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Protection by dexamethasone of the functional desensitization to β_2-adrenoceptor-mediated responses in human lung mast cells

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1 The β-adrenoceptor agonist, isoprenaline, inhibited the IgE-mediated release of histamine from human lung mast cells (HLMC) in a dose-dependent manner. Maximal inhibitory effects were obtained with 0.1 μM isoprenaline. However, the inhibition of histamine release from HLMC by isoprenaline (0.1 μM) was highly variable ranging from 33 to 97% inhibition (mean, $59 \pm 3\%$, $n = 27$).

2 Long-term (24 h) incubation of HLMC with isoprenaline led to a subsequent reduction in the ability of a second exposure of isoprenaline to inhibit IgE-mediated histamine release from HLMC. The impairment in the ability of isoprenaline (0.1 μM) to inhibit histamine release following desensitizing conditions (1 μM isoprenaline for 24 h) was highly variable amongst HLMC preparations ranging from essentially negligible levels of desensitization in some preparations to complete abrogation of the inhibitory response in others (mean, $65 \pm 6\%$ desensitization, $n = 27$).

3 The ability of HLMC to recover from desensitization was investigated. Following desensitizing conditions (1 μM isoprenaline for 24 h), HLMC were washed and incubated for 24 h in buffer and the effectiveness of isoprenaline (0.1 μM) to inhibit IgE-mediated histamine release from HLMC was assessed. The extent of recovery was highly variable with some HLMC preparations failing to recover and others displaying a complete restoration of responsiveness to isoprenaline (mean, $40 \pm 6\%$ recovery, $n = 23$).

4 The effects of the glucocorticoid, dexamethasone, were also investigated. Long-term (24–72 h) treatments with dexamethasone (0.1 μM) had no effect on IgE-mediated histamine release from HLMC. Additionally, long-term (24–72 h) treatments with dexamethasone (0.1 μM) had no effect on the effectiveness of isoprenaline to inhibit histamine release. However, long-term (24–72 h) treatments with dexamethasone (0.1 μM) protected against the functional desensitization induced by incubation (24 h) of HLMC with isoprenaline (1 μM). The protective effect was time-dependent and pretreatment of HLMC with dexamethasone for either 24, 48 or 72 h prevented desensitization by either 15 ± 7, 19 ± 5 or $51 \pm 10\%$, respectively ($n = 5–7$).

5 HLMC preparations which were relatively refractory to isoprenaline even after withdrawal (24 h) from desensitizing conditions responded more effectively to isoprenaline (0.1 μM) if dexamethasone (0.1 μM) was also included during the recovery period ($19 \pm 9\%$ recovery after 24 h in buffer; $50 \pm 8\%$ recovery after 24 h with dexamethasone, $n = 5$).

6 These data indicate that the responses of different HLMC preparations to isoprenaline, the susceptibility of HLMC to desensitization and the ability of HLMC to recover from desensitizing conditions varies markedly. Dexamethasone, which itself has no direct effects on IgE-mediated histamine release from HLMC, protected HLMC from the functional desensitization to β-adrenoceptor agonists. Because β_2-adrenoceptor agonists and glucocorticoids are important in the therapeutic management of asthma and as the HLMC is probably important in certain types of asthma, these findings may have wider clinical implications.

Keywords: Mast cells; β_2-adrenoceptors; glucocorticoids; desensitization

Introduction

Bronchodilator β_2-adrenoceptor agonists continue to be important in the therapeutic management of asthma (Tattersfield, 1992). Whilst the major action of these compounds is to relax smooth muscle in the airways (bronchodilator effect), it is possible that β_2-adrenoceptor agonists may also act in asthma to inhibit mast cell responses (non-bronchodilator effect). Certainly, a large number of *in vitro* studies indicates that β_2-adrenoceptor agonists are very 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).

In recent years, concerns have been voiced about the safety of β_2-adrenoceptor agonists in the treatment of asthma (Barnes & Chung, 1992). Increased fatalities have been correlated with continued use of these compounds (Sears *et al*., 1990; Grainger *et al*., 1991; Spitzer *et al*., 1992). Although the reasons for these fatalities have not been determined, one possible contributory factor may include the acquisition of tolerance to β_2-adrenoceptor agonists. Although tolerance to the bronchodilator effects of β_2-adrenoceptor agonists is not thought to be important at conventional therapeutic doses (Svedmyr, 1990), tolerance to the extra-pulmonary side-effects of β_2-adrenoceptor agonists can be demonstrated (Holgate *et al*., 1980). Recent clinical studies have indicated that administration of β_2-adrenoceptor agonists may induce tolerance to the mast cell-stabilizing properties without impairing the bronchodilator properties of this class of drug (O'Connor *et al*., 1992;

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Cockcroft *et al*., 1993). This could promote a potentially undesirable situation in which mast cell mediator release would proceed unchecked and be masked by the symptomatic relief (by bronchodilatation) that β_2-adrenoceptor agonists would continue to provide.

Glucocorticoids are also important in the therapeutic management of asthma where they act to stem airways inflammation (Schleimer, 1990). In addition to their anti-inflammatory properties, it is possible that glucocorticoids may act to promote β_2-adrenoceptor responses (Svedmyr, 1990). For example, *in vivo* studies indicate that tolerance to β_2-adrenoceptor agonists may be reversed by glucocorticoid administration (Holgate *et al*., 1977).

At the molecular level, tolerance to β_2-adrenoceptor agonists may reflect β_2-adrenoceptor desensitization (Haudsorff *et al*., 1990). A large number of *in vitro* studies has indicated that exposure of β_2-adrenoceptors to agonists induces receptor desensitization (Conolly & Greenacre, 1976; Davis & Conolly, 1980; Galant *et al*., 1980; Avner & Jenne, 1982; Hui *et al*., 1982; Hasegawa & Townley, 1983; Van der Heijden *et al*., 1984). Desensitization is a complex process involving the rapid uncoupling of receptors followed by processes that can include sequestration and degradation of receptors (Haudsorff *et al*., 1990). Whereas short-term (minutes) exposure to an agonist may induce a form of desensitization that rapidly reverses (Hertel & Staehelin, 1983), long-term (hours) exposure to agonists promotes a form of desensitization that may take days to reverse (Doss *et al*., 1981; Scarpace *et al*., 1985).

We have previously shown that long-term incubation of HLMC with the β-adrenoceptor agonist, isoprenaline, induces a functional desensitization to β-adrenoceptor agonists (Chong *et al*., 1995). This ability to induce desensitization was also shared by the β_2-adrenoceptor-selective agonists, salbutamol, terbutaline and fenoterol. The extent of the desensitization was dependent on the concentration of isoprenaline and the length of exposure of HLMC to the agonist. A major aim of the present study was to establish how effectively HLMC resensitize to β-adrenoceptor-mediated responses following withdrawal of HLMC from desensitizing conditions and to determine whether the glucocorticoid, dexamethasone, would accelerate the resensitization process.

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: $CaCl_2.2H_2O$ 1 mM, $MgCl_2.6H_2O$ 1 mM, glucose 5.6 mM, bovine serum albumin (BSA) 1 mg ml^{-1} and 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

Dexamethasone (100 mM) was dissolved in dimethyl sulphoxide (DMSO) and stored frozen in appropriate aliquots. Stock solutions (10 mM) of (−)-isoprenaline bitartrate were prepared weekly in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl) and stored at 4°C.

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} tissue) containing collagenase Ia (350 u ml^{-1} PBS) 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 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 by use of an alcian blue stain (Gilbert & Ornstein, 1975). 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.

Mediator release

Histamine release experiments were performed in +PBS buffer. Histamine release was initiated immunologically with anti-IgE. The concentration of anti-IgE (1:1000) employed in these experiments induced between 63 and 95% (mean, 79 ± 4%) of the maximal response obtained with an optimal releasing concentration of 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). When isoprenaline was employed, cells were incubated with isoprenaline 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 ($\leqslant 7$%). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release.

In experiments in which long-term incubations were performed, RPMI 1640 buffer supplemented with penicillin/streptomycin (10 μg ml^{-1}) and gentamicin (50 μg ml^{-1}) was employed. Cells were incubated at a density of 0.1×10^6 HLMC ml^{-1} in 24 well plates with, usually, 0.5×10^6 HLMC per condition with or without isoprenaline and with or without dexamethasone. For incubations exceeding 24 h, cells were washed at every 24 h interval and reconstituted in fresh buffer with or without drug as appropriate. After completion of the incubations, the cells were washed and reconstituted in +PBS for mediator release experiments. Incubations of HLMC with either dexamethasone or 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, 6 ± 1, 7 ± 1 and 6 ± 1% at times 0, 24, 48 and 72 h, respectively. The percentage recovery of HLMC following long-term incubations was 94 ± 4% after 24 h, 74 ± 5% after 48 h and 31 ± 6% after 72 h.

Materials

The following were purchased from the sources indicated: anti-human IgE, BSA, collagenase, DNAse, dexamethasone, DMSO, HSA, (−)-isoprenaline (all Sigma, Poole, U.K.); calcium chloride and magnesium chloride (BDH, Poole, U.K.); RPMI 1640, gentamicin, penicillin/streptomycin (Gibco RBL).

Statistics

The statistical significance of drug-related effects was analysed by comparing control and treated cells by use of two-way ANOVA with respect to concentration and treatments. In all instances, the effects of long-term isoprenaline treatment had

no effect on the levels of control histamine release from HLMC activated with anti-IgE. In all experiments, the transformed data (ie % inhibition values) were subjected to statistical analyses. Values were considered significant at the $P<0.05$ level.

Results

Isoprenaline ($10^{-8}-10^{-5}$ M) inhibited the IgE-mediated release of histamine from HLMC (Figure 1). Long-term incubation (24 h) of HLMC with isoprenaline ($10^{-8}-10^{-5}$ M) led to a dose-dependent reduction in the subsequent ability of a second isoprenaline ($10^{-8}-10^{-5}$ M) exposure to inhibit histamine release from HLMC (Figure 1).

In experiments designed to determine whether HLMC would reacquire their responsiveness to isoprenaline following desensitizing conditions, HLMC were exposed (24 h) to buffer or isoprenaline (1 μM) or alternatively were exposed (24 h) to isoprenaline (1 μM), washed and then incubated in buffer for 24 h. After this time cells exposed to each of the three conditions were washed and incubated with isoprenaline ($10^{-9}-10^{-5}$ M) for 10 min before challenge with anti-IgE (1:1000). The mean data indicate that there was some restoration of responsiveness of HLMC to isoprenaline following a period of recovery from desensitizing conditions (Figure 2). However, inspection of the individual data for each of the 27 HLMC preparations used to construct Figure 2 revealed a more complex picture (Table 1). Some HLMC preparations (set 1, $n=4$) failed to desensitize appreciably, other HLMC preparations (set 2, $n=17$) desensitized well and recovered to some degree following removal from desensitizing conditions, whereas other preparations (set 3, $n=6$) desensitized well and failed to recover even after removal from desensitizing conditions. In 7 of the 17 HLMC preparations that comprise set 2, recovery was followed for 48 h. As a generalization, HLMC that were allowed to recover for 48 h following desensitizing conditions (1 μM isoprenaline for 24 h) were more responsive to isoprenaline (0.1 μM) than HLMC that were allowed to recover for 24 h and this increase was statistically significant ($P<0.01$) (59±7% recovery after 24 h; 75±7% recovery after 48 h, $n=7$).

In a further series of experiments the effects of dexamethasone on HLMC responses were evaluated. Long-term (24–72 h) incubation with dexamethasone (0.1 μM) alone had no

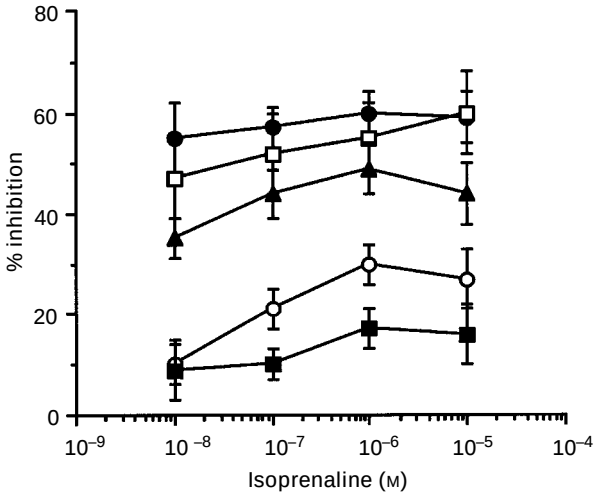


Figure 1 Dose-dependence of the isoprenaline-induced functional desensitization of HLMC. Cells were incubated for 24 h in the absence (●) and presence of isoprenaline at concentrations of 10^{-8} M (□), 10^{-7} M (▲), 10^{-6} M (○) and 10^{-5} M (■). After this incubation, the cells were washed and incubated for 10 min with isoprenaline ($10^{-8}-10^{-5}$ M) before challenge with anti-IgE (1:1000) for a further 25 min for histamine release. Results are expressed as the % inhibition of the control histamine release which ranged from 36±5% to 28±5%. Values are means and vertical lines show s.e.mean ($n=9$). Desensitizing concentrations of 10^{-7} M and above caused statistically significant ($P<0.0001$) reductions in the effectiveness of isoprenaline to inhibit histamine release.

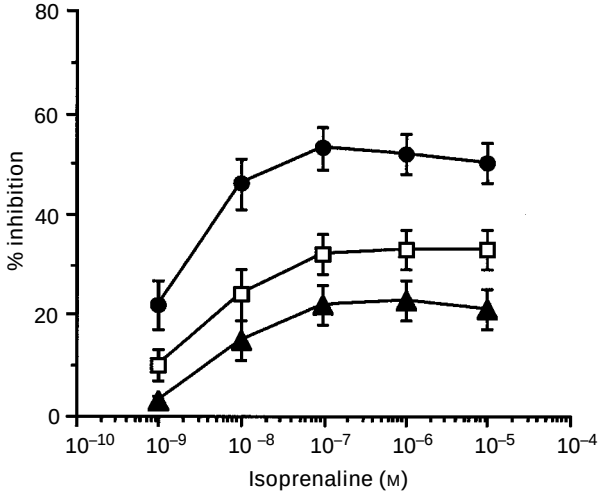


Figure 2 Recovery from desensitization. HLMC were incubated either for 48 h in buffer (●; untreated), for 24 h in buffer and then for 24 h with 1 μM isoprenaline (▲; desensitized) or for 24 h with 1 μM isoprenaline and then for 24 h in buffer (□; recovery). After this time, HLMC under each of the conditions were washed and incubated with isoprenaline ($10^{-9}-10^{-5}$ M) 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 43±2% (untreated), 43±2% (desensitized) and 42±2% (recovery). Values are means and vertical lines show s.e.mean, $n=27$. Statistically significant ($P<0.0001$) reductions in the effectiveness of isoprenaline to inhibit histamine release were observed between untreated and desensitized sets. HLMC that were allowed to recover responded more effectively to isoprenaline when compared to the desensitized set ($P<0.0001$).

Table 1 Variability in the susceptibility of HLMC preparations to desensitization

		% inhibition		
Set	n	*Control*	*Desensitization*	*Recovery*
1	4	63±12	55±10	54±10
2	17	59±3	16±4	38±4
3	6	56±8	13±4	16±5

HLMC preparations demonstrated marked differences both in the susceptibility to desensitization and in the ability to recover from desensitizing conditions. The table represents an alternative representation of the data used to construct Figure 2. HLMC preparations were incubated in either buffer (control), with isoprenaline (1 μM) for 24 h (desensitization) or subjected to desensitizing conditions and then allowed to recover for 24 h (recovery). The effectiveness of isoprenaline (0.1 μM) to inhibit IgE-mediated histamine release was assessed after these treatments. The data indicate that certain HLMC preparations are resistant to desensitization (set 1), certain HLMC preparations desensitize and recover following withdrawal from desensitizing conditions (set 2) and certain HLMC preparations desensitize but fail to recover following withdrawal from desensitizing conditions (set 3). Desensitization ranged from 0 to 23%, 38 to 100% and 53 to 88% in sets 1, 2 and 3, respectively. Resensitization was negligible in set 1 and ranged from 28 to 100% and 0 to 13% in sets 2 and 3, respectively. Values are expressed as the % inhibition by isoprenaline (0.1 μM) of control histamine release which ranged from 39±3 to 44±5% for all sets under each of the conditions. Values are means±s.e.mean.

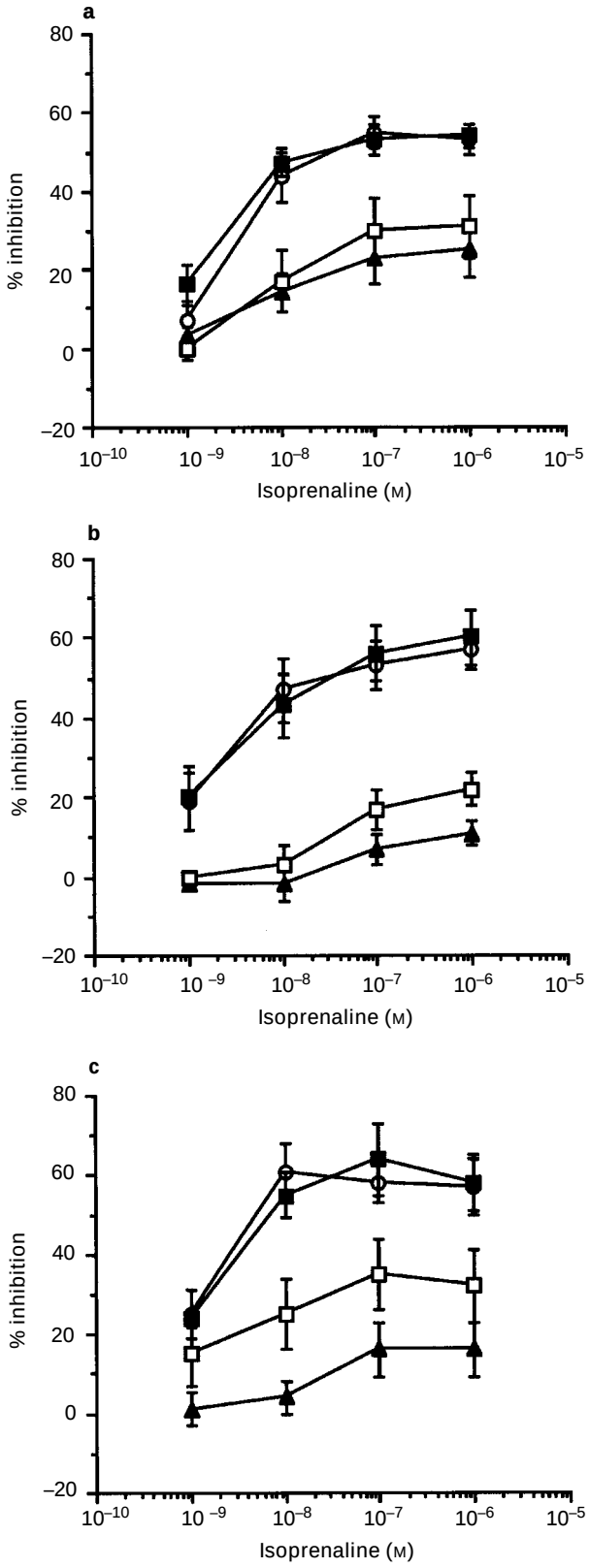
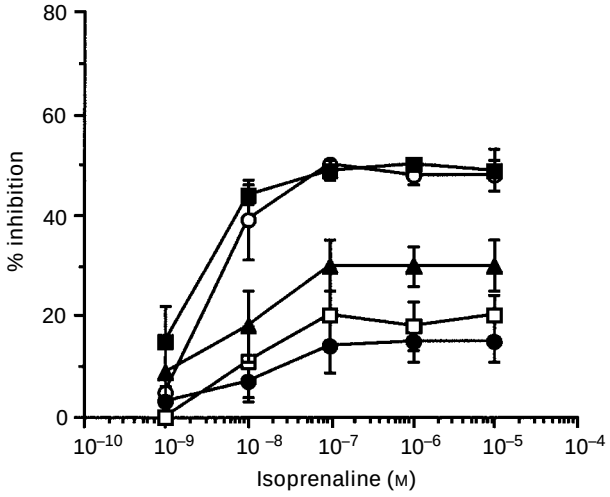


Figure 3 Protection by dexamethasone of the desensitization. The effects of 24 h (a), 48 h (b) and 72 h (c) pretreatments with dexamethasone (0.1 μM) on the functional desensitization induced by isoprenaline (1 μM) were determined. HLMC were incubated either (i) with buffer (○) for 24 to 72 h, (ii) with dexamethasone (■) for 24 to 72 h, (iii) with isoprenaline for the final 24 h of any given 24 to 72 h incubation protocol (▲) or, (iv) with dexamethasone for any given 24 to 72 h incubation protocol (□) and together with isoprenaline for the final 24 h. After these incubations, HLMC under each condition were washed and incubated with isoprenaline (10^{-9}–10^{-6} M) for 10 min before challenge with anti-IgE (1:1000). Values are expressed as the % inhibition of the control histamine release which ranged from 45±3 to 39±2% for (a), 41±5 to 34±3% for (b) and 47±1 to 43±4% for (c). Values are means and vertical lines show s.e.mean, $n=7$ (a), $n=6$ (b) and $n=5$ (c). Dexamethasone incubations of 48 and 72 h protected HLMC from desensitization to a statistically significant ($P<0.0001$) extent.



dexamethasone (0.1 μM) of the desensitization induced by isoprenaline (1 μM). HLMC were incubated either for 48 h with buffer (○), for 24 h with buffer and then for 24 h with dexamethasone (■), for 24 h with buffer and then for 24 h with isoprenaline (●; desensitized), for 24 h with isoprenaline and then for 24 h with buffer (□; recovery) or for 24 h with isoprenaline and then for 24 h with dexamethasone (▲; recovery in the presence of dexamethasone). After these treatments, HLMC were washed and then incubated for 10 min with isoprenaline (10^{-9}–10^{-5} M) before challenge with anti-IgE (1:1000) for a further 25 min. Values are expressed as the % inhibition of the control histamine release which ranged from 45±3 to 40±3%. Values are means and vertical lines show s.e.mean, $n=5$. The presence of dexamethasone during the recovery period (▲) increased the inhibitory effects of isoprenaline, when compared to recovery in the absence of dexamethasone (□), to a statistically significant ($P<0.0001$) degree.

effect on the stimulated release of histamine from HLMC. The effect of dexamethasone on the functional desensitization induced by prolonged exposure to isoprenaline was determined. Initially, HLMC were incubated (24 h) either (a) in buffer, (b) with dexamethasone (0.1 μM), (c) with isoprenaline (1 μM) or (d) with isoprenaline and dexamethasone together. Each set was washed and incubated (10 min) with isoprenaline (10^{-9}–10^{-6} M) and then challenged with anti-IgE for histamine release (Figure 3a). Long-term treatment with dexamethasone had no effect on the ability of isoprenaline to inhibit histamine release. However, dexamethasone did protect slightly against the isoprenaline-induced desensitization although this effect was not statistically significant ($P>0.05$). Further experiments were performed employing longer incubations with dexamethasone (Figure 3b). HLMC were incubated either (a) for 48 h in buffer, (b) with dexamethasone (0.1 μM) for 48 h, (c) with buffer for the first 24 h and with isoprenaline (1 μM) for the final 24 h or (d) with dexamethasone for 48 h and together with isoprenaline for the final 24 h. Long-term treatment with dexamethasone had no effect on the ability of isoprenaline to inhibit histamine release. However, dexamethasone did protect against the isoprenaline-induced desensitization and although the protection was modest it was statistically significant ($P<0.001$). The effect of a longer incubation with dexamethasone was also investigated (Figure 3c). HLMC were incubated either (a) for 72 h in buffer, (b) with dexamethasone (0.1 μM) for 72 h, (c) with buffer for the first 48 h and with isoprenaline (1 μM) for the final 24 h or (d) with dexamethasone for 72 h and together with isoprenaline for the final 24 h. Again, long-term treatment with dexamethasone had no effect

on the ability of isoprenaline to inhibit histamine release, whereas dexamethasone did protect against the isoprenaline-induced desensitization, an effect that was statistically significant ($P<0.0001$). Treatments of HLMC with dexamethasone for either 24, 48 or 72 h prevented the isoprenaline-induced desensitization of HLMC responses to 0.1 μM isoprenaline by either 15 ± 7, 19 ± 5 or $51\pm10\%$, respectively.

In addition to protection experiments, the effects of dexamethasone on the reconstitution of HLMC responsiveness to isoprenaline were determined. In a total of 11 experiments, 6 experiments failed to generate any meaningful data either because HLMC were refractory to desensitization or because those preparations that were sensitive to desensitizing conditions recovered effectively and responded well to isoprenaline following removal from desensitizing conditions. The remaining 5 experiments utilized HLMC preparations that desensitized readily following long-term treatment with isoprenaline and failed to recover following removal from desensitizing conditions for 24 h (ie set 3 in Table 1). In this subset of HLMC preparations, the presence of dexamethasone (0.1 μM) during the 24 h recovery period enhanced the responsivity of HLMC to isoprenaline, by a statistically significant ($P<0.0001$) extent compared to HLMC that were allowed to recover in the absence of dexamethasone (Figure 4).

Discussion

Bronchodilator β_2-adrenoceptor agonists continue to be important in the therapeutic management of asthma (Tattersfield, 1992). However, concerns surrounding the safety of this class of drug have been voiced (Barnes & Chung, 1992). Clinical studies indicate an association between continued use of β_2-adrenoceptor agonists and increased morbidity and mortality in asthmatics (Sears *et al*., 1990; Grainger *et al*., 1991; Spitzer *et al*., 1992). Although the reasons for these unfavourable sequelae have not been delineated, the possibility exists that tolerance to β_2-adrenoceptor agonists may constitute a contributory factor.

At the molecular level, tolerance may reflect the desensitization of β_2-adrenoceptors (Haudsorff *et al*., 1990). A large body of work has demonstrated that both short and long-term exposure of β_2-adrenoceptors to agonists induces receptor desensitization (Haudsorff *et al*., 1990). Our own previous studies indicate that long-term exposure of HLMC to β_2-adrenoceptor agonists induces a state of functional desensitization to β_2-adrenoceptor agonists (Chong *et al*., 1995). In the present study we have established that the extent of the functional desensitization is highly variable amongst HLMC preparations. Thus, certain HLMC preparations do not desensitize readily whereas others are highly susceptible to desensitization. Whilst the reasons for this wide range in susceptibility to desensitization are unknown, recent studies by others indicate that genotypic differences may contribute to the extent of β_2-adrenoceptor desensitization (Green *et al*., 1995). Thus, β_2-adrenoceptors that express the gly 16 genotype are more susceptible to desensitization whereas β_2-adrenoceptors that express the glu 27 genotype are resistant to down-regulation compared to 'wild-type' (arg 16, gln 27) receptors.

Our studies indicate that most HLMC preparations resensitize, at least to some degree, following withdrawal from desensitizing conditions. However, as a generalization, very few HLMC preparations that have been subjected to desensitizing conditions display full responsiveness to isoprenaline even following a 48 h recovery period. A number of studies has investigated resensitization although most of these studies are not immediately amenable to comparison with the present system because of differences in experimental conditions (Doss *et al*., 1981; Hertel & Staehelin, 1983; Stadel *et al*., 1983; Scarpace *et al*., 1985). However, in one study with astrocytoma cells in which the conditions were similar to those employed in the present study, a 12 h incubation with 0.1 μM isoprenaline induced a 95% loss in β_2-adrenoceptors and between 48 and 72 h were required for full restoration of β_2-adrenoceptor numbers (Doss *et al*., 1981). Although most HLMC preparations do show some recovery following withdrawal from desensitizing conditions, a subset of preparations desensitize readily following long-term exposure to isoprenaline and fail to show any degree of recovery from desensitization. Should similar processes be operative *in vivo*, then these findings may be important clinically. Any loss of mast cell-stabilizing activity by β_2-adrenoceptor agonists would be likely to impair the continued therapeutic effectiveness of this class of drug in asthma, an effect that may be accentuated in individuals whose mast cells either succumb to a tolerant state more readily or who experience difficulties in recovering from a tolerant state.

The effects on HLMC of the glucocorticoid, dexamethasone, were also investigated. In accord with previous findings (Schleimer *et al*., 1983), dexamethasone was found to be ineffective as an inhibitor of mediator release from HLMC. However, dexamethasone was found to protect against desensitization and to reverse the desensitized state in HLMC. Numerous *in vitro* studies have shown that glucocorticoids possess a generally permissive effect on β_2-adrenoceptor function (Svedmyr, 1990). For example, treatment of human lung tissue with dexamethasone has been shown to increase mRNA for β-adrenoceptors and to increase β-adrenoceptor numbers (Mak *et al*., 1995). Whether these studies on human lung tissue are representative of processes that may occur in the HLMC following dexamethasone administration is not presently known. Taken as a whole, these data suggest that glucocorticoids, as well as having recognised anti-inflammatory properties, may exert additional therapeutically desirable effects by acting to preserve β_2-adrenoceptor-mediated responses in HLMC.

How far these experiments represent the *in vivo* situation is difficult to determine. In the present study, the levels of mediator release are higher than would be expected *in vivo* and high concentrations of β-adrenoceptor agonist have been employed to counter these levels of secretion. The lower levels of mediator release that would be expected *in vivo* would be modulated by lower concentrations of β-adrenoceptor agonist. Moreover, the concentrations of β-adrenoceptor agonist that might be required to induce desensitization to these lower inhibitory concentrations of agonist may, as a consequence, be lower. It is possible, therefore, that the present model may serve as a valid, if exaggerated, representation of HLMC responses *in vivo*. These considerations apart, the potential importance of HLMC desensitization in limiting the therapeutic effectiveness of β_2-adrenoceptor agonists is highlighted by several clinical studies (O'Connor *et al*., 1992; Cockcroft *et al*., 1993). These studies have demonstrated that, following administration of a β_2-adrenoceptor agonist, tolerance to the mast cell-stabilizing effects of β_2-adrenoceptor agonists can be induced without affecting the bronchodilator effects of the agonist.

In summary, we have determined that a large degree of variability exists amongst HLMC preparations in responses to the β-adrenoceptor agonist, isoprenaline, sensitivity to desensitization and recovery from desensitization. These findings may be important in highlighting limitations that may be associated with the therapeutic utility of β_2-adrenoceptor agonists. Additionally, we have established that dexamethasone acts both to protect and to reverse desensitization to β_2-adrenoceptor-mediated responses in HLMC. This finding identifies an additional potential role for glucocorticoids that may be of wider clinical significance in the context of asthma therapy.

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