-10^{-5}$ M) before challenge with anti-IgE. There were rightward shifts in the dose-response curves for both isoprenaline and salbutamol following BAAM treatment. The pD_2 values for isoprenaline were 8.4 ± 0.1 and 7.7 ± 0.1 ($P<0.0005$) in the absence and presence of BAAM, respectively, and for salbutamol, 7.2 ± 0.1 and 6.8 ± 0.1 ($P<0.01$). BAAM caused statistically significant ($P<0.0001$) reductions in the maximal responses obtained with isoprenaline and salbutamol of $23\pm2\%$ (range, 0 to 42%) and $41\pm3\%$ (range, 6 to 60%), respectively.

In these experiments, the salbutamol inhibition of histamine release was variable ranging from 65 to 100% of the maximal response observed with isoprenaline. Moreover, the suscept-

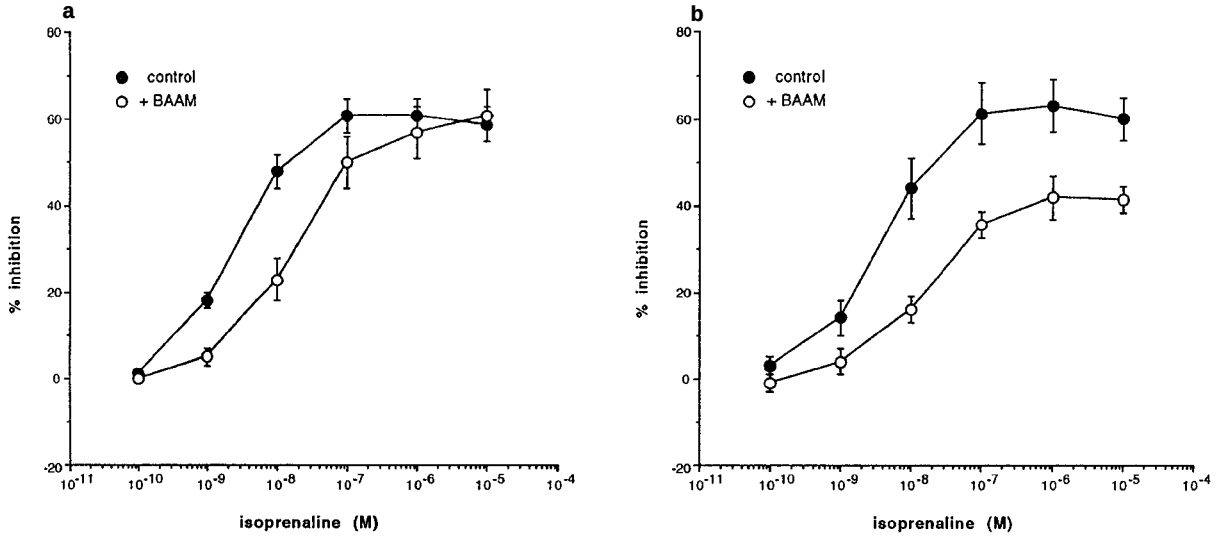

Figure 5 Variability in the effect of BAAM on the isoprenaline inhibition of histamine release. HLMC were incubated with or without BAAM (1 nM) for 25 min after which the cells were washed extensively and then incubated for 10 min with isoprenaline before challenge with anti-IgE (1 : 1000) for 25 min. In 6 out of 16 experiments, BAAM had no effect on the maximal response to isoprenaline (a) whereas, in the remainder, BAAM reduced the maximal response to isoprenaline (b). Results are expressed as the % inhibition of the control histamine releases which were, $28\pm3\%$ for (a) and $28\pm5\%$ for (b) in the absence and $26\pm3\%$ for (a) and $29\pm5\%$ for (b) in the presence of BAAM. Values are means and vertical lines show s.e.mean.

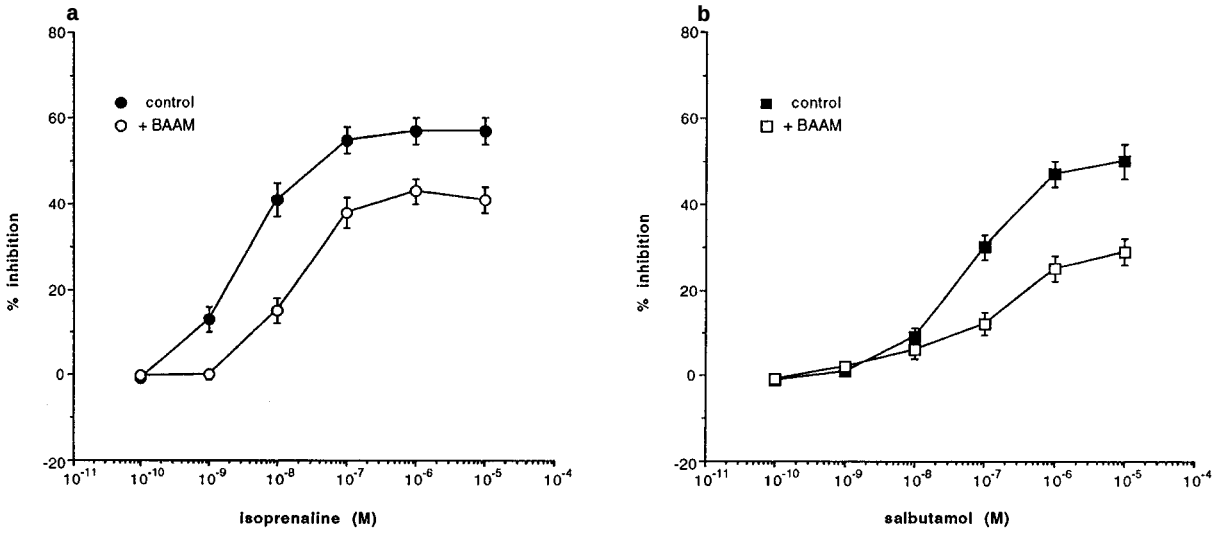

Figure 6 Effect of BAAM on the isoprenaline and salbutamol-mediated inhibition of histamine release. HLMC were incubated with or without BAAM (10 nM) for 25 min after which the cells were washed extensively and then incubated for 10 min with either isoprenaline (a) or salbutamol (b) before challenge with anti-IgE (1 : 1000) for 25 min. Results are expressed as the % inhibition of the control histamine releases which were, $28\pm3\%$ in the absence and $30\pm3\%$ in the presence of BAAM. Values are means and vertical lines show s.e.mean, $n=17$.

ibility of the salbutamol inhibition to BAAM was variable ranging from 10 to 74% reduction in the maximal response (observed with isoprenaline). There was a good correlation ($r=-0.77$, $P<0.005$) between the partial/full agonistic nature of salbutamol and the extent of the reduction in the maximal response obtained with salbutamol following BAAM treatment (Figure 7). In addition, there was some correlation ($r=-0.55$, $P<0.01$) between the partial/full agonistic nature of salbutamol and the extent of the reduction in the maximal response obtained with isoprenaline following BAAM treatment. Thus, in those preparations in which salbutamol acted as a full agonist, there was a larger receptor reserve.

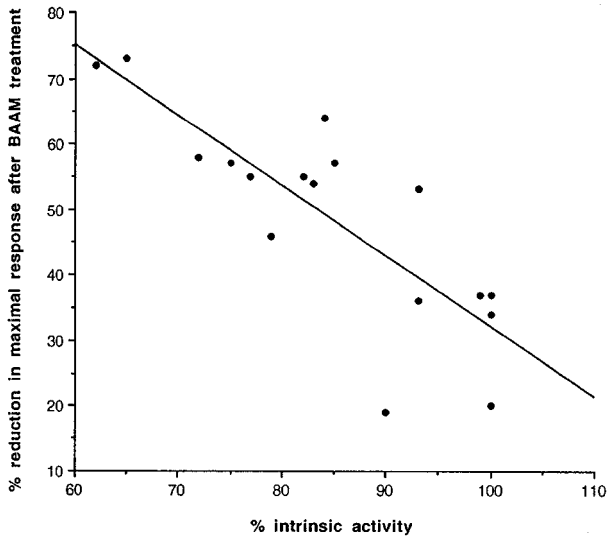

Figure 7 Correlation between the % intrinsic activity of salbutamol (calculated as a % of the maximal response obtained with isoprenaline) and the % reduction of the maximal response observed with salbutamol following BAAM (10 nM) treatment. Data points were obtained from the individual experiments used to construct Figure 6. The correlation coefficient is -0.77 ($P<0.005$).

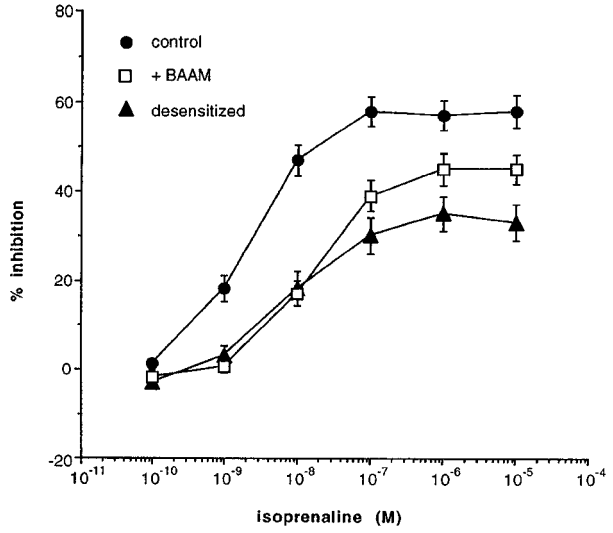

Figure 8 Functional desensitization of β-adrenoceptor-mediated responses in HLMC. HLMC were incubated either in buffer or with 1 μM isoprenaline for 24 h or, alternatively, cells were incubated for 23 h and 35 min in buffer and then with 10 nM BAAM for 25 min. After this time, cells subjected to each of these treatments were washed extensively and incubated for 10 min with isoprenaline before challenge with anti-IgE (1:1000). Values are expressed as the % inhibition of the control histamine releases which were, 31±3% (circles), 26±3% (triangles) and 31±3% (squares). Values are means and vertical lines show s.e.mean, $n=21$.

The influence of receptor reserve on the functional desensitization of β-adrenoceptor-mediated responses was also investigated. HLMC were incubated in either buffer or with isoprenaline (1 μM) for 24 h, or alternatively, cells were incubated for 23 h and 35 min in buffer and then with BAAM (10 nM) for 25 min. After this time, cells subjected to each of these treatments were washed extensively and incubated (10 min) with isoprenaline (10^{-10}–10^{-5} M) before challenge with anti-IgE (Figure 8). The data show that desensitizing conditions caused a subsequent reduction (40±5% desensitization) in the maximal inhibitory activity of isoprenaline. However, the extent of desensitization was variable between preparations, ranging from negligible (<10% desensitization) to extensive (93% desensitization). In addition, BAAM reduced, to a statistically significant ($P<0.001$) degree, the maximal inhibitory response of isoprenaline by 24±3% (range, 0 to 52% reduction). Desensitizing and BAAM treatments altered, to a statistically significant ($P<0.001$ at least) degree, the pD_2 value for the isoprenaline inhibition from 8.6±0.1 (control) to 7.8±0.1 and 7.7±0.1, respectively.

There was a reasonable correlation ($r=0.59$, $P<0.005$) between the extent of desensitization and sensitivity of the isoprenaline response to BAAM (Figure 9). Thus, HLMC with a larger receptor reserve appeared to be more resistant to desensitization.

Discussion

In the therapeutic management of asthma, bronchodilator β-adrenoceptor agonists may act not only to relax airways smooth muscle but to stabilize inflammatory cell activity (Tattersfield, 1992; Barnes, 1995). The mast cell may be an important target of this class of drug because a large number of *in vitro* studies has shown that β-adrenoceptor agonists are effective inhibitors of the stimulated release of mediators from

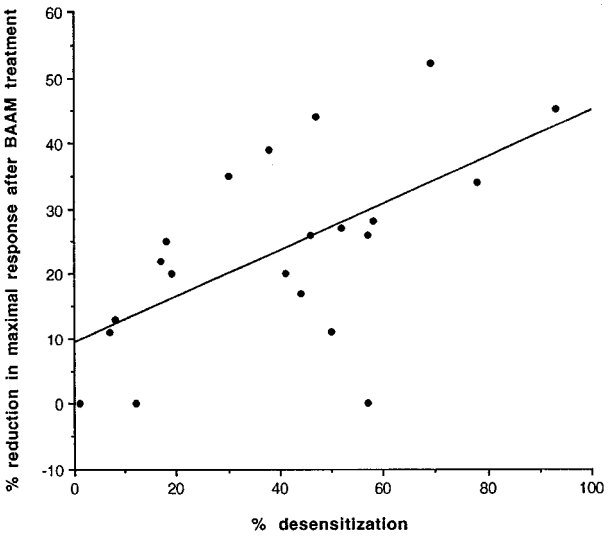

Figure 9 Correlation between the reduction (% desensitization) in the maximal response obtained with isoprenaline following long-term (24 h) treatment with isoprenaline (1 μM) and the % reduction in the maximal response obtained following treatment with BAAM (10 nM). Data points were obtained from the 21 experiments used to construct Figure 8. The correlation coefficient is 0.59 ($P<0.005$).

HLMC (Assem & Schild, 1969; Orange *et al*., 1971; Butchers *et al*., 1980; Church & Hiroi, 1987; Peachell *et al*., 1988; Undem *et al*., 1988).

In the present study, we have investigated the responses of different HLMC preparations to isoprenaline and salbutamol. Isoprenaline was more potent than salbutamol as an inhibitor of the stimulated release of histamine from HLMC. Moreover, the mean data indicated that salbutamol was a partial agonist relative to isoprenaline. However, there was considerable variation in the response of HLMC to salbutamol which was a full agonist in some preparations and a partial agonist in others. In view of studies showing that the intrinsic activity of partial agonists may increase if a larger receptor reserve exists (Hoyer & Boddeke, 1993; Kenakin, 1984; MacEwan *et al*., 1995), these data could suggest that variable receptor reserves exist between different HLMC preparations.

Studies were performed with the irreversible antagonist BAAM which has been used widely to investigate β-adrenoceptor reserve (Mahan & Insel, 1986; Minneman & Mowry, 1986; Undem *et al*., 1988; MacEwan *et al*., 1995). BAAM treatment antagonized the response of HLMC to isoprenaline without affecting the inhibitory effects of PGE_2, an agonist which is known to cause increases in HLMC adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) (Peachell *et al*., 1988) and which is, therefore, likely to act through EP receptors to inhibit HLMC. In this limited experimental context, these data suggest that BAAM is selective for β-adrenoceptors and does not inactivate alternative cell surface receptors. Moreover, ICI 118551, a β_2-selective antagonist, protected against the antagonism by BAAM of the isoprenaline inhibition suggesting a selective effect of BAAM at β_2-adrenoceptors.

Our studies indicated that treatments of HLMC with 1, 10 and 100 nM BAAM led to progressive rightward shifts in the dose-response curves for isoprenaline with a dose-dependent depression in the maximal response. Our estimate of the K_D for isoprenaline (260 nM) is consistent with previous estimates for isoprenaline at β_2-adrenoceptors in mast cells (Undem *et al*., 1988) and in alternative systems (Dulis & Wilson, 1980; Mattson *et al*., 1983; Minneman *et al*., 1983; McPherson *et al*., 1985). The fraction of receptors remaining following a 25 min treatment with 1, 10 and 100 nM BAAM was 0.25, 0.1 and 0.02, respectively. These experiments indicate that with 98% of the receptors inactivated, isoprenaline can inhibit histamine release to levels greater than 50% of the maximal response.

Evaluation of the data from individual experiments indicated that a given BAAM treatment would shift the dose-response curve to isoprenaline to a similar degree in all experiments. This suggests that similar proportions of receptors are inactivated amongst different HLMC preparations following a given BAAM treatment. However, interestingly, there was substantial variability, between preparations, in the extent to which BAAM reduced the maximal response observed with isoprenaline. In some experiments, pretreatment with BAAM (at a concentration of 1 nM) did not depress the maximal response to isoprenaline (indicative of a larger receptor reserve), whereas in other preparations there was a substantial depression in the maximal response. These data provide further evidence that variable receptor reserves exist amongst HLMC preparations.

Attempts were made to establish the influence of receptor reserve on functional responses in HLMC. Isoprenaline was more potent as an inhibitor of histamine release in those preparations which possessed a larger receptor reserve. Moreover, salbutamol was a full agonist, relative to isoprenaline, in those preparations with a larger receptor reserve. These data indicate that the response of individual HLMC preparations to β-adrenoceptor agonists is influenced by receptor reserve. Should similar processes be operative *in vivo* then receptor reserve could be influential in determining the extent of mast cell-stabilization by β_2-adrenoceptor agonists in the management of asthma.

In addition to influencing β-adrenoceptor-mediated inhibition of HLMC, receptor reserve appeared also to play some part in the desensitization of β-adrenoceptor-mediated responses. In accord with our previous studies (Chong *et al*., 1995; 1997), the extent of desensitization was highly variable amongst HLMC preparations and a reasonably good correlation was established between receptor reserve and the extent of desensitization – a larger receptor reserve generally associated with resistance to desensitization. In the clinical context, therefore, receptor reserve may be influential in determining the extent to which mast cells succumb to a tolerant state following continued exposure of mast cells to β_2-adrenoceptor agonists.

In addition to receptor reserve, it is quite likely that other factors may be important in determining the extent of desensitization of β_2-adrenoceptor-mediated resonses in HLMC because, in a small number of HLMC preparations, a correlation between receptor reserve and the extent of desensitization was less convincing. It is of interest that studies by others (Green *et al*., 1994; 1995a,b) have shown that genetic polymorphisms in the β_2-adrenoceptor may be linked to both resistance and sensitivity to desensitization and it is possible that genetic differences in the β_2-adrenoceptor could contribute to the wide-ranging differences in desensitization observed in the HLMC. However, desensitization is a complex process that may involve the uncoupling, sequestration and degradation of receptors (Hausdorff *et al*., 1990). Phosphorylations of the β_2-adrenoceptor mediated by β-adrenoceptor kinase and cyclic AMP-dependent protein kinase may contribute to these processes, although alterations in gene expression may also be involved following longer-term exposure to desensitizing conditions (Hausdorff *et al*., 1990). At present, it is not known which of these mechanisms is involved in regulating the desensitization of β_2-adrenoceptor-mediated responses in HLMC. It is possible that heterogeneity in any of the machinery (e.g. kinases, phosphatases) involved in mediating desensitization, as well as intrinsic properties of the receptor, could contribute to the wide differences in the extent of desensitization observed in HLMC.

It is well-known that mast cells isolated from different species and from different sites of the same species can vary extensively in their responses to exogenous agents (Pearce, 1983). In the present study, parenchymal mast cells have been employed and it is uncertain whether the responses of these mast cells reflect identically the responses of alternative subsets of pulmonary mast cells such as bronchial mast cells and mast cells derived from bronchoalveolar lavage. The possibility exists, therefore, that the observations made and the conclusions drawn from the present study, employing parenchymal mast cells, may not necessarily hold for alternative subsets of pulmonary mast cells.

The present study has shown that a receptor reserve exists for the β-adrenoceptor-mediated inhibition of histamine release from HLMC and that the size of this reserve varies between HLMC preparations. The factors which may be responsible for this variability in receptor reserve are unknown. Differences in receptor density and/or the efficiency of receptor-coupling mechanisms could contribute to variable receptor reserves between different

mast cell preparations (Kenakin, 1984). It is possible that endogenous mediators generated in response to events in the lung, drug treatments and perhaps genetic predispositions could all constitute contributory factors influencing receptor reserve. Whatever the mechanisms that may be involved, the present study has shown that the size of the receptor reserve may influence the sensitivity of HLMC to β-adrenoceptor agonists and the susceptibility of individual HLMC preparations to desensitization.

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