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Salmeterol inhibition of mediator release from human lung mast cells by β-adrenoceptor-dependent and independent mechanisms

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1 The long-acting β_2-adrenoceptor agonist, salmeterol ($10^{-9}-10^{-5}$ M), inhibited the IgE-mediated release of histamine from human lung mast cells (HLMC) in a dose-dependent fashion. Additional β-adrenoceptor agonists were studied and the rank order of potency for the inhibition of histamine release from HLMC was isoprenaline > salmeterol > salbutamol. Approximate EC_{50} values for the inhibition of histamine release were 10 nM for isoprenaline and 100 nM for salbutamol. An EC_{50} value for salmeterol could not be calculated because maximal responses to salmeterol were not observed over the concentration range employed.

2 Both salmeterol and isoprenaline inhibited the generation of sulphopeptidoleukotrienes (sLT) more potently and more efficaciously than the release of histamine from immunologically-activated HLMC. Salmeterol ($EC_{50} < 0.1$ nM) was more potent than isoprenaline (EC_{50} 0.4 nM) at attenuating sLT generation.

3 The β-adrenoceptor antagonist, propranolol (1 μM), and the selective β_2-adrenoceptor antagonist, ICI 118,551 (0.1 μM), both caused rightward shifts in the dose-response curve for the inhibition of histamine release by isoprenaline. The antagonism of salmeterol effects by propranolol and ICI 118,551 was more complex. At lower concentrations (< 1 μM) of salmeterol, both antagonists shifted the dose-reponse curve to salmeterol rightward. At a higher concentration (10 μM) of salmeterol, neither ICI 118,551 nor propranolol was an effective antagonist of the salmeterol-mediated inhibition of histamine release.

4 Prolonged exposure (4 h) of HLMC to isoprenaline (1 μM) caused an approximately 50% reduction in the effectiveness of a second exposure to isoprenaline (10 μM) at inhibiting the release of histamine, whereas this pretreatment did not affect the salmeterol (10 μM) inhibition of histamine release.

5 Isoprenaline ($10^{-9}-10^{-5}$ M) caused a dose-dependent increase in total cell cyclicAMP levels in purified HLMC which paralleled the inhibition of histamine release. Salmeterol ($10^{-9}-10^{-5}$ M) was considerably less potent than isoprenaline at increasing HLMC cyclicAMP levels.

6 In summary, these data indicate that salmeterol is an effective inhibitor of the stimulated release of mediators from HLMC. The present data also suggest that salmeterol may act to inhibit mediator release from HLMC by β-adrenoceptor-dependent and independent mechanisms.

Keywords: Human lung mast cells; salmeterol; β-adrenoceptors; mediator release

Introduction

Bronchodilator β_2-adrenoceptor agonists continue to be of importance in the therapeutic management of asthma (Tattersfield, 1992). Whilst the primary effect of bronchodilators is to relax airways smooth muscle, additional effects may include the stabilization of inflammatory cell activity (Tattersfield, 1992). The release of spasmogenic and pro-inflammatory mediators from cells in the airways could contribute to both the progression and the severity of asthma. Suppression of the release of these mediators by β-adrenoceptor agonists could represent an important component of the anti-asthma activity of these drugs.

Human lung mast cells (HLMC) could be an important target for the anti-asthma actions of β_2-adrenoceptor agonists, because HLMC are particularly sensitive to the inhibitory effects of this class of drug (Assem & Schild, 1969; Orange *et al*., 1971; Butchers *et al*., 1980; Church & Hiroi, 1987; Peachell *et al*., 1988; Undem *et al*., 1988). In contrast, β_2-adrenoceptor agonists are relatively ineffective at attenuating the activity of alternative cell types such as macrophages (Fuller *et al*., 1988) and eosinophils (Yukawa *et al*., 1990), both of which have been implicated in the pathogenesis of asthma.

In recent years, the longer-acting β_2-adrenoceptor agonist, salmeterol, has been introduced clinically (Tattersfield, 1993). Structurally, salmeterol is related to salbutamol the difference being that salmeterol also possesses an extended lipophilic side chain (Johnson *et al*., 1993). The suggestion has been made that, due to the lipophilicity of salmeterol, non-specific physicochemical interactions with the plasma membrane may be responsible for the long-acting effects of salmeterol (Anderson *et al*., 1994). Alternatively, studies indicate that the lipophilic chain of salmeterol interacts specifically with a site (termed the 'exosite') adjacent to or even within the β_2-adrenoceptor (Coleman *et al*., 1996). Following disengagement of the active part of the molecule from the receptor, salmeterol does not diffuse away because the interaction of the lipophilic side chain ensures that the molecule remains attached to the exosite and the potential exists for repeated interactions with the β_2-adrenoceptor.

Salmeterol has been shown to be a potent relaxant of airways smooth muscle *in vivo* (Ball *et al*., 1991; Nials *et al*., 1993; Gorenne *et al*., 1995) and it has also been shown to inhibit the release of mediators from antigenically-activated human lung fragments (Butchers *et al*., 1991) and HLMC (Lau *et al*., 1994). Although these effects of salmeterol on mast cells have been characterized as being β_2-adrenoceptor-mediated, other studies in alternative systems indicate that salmeterol may have non-β_2-

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adrenoceptor-dependent effects (Baker & Fuller, 1990; Nials *et al*., 1997). The present study was performed to determine the mechanism by which salmeterol inhibits HLMC responses.

Methods

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 (mM): $CaCl_2.2H_2O$ 1, $MgCl_2.6H_2O$ 1, glucose 5.6 and bovine serum albumin (BSA) 1 mg ml^{-1}; DNase 15 μg ml^{-1}. +PBS was −PBS additionally supplemented with (mM): $CaCl_2.2H_2O$ 1, $MgCl_2.6H_2O$ 1, glucose 5.6 and human serum albumin (HSA) 30 μg ml^{-1}. The pH of all PBS buffers was titrated to 7.3.

Preparation of drugs

Salbutamol and propranolol were prepared daily in −PBS buffer as 10 mM solutions. Salmeterol was prepared daily (100 mM) in dimethyl sulphoxide (DMSO). Isoprenaline (10 mM) was dissolved in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl) and this stock solution was made weekly and stored at 4°C. ICI 118,551 was prepared as a 1 mM stock solution in distilled water. Preliminary experiments indicated that the vehicles used to prepare drugs, had no effect on mediator release from activated HLMC.

Cell isolation

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 Ia (350 u ml^{-1} of PBS) and agitated for 75 min with 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 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. Of the total cells, 3–13% were mast cells. This method generated 2 to 9×10^5 HLMC g^{-1} tissue. HLMC prepared in this manner were used in mediator release experiments. In those experiments in which adenosine 3′:5′-cyclic monophosphate (cyclicAMP) levels were measured, HLMC were purified by countercurrent elutriation (Beckman J6B centrifuge, JE-5.0 elutriator head) and flotation over Percoll density gradients by methods that have been described in detail elsewhere (Schulman *et al*., 1982; Ishizaka *et al*., 1983). Mast cells were visualized by microscopy with an alcian blue stain (Gilbert & Ornstein, 1975).

Mediator release

Histamine release experiments were performed in +PBS buffer. Histamine release was initiated immunologically with anti-IgE. 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) and, when appropriate, an aliquot of the supernatant was taken and stored frozen for sulphopeptidoleukotriene (sLT) analysis by enzyme immunoassay (EIA). When the effects of agonists were investigated, cells were incubated with drug for 15 min (or for time periods as indicated in the text) at 37°C before the addition of stimulus and then samples were processed as indicated above. Preliminary studies investigating the effects of preincubation of salmeterol on the inhibition of histamine release from HLMC indicated that there was very little difference in inhibitory activity following preincubations of 15, 30, 60 and 120 min. Total histamine content was determined by lyzing 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 the subtraction of spontaneous histamine release. All experiments were performed in duplicate.

Assay for cyclicAMP

Total cell cyclicAMP levels were assayed in purified HLMC by the employment of methods that have been described elsewhere (Peachell *et al*., 1988). HLMC (0.1×10^6 cells) were incubated in the presence or absence of a β-adrenoceptor agonist for 15 min in a total volume of 0.1 ml. After this incubation, the reaction was stopped by the addition of 0.9 ml of acidified ethanol (1 ml 1 M HCl in 99 ml of ethanol). The samples were vortexed and snap frozen in liquid nitrogen. The samples were thawed and centrifuged (14 000 *g*, 4 min, 4°C) and 0.9 ml of the supernatant removed. The supernatants were evaporated to dryness with a rotary evaporator. The dried samples were reconstituted in 0.2 ml of 0.05 M sodium acetate (pH 6.2) supplemented with HSA (100 μg ml^{-1}) and stored at −80°C. Samples were assayed by use of a commercially-available EIA.

Materials

The following were purchased from the sources indicated: anti-human IgE, BSA, collagenase, DMSO, DNAse, HSA, (−)-isoprenaline bitartrate, Percoll, propranolol, salbutamol, (all Sigma, Poole, U.K.); ICI 118,551 (erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol; Cambridge Research Biochemicals, Northwich, U.K.); sLT and cyclicAMP EIA kits (Amersham, Little Chalfont, U.K.). Salmeterol was a kind gift from Dr R.A. Coleman (GlaxoWellcome, Stevenage, U.K.).

Statistics

Statistical significance was determined by means of Student's *t* test for paired data. Values of $P<0.05$ were taken as significant.

Results

Several studies have shown that β-adrenoceptor agonists are effective inhibitors of the stimulated release of histamine from human lung fragments and 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, isoprenaline, salmeterol and salbutamol were found to inhibit the release of histamine from HLMC stimulated with anti-IgE in a dose-dependent fashion (Figure 1). The rank order of potency for the inhibition was, isoprenaline > salmeterol > salbutamol. Approximate EC_{50} values for the inhibition of histamine release were 10 nM for isoprenaline and 100 nM for salbutamol. An EC_{50} value for salmeterol could not be estimated reliably because maximal responses to salmeterol were not observed over the concentration range employed.

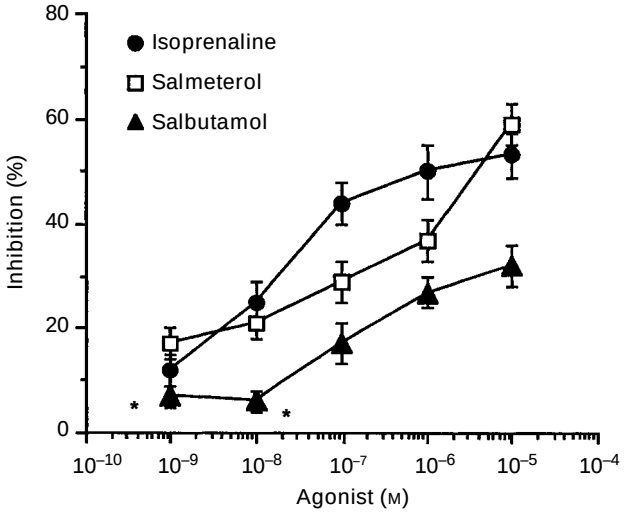

Figure 1 Effect of β-adrenoceptor agonists on HLMC. Cells were incubated with either isoprenaline, salmeterol or salbutamol for 15 min before challenge with anti-IgE (1 : 1000). Values show the concentration-dependent inhibition of the control histamine release which was $28 \pm 3\%$. Values are means and vertical lines s.e.mean, $n = 19$ for salmeterol and isoprenaline. In 6 of these 19 experiments, salbutamol was also included. Statistically significant ($P < 0.05$) levels of inhibition were obtained at all concentrations of agonists except those indicated by an asterisk.

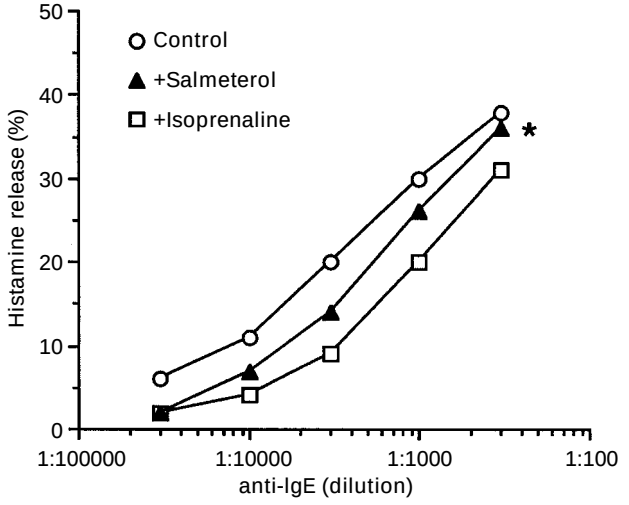

Figure 2 Effects of salmeterol and isoprenaline on varying levels of histamine release. HLMC were incubated in either buffer (control), or the agonists (0.1 μM) salmeterol or isoprenaline for 15 min before challenge with various concentrations of anti-IgE. Values are means, $n = 8$. Error bars (which ranged from 1 to 5%) have been omitted for reasons of clarity. Both salmeterol and isoprenaline caused statistically significant ($P < 0.05$) reductions in histamine release at all concentrations of anti-IgE, except that indicated by an asterisk.

The inhibition of histamine release by salmeterol and isoprenaline was inversely related to the level of control histamine release. Thus lower levels of control histamine release induced by lower concentrations of anti-IgE were modulated to a greater extent than higher levels of secretion (Figure 2). For example, at a low (1 : 3000) releasing concentration of anti-IgE, isoprenaline (0.1 μM) and salmeterol (0.1 μM) inhibited histamine release by $57 \pm 8\%$ ($P < 0.05$) and $34 \pm 9\%$ ($P < 0.05$), respectively, whereas at a higher (1 : 300) concentration of anti-IgE, isoprenaline and salmeterol inhibited histamine release by $23 \pm 8\%$ ($P < 0.05$) and $9 \pm 3\%$ ($P > 0.05$), respectively.

Salmeterol and isoprenaline inhibited not only the IgE-mediated release of histamine but the generation of sLT also (Figure 3). The inhibitory effects of both agonists were dose-dependent and both drugs were more potent and more efficacious as inhibitors of sLT generation than histamine release. Approximate EC_{50} values for the inhibition of sLT

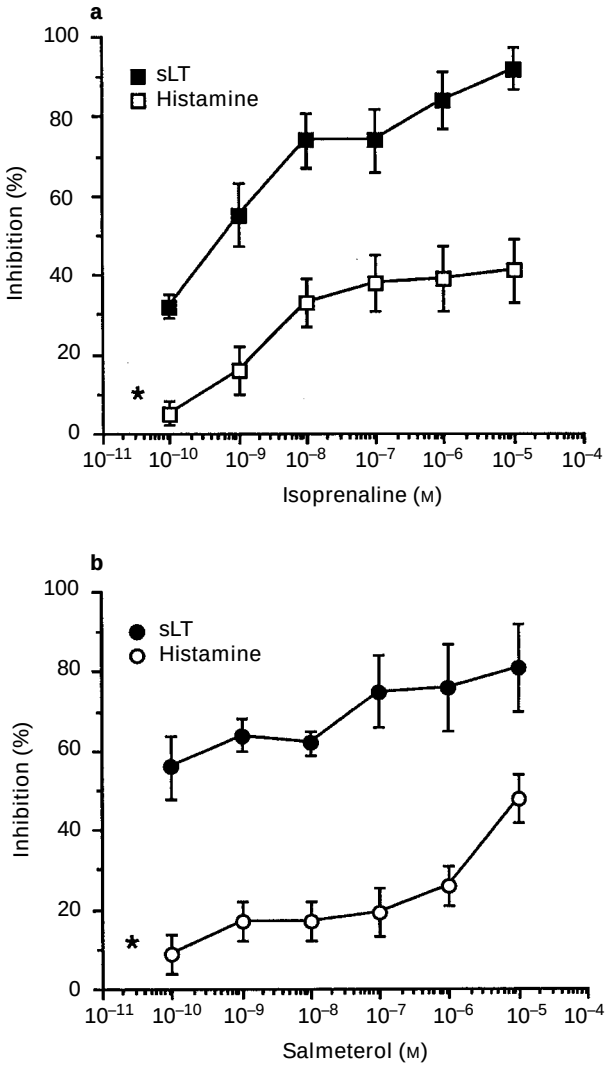

Figure 3 Effect of isoprenaline (a) and salmeterol (b) on sLT generation and histamine release from HLMC. Cells were incubated with either isoprenaline or salmeterol for 15 min before challenge with anti-IgE (1 : 1000) for a further 25 min for the release of histamine or the generation of sLT. Results are expressed as the % inhibition of the control release which was $46 \pm 6\%$ for histamine and 6 ± 2 ng per 10^6 HLMC for sLT. Values are means and vertical lines s.e.mean, $n = 6$. The inhibition of histamine release and sLT generation was statistically significant ($P < 0.05$ at least) at all concentrations of agonists, except those indicated by an asterisk.

generation were 0.4 nM for isoprenaline and <0.1 nM for salmeterol. In further studies with β-adrenoceptor agonists (10 pM), salmeterol inhibited IgE-mediated sLT generation by $63\pm12\%$ ($P<0.05$), whereas neither isoprenaline ($14\pm10\%$, $P>0.05$) nor salbutamol ($2\pm9\%$, $P>0.05$) was effective ($n=4$).

To determine whether salmeterol acts to inhibit histamine release from HLMC by interacting with β-adrenoceptors, the effects of the non-selective β-adrenoceptor antagonist, propranolol, and the selective β_2-adrenoceptor antagonist, ICI 118,551, were studied. Propranolol (1 μM) caused a rightward shift in the dose-response curve for the inhibition of histamine release by isoprenaline although the degree of shift in the curve was not constant ranging from a 15 to 300 fold shift, respectively, at the low (1 nM) and at the higher (10 μM) end of the isoprenaline dose-response curve (Figure 4a). ICI 118,551 (0.1 μM) caused a 100 fold rightward shift in the isoprenaline dose-response curve (Figure 5a). Both antagonists reversed the salmeterol inhibition of histamine release but only at lower concentrations of the agonist (Figures 4b and 5b). At the highest concentration (10 μM) of agonist, increasing concentrations of ICI 118,551 ($10^{-7}-10^{-5}$ M) reversed the isoprenaline inhibition of histamine release but not the salmeterol inhibition (Table 1).

In order to establish whether salmeterol and isoprenaline might be acting at the same receptor, cross-desensitization experiments were performed. HLMC were incubated for 4 h with isoprenaline (1 μM), the cells washed and then incubated with either isoprenaline (10 μM) or salmeterol (10 μM) for 15 min before challenge with anti-IgE. The data indicate that whereas the 4 h isoprenaline pretreatment induced a functional desensitization to the isoprenaline-mediated inhibition of histamine release, the pretreament had no effect on the salmeterol inhibition of histamine release (Table 2).

Should salmeterol act to inhibit histamine release from HLMC by interacting with β-adrenoceptors then salmeterol might be expected to increase intracellular cyclicAMP levels in purified HLMC. HLMC were incubated with increasing

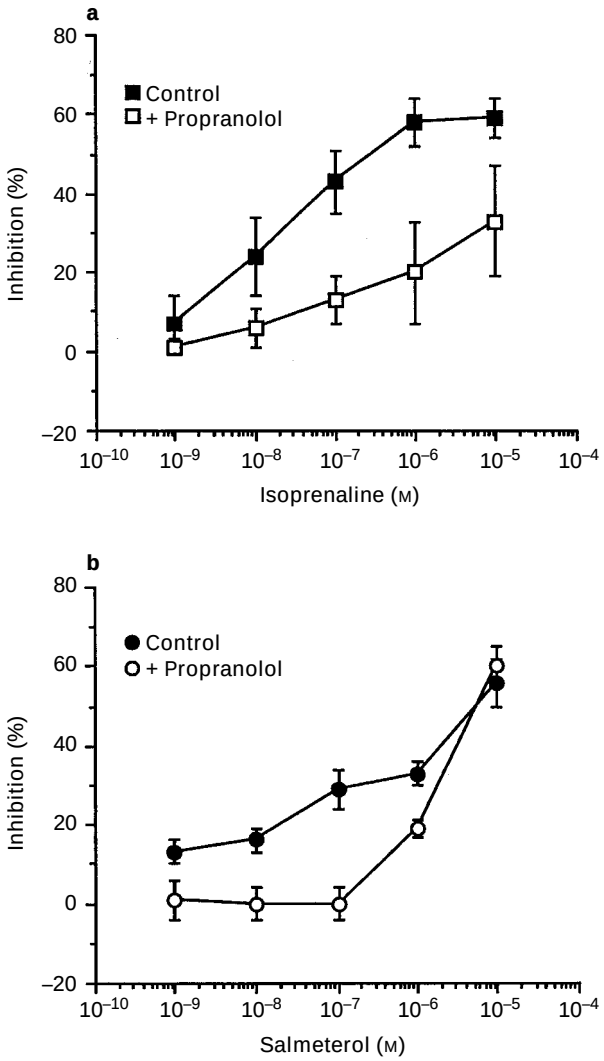

Figure 4 Effect of propranolol on the isoprenaline (a) and salmeterol (b) inhibition of histamine release. HLMC were incubated either with or without (control) propranolol (1 μM) for 5 min, before incubation with either isoprenaline or salmeterol for a further 15 min before challenge with anti-IgE for histamine release. Values are expressed as the % inhibition of the control histamine release which was $26\pm4\%$ in the absence and $25\pm5\%$ in the presence of propranolol, $n=5$. Vertical lines show s.e.mean.

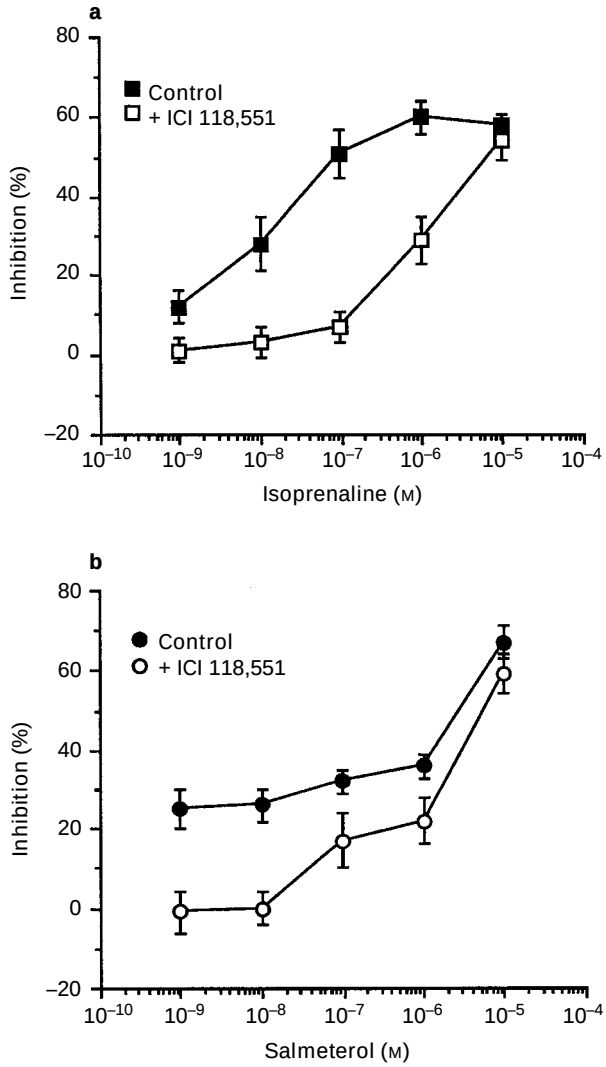

Figure 5 Effect of ICI 118,551 on the isoprenaline (a) and salmeterol (b) inhibition of histamine release. HLMC were incubated either with or without (control) ICI 118,551 (0.1 μM) for 5 min, before incubation with either isoprenaline or salmeterol for a further 15 min before challenge with anti-IgE for histamine release. Values are expressed as the % inhibition of the control histamine release which was $21\pm3\%$ in the absence and $23\pm3\%$ in the presence of ICI 118,551, $n=5$. Vertical lines show s.e.mean.

concentrations of either salmeterol or isoprenaline for 15 min and then samples were analysed for cyclicAMP (Figure 6a). The effect of this incubation period on the release of histamine from the same cell preparations was determined in parallel (Figure 6b). Whereas isoprenaline was very effective at elevating cyclicAMP levels, salmeterol was substantially less effective.

Salmeterol has been identified as a long-acting β_2-adrenoceptor agonist (Johnson *et al*., 1993). In studies with human lung fragments, inhibition of histamine release by salmeterol persisted for up to 20 h even after the fragments had been washed (Butchers *et al*., 1991). In order to determine whether salmeterol has a longer duration of action than isoprenaline for the inhibition of histamine release, HLMC were incubated for 20 min with an agonist (1 μM), washed and then incubated for 16 h in buffer. Under these conditions, the inhibitory activities of both isoprenaline and salmeterol on IgE-mediated histamine release were lost (data not shown, $n=6$). In further studies, HLMC were incubated for 20 min with an agonist (1 μM), washed and then incubated for 4 h in buffer. After this treatment, the inhibitory activities of both isoprenaline and salmeterol on IgE-mediated histamine release were lost (Table 3).

Discussion

Bronchodilator β_2-adrenoceptor agonists continue to be important in the therapeutic management of asthma (Tattersfield, 1992). In recent years, a longer-acting β_2-adrenoceptor agonist, salmeterol, has been introduced. Whilst it has been demonstrated that salmeterol is an effective bronchodilator and that this effect is likely to be β_2-adrenoceptor-mediated, other studies suggest that salmeterol,

Table 1 Effect of ICI 118,551 on the inhibition by isoprenaline and salmeterol of histamine release

	Inhibition (%)	
ICI 118,551 (μM)	*Isoprenaline*	*Salmeterol*
–	51±6	58±10
0.1	44±5	56±8
1	15±6	58±7
10	3±2	61±7

HLMC were incubated without (–) or with increasing concentrations of ICI 118,551 for 5 min before addition of agonist (10 μM) for an additional 15 min before challenge with anti-IgE (1:1000) for a further 25 min. Values are expressed as the % inhibition of the control histamine release which was 17±2%. Values are means±s.e.mean, $n=6$.

Table 2 Effect of desensitizing conditions on the salmeterol inhibition of histamine release

	Inhibition (%)	
Condition	*Isoprenaline*	*Salmeterol*
Control	39±5	32±4
Desensitized	22±3*	31±4

HLMC were incubated for 4 h in either buffer (control) or with 1 μM isoprenaline (desensitized), then washed and incubated with either isoprenaline (10 μM) or salmeterol (10 μM) for 15 min before challenge with anti-IgE (1:1000) for 25 min. Results are the % inhibition of the control histamine release which was 34±6% for control cells and 31±6% for desensitized cells. The asterisk denotes a statistically significant reduction in the ability of isoprenaline to inhibit histamine release following desensitizing conditions. Values are mean±s.e.mean, $n=13$.

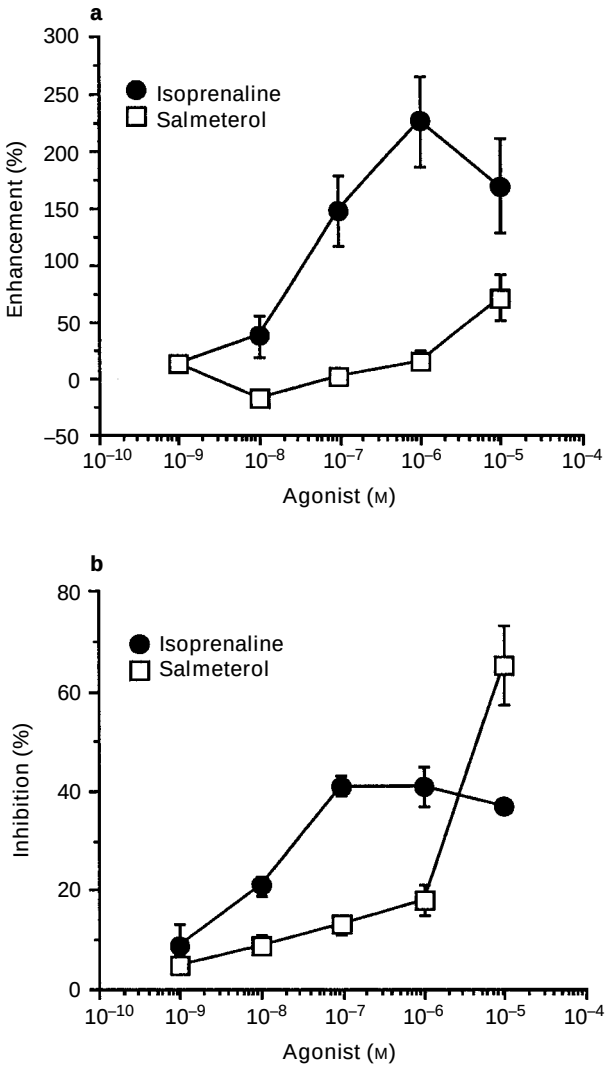

Figure 6 Effect of isoprenaline and salmeterol on cyclicAMP levels (a) and histamine release (b) in HLMC. (a) HLMC were incubated for 15 min with either isoprenaline or salmeterol after which time the reaction was terminated and cyclicAMP levels measured. Values are expressed as the % enhancement in cyclicAMP levels over the basal cyclicAMP level which was 0.8±0.2 pmol cyclicAMP per 10^6 cells. (b) Histamine release was monitored in parallel. HLMC were incubated with either isoprenaline or salmeterol for 15 min before challenge with anti-IgE (1:1000) for a further 25 min. Values are expressed as the % inhibition of the control histamine release which was 25±3%. Values are means and vertical lines s.e.mean, $n=3$. HLMC purites were 72, 75 and 88%.

Table 3 Loss of salmeterol inhibitory activity with washing

	Inhibition (%)	
Condition	*Isoprenaline*	*Salmeterol*
Control	59±9	47±9
Washed	10±9	2±2

HLMC were incubated either for 20 min with an agonist (1 μM), the cells washed and incubated in buffer for 4 h (washed) or for 4 h in buffer, the cells washed and then incubated for 20 min with an agonist (control). After these pretreatments, the cells were challenged with anti-IgE (1:1000) for 25 min. Results are expressed as the % inhibition of the control histamine release which was 19±4%. Values are means±s.e.mean, $n=4$.

at least at high concentrations, may also exert effects independent of β_2-adrenoceptors (Baker & Fuller, 1990; Nials *et al*., 1997).

In the present study, a number of β-adrenoceptor agonists were found to inhibit the IgE-mediated release of histamine from HLMC. Although isoprenaline is a non-selective agonist, that salbutamol inhibited histamine release suggests that the inhibitory activity is mediated through β_2-adrenoceptors. The dose-response curves for isoprenaline and salbutamol paralleled each other and salbutamol appeared to be less potent than isoprenaline. Although salmeterol inhibited the release of histamine dose-dependently, the shape of the dose-response curve deviated appreciably from those obtained for both isoprenaline and salbutamol. These findings perhaps identify differences between salmeterol and alternative β-adrenoceptor agonists indicating that additional, if not alternative, mechanisms may be involved for the inhibition of histamine release by salmeterol.

Both salmeterol and isoprenaline inhibited the stimulated generation of sLT from activated HLMC. Salmeterol was considerably more potent as an inhibitor of the generation of sLT than might have been anticipated on the basis of the effects of salmeterol on histamine release. Moreover, salmeterol was a very effective inhibitor of sLT generation even at picomolar concentrations, when alternative agonists were ineffective at these concentrations. Again, these data suggest that salmeterol exerts effects not observed with alternative β-adrenoceptor agonists. Clearly, the suppression of sLT generation could constitute a significant component of the anti-asthma actions of salmeterol.

Studies with β-adrenoceptor antagonists indicated that salmeterol acts to inhibit the release of histamine from HLMC by both β-adrenoceptor-dependent and independent processes. Propranolol and ICI 118,551 were capable of reversing the inhibitory effects of lower (<1 μM) concentrations of salmeterol, whereas at higher concentrations of salmeterol (10 μM), both propranolol and ICI 118,551 were completely ineffective. These data strongly suggest that salmeterol, at higher concentrations, exerts inhibitory effects on HLMC secretion that may not be β-adrenoceptor-mediated. These findings are supported by attempts to desensitize salmeterol responses by pretreating (4 h) HLMC with isoprenaline. Pretreatment with isoprenaline had no effect on the ability of salmeterol to inhibit histamine release, whereas our previous studies have shown that treatment of HLMC with either isoprenaline, salbutamol, fenoterol or terbutaline induces a functional desensitization to each of these agonists (Chong *et al*., 1995).

Receptor-mediated activation of adenylate cyclase causes increases in cyclicAMP. Previous studies have shown that isoprenaline induces increases in total cell cyclicAMP levels in purified HLMC (Peachell *et al*., 1988). Thus, if salmeterol is acting through β-adrenoceptors to inhibit HLMC responses, then it might be anticipated that salmeterol would also increase cyclicAMP levels in HLMC. In the present study, isoprenaline induced increases in cyclicAMP in HLMC comparable to those previously obtained and although salmeterol also elevated cyclicAMP levels, it was considerably less efficacious and potent than isoprenaline in this regard. Although it might be expected that a partial agonist would be less able to elevate cyclicAMP levels than a full agonist, there was no obvious correlation between increases in cyclicAMP and the inhibition of histamine release with salmeterol. In contrast, there was an evident correlation between the isoprenaline-induced increases in total cell cyclicAMP and the inhibition of histamine release by isoprenaline.

One characteristic feature of the action of salmeterol, both *in vivo* and *in vitro*, is a long duration of action (Ullman & Svedmyr, 1988; Ball *et al*., 1991; Nials *et al*., 1993; Tattersfield, 1993). In studies investigating smooth muscle relaxation (Nials *et al*., 1993) and inhibition of mediator release from lung fragments (Butchers *et al*., 1991), the relaxant and inhibitory effects of salmeterol persisted even after the tissues had been washed. However, in the present study washing removed the inhibitory effects of salmeterol on HLMC. The reasons for this apparent discrepancy are not known although differences in the nature of the respective systems may have some bearing on the experimental outcomes. It is possible that, relatively speaking, it may be easier to wash away salmeterol from cells in suspension as opposed to an intact tissue which could serve as a reservoir for a lipophilic drug like salmeterol. However, alternative studies in cell lines have shown that the effects of salmeterol persist following washing of the cells suggesting that, at least in these cell systems, the drug is retained by the cells following washing (McCrea & Hill, 1993; Clark *et al*., 1996). Should salmeterol be retained by isolated HLMC following washing, then the drug would be available for repeated and continued interactions with β-adrenoceptors which could lead to desensitization. Thus, desensitization of β-adrenoceptors in HLMC could serve as a possible explanation for the loss in the inhibitory activity of salmeterol following washing of the cells especially if, as in the present study, the cells are exposed to a high concentration of salmeterol.

The present study has provided evidence that salmeterol inhibits mediator release from HLMC. However, it has proved difficult to establish a unilateral mechanism by which salmeterol acts to inhibit HLMC responses. At high concentrations, salmeterol may act in a non-β-adrenoceptor-mediated manner to inhibit the responses of HLMC. This finding is not unexpected in view of data from alternative systems demonstrating a non-receptor-mediated mechanism of action for salmeterol at higher concentrations (Baker & Fuller, 1990; Nials *et al*., 1997). However, a striking finding of the present study is the ability of picomolar concentrations of salmeterol to inhibit the generation of sLT. In fact, salmeterol was more potent than isoprenaline as an inhibitor of sLT generation which is a surprising finding given that isoprenaline was more potent as an inhibitor of histamine release and that the intrinsic efficacy of isoprenaline at β_2-adrenoceptors is greater than that of salmeterol (Dougall *et al*., 1991; Nials *et al*., 1994). Thus, the intriguing possibility exists that non-β-adrenoceptor-dependent mechanisms are involved in the inhibition of mediator release at both very low and high concentrations of salmeterol. In attempts to elucidate further the mechanism of action of salmeterol on HLMC, studies to establish the effects of the enantiomeric forms of salmeterol as well as the extended lipophilic side chain of salmeterol could prove informative.

In summary, the present study has provided evidence that salmeterol inhibits mediator release from HLMC. The mechanism by which salmeterol acts to inhibit HLMC responses is complex, perhaps involving both β-adrenoceptor-dependent and independent mechanisms.

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