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Prostaglandin E_2 activates EP_2 receptors to inhibit human lung mast cell degranulation

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1 The prostanoid, PGE_2, is known to inhibit human lung mast cell activity. The aim of the present study was to characterize the EP receptor that mediates this effect.

2 PGE_2 (pEC_{50}, 5.8 ± 0.1) inhibited the IgE-mediated release of histamine from mast cells in a concentration-dependent manner. Alternative EP receptor agonists were studied. The EP_2-selective agonist, butaprost (pEC_{50}, 5.2 ± 0.2), was an effective inhibitor of mediator release whereas the EP_1/EP_3 receptor agonist, sulprostone, and the EP_1-selective agonist, 17-phenyl-trinor-PGE_2, were ineffective.

3 The DP agonist PGD_2, the FP agonist $PGF_{2\alpha}$, the IP agonist iloprost and the TP agonist U-46619 were ineffective inhibitors of IgE-mediated histamine release from mast cells.

4 PGE_2 induced a concentration-dependent increase in intracellular cAMP levels in mast cells.

5 The effects of the EP_1/EP_2 receptor antagonist, AH6809, and the EP_4 receptor antagonist, AH23848, on the PGE_2-mediated inhibition of histamine release were determined. AH6809 (pK_B, 5.6 ± 0.1) caused a modest rightward shift in the PGE_2 concentration–response curve, whereas AH23848 was ineffective.

6 Long-term (24 h) incubation of mast cells with either PGE_2 or butaprost (EP_2 agonist), but not sulprostone (EP_1/EP_3 agonist), caused a significant reduction in the subsequent ability of PGE_2 to inhibit histamine release.

7 Collectively, these data suggest that PGE_2 mediates effects on human lung mast cells by interacting with EP_2 receptors.
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Abbreviations: DMSO, dimethyl sulphoxide; E_{max}, maximal response; IBMX, isobutyl methylxanthine; PBS, phosphate-buffered saline

Introduction

Prostanoids are critically important endogenous regulators of a wide variety of physiological processes. This is highlighted by the manifold actions ascribed to PGE_2. For example, PGE_2 is known to be gastroprotective (Peskar *et al*., 2003), to induce both contraction and relaxation of different types of smooth muscle (Norel *et al*., 1999) and to mediate both pro- and anti-inflammatory effects (Tilley *et al*., 2001). A component of the anti-inflammatory properties of PGE_2 could be the stabilization of cell activity and, in this context, inhibitory effects of PGE_2 on mast cells could be important (Drury *et al*., 1998; Gauvreau *et al*., 1999; Hartert *et al*., 2000).

The mast cell has long been recognized as central to the mediation of allergic disorders such as asthma (Holgate *et al*., 1986). Activation of the mast cell, by allergens or other stimuli, leads to the generation and/or release of a wide variety of autacoids such as histamine, eicosanoids, cytokines and enzymes (Williams & Galli, 2000). These generated mediators influence the immediate environment. In the context of asthma, the generation of mediators from lung mast cells can result in both bronchoconstriction and inflammation (Bingham & Austen, 2000). In addition to these more immediate effects, it is quite probable that, over the longer term, mast cell-derived products contribute to airway remodelling (Elias *et al*., 1999; Holgate *et al*., 2003). Mast cell-derived mediators can, therefore, alter lung physiology over both the short and longer term. As PGE_2 can prevent the release of mediators from mast cells, the prostanoid may play an important role as a physiological regulator of mast cell activity.

PGE_2 mediates effects by interacting with EP receptors. Four EP receptors have been identified that are G-protein coupled (Coleman *et al*., 1994; Breyer *et al*., 2001). EP_1 receptors mediate elevations of intracellular calcium, EP_2 and EP_4 receptors activate adenylyl cyclase, whereas EP_3 receptors have been shown to inhibit and to activate adenylyl cyclase as well as drive calcium mobilization (Irie *et al*., 1993; An *et al*., 1994; Breyer *et al*., 2001). Agonists and antagonists to these receptors have been developed although selectivity of action can be an issue with some of these ligands (Kiriyama *et al*., 1997; Abramovitz *et al*., 2000; Wilson *et al*., 2004). However, judicious use of these compounds can go a long way in identifying the EP receptor that mediates a response. The primary aim of the present study was to identify the EP receptor that modulates human lung mast cell activity.

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Methods

Buffers

Tyrode's buffer contained (mM): NaCl 137, HEPES 1.2, KCl 2.7, $NaH_2PO_4 \cdot H_2O$ 0.04, glucose 5.6. Tyrode's-FBS was Tyrode's which additionally contained: $CaCl_2 \cdot 2H_2O$ 0.5 mM, $MgCl_2 \cdot 6H_2O$ 1 mM, FBS 2%, DNase 15 $\mu g\,ml^{-1}$. Phosphate-buffered saline (PBS) contained (mM): NaCl 137, $Na_2HPO_4 \cdot 12H_2O$ 8, KCl 2.7, KH_2PO_4 1.5, $CaCl_2 \cdot 2H_2O$ 1, $MgCl_2 \cdot 6H_2O$ 1, glucose 5.6, HSA 30 $\mu g\,ml^{-1}$. The pH of Tyrode's buffers and PBS was titrated to 7.3.

Preparation of compounds

Stock solutions (10 mM) of PGE_2, PGD_2, $PGF_{2\alpha}$, 17-phenyl-trinor-PGE_2, butaprost methyl ester, sulprostone and U-46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$) were prepared in ethanol and stored at $-20^{\circ}C$. Iloprost was provided as a stock solution (13.9 mM) in methyl acetate and stored at $-20^{\circ}C$. AH6804 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) and AH23848 ([1α(z),2β,5α]-($\pm$)-7-[5-[[1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) were prepared as stock solutions of 10 and 100 mM, respectively, in dimethyl sulphoxide (DMSO) and stored at $-20^{\circ}C$. Stock solutions (10 mM) of (−)-isoprenaline bitartrate were prepared weekly in 0.05% sodium metabisulphite (dissolved in 0.9% NaCl) and stored at $4^{\circ}C$. Forskolin (100 mM) was made up as a stock in DMSO and stored at $-20^{\circ}C$. Formoterol fumarate (10 mM) was prepared weekly as a stock in DMSO and stored at $4^{\circ}C$. Isobutyl methylxanthine (IBMX) and dibutyryl-cAMP (Bu_2-cAMP) were made up daily as stock solutions (2 mM) in PBS buffer. Lyophilized polyclonal goat anti-human IgE antibody was reconstituted in distilled water and stored at $4^{\circ}C$.

Lung tissue

Non-lesional tissue from lung resections of patients was obtained following surgery. Most of the patients were undergoing surgery for carcinoma. The male to female split was 60–40 and 90% of the patients were white caucasians. The provision of lung tissue and the use of the tissue in this study were approved by the Local Research Ethics' Committee.

Isolation of mast cells

Mast cells were isolated from human lung tissue by a modification of the method described (Ali & Pearce, 1985). The tissue was stripped of its pleura and chopped vigorously for 15 min with scissors in a small volume of Tyrode's buffer. The chopped tissue was washed over a nylon mesh (100 μm pore size; Incamesh Filtration, Warrington, U.K.) with 0.5–1 l of Tyrode's buffer to remove lung macrophages. The tissue was reconstituted in Tyrode's-FBS (10 ml g^{-1} of tissue) containing collagenase Ia (15 mg per 100 ml of Tyrode's-FBS) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at $37^{\circ}C$. The supernatant was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of Tyrode's-FBS buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with Tyrode's-FBS (300–600 ml). The pooled filtrates were sedimented ($120 \times g$, room temperature, 8 min), the supernatant discarded and the pellets reconstituted in Tyrode's-FBS (100 ml). The pellet was washed a further two times. The dispersion procedure generated $0.2–1 \times 10^6$ mast cells per g of lung tissue at 5–20% purity as assessed by alcian blue staining (Gilbert & Ornstein, 1975). These cell preparations were used in histamine release experiments. Mast cell-enriched preparations (>30% purity) were generated by countercurrent elutriation (Beckman J6B centrifuge, JE-5.0 elutriator head) and further purification ($\geq$74%) was achieved by flotation of mast cell-enriched preparations over Percoll density gradients using slight modifications of the methods that have been described in detail elsewhere (Schulman *et al*., 1982; Ishizaka *et al*., 1983). Purified mast cells were used in cAMP assays.

Histamine release

Histamine release experiments were performed in PBS. Histamine release from mast cells was initiated immunologically with a maximal releasing concentration of anti-IgE (1 : 300). Secretion was allowed to proceed for 25 min at $37^{\circ}C$ after which time the cells were pelleted by centrifugation ($400 \times g$, room temperature, 3 min). Histamine released into the supernatant was determined by a modification of the automated fluorometric method of Siraganian (1974). When prostanoids or alternative cAMP-active compounds were employed, cells were incubated with inhibitor for 10 min at $37^{\circ}C$ before the addition of stimulus and then samples were processed as indicated above. Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in buffer alone served as a measure of spontaneous histamine release (<6%). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release.

When long-term incubations were performed, RPMI 1640 buffer supplemented with penicillin/streptomycin (10 $\mu g\,ml^{-1}$) and gentamicin (50 $\mu g\,ml^{-1}$) was employed. Cells were incubated (24 h) at a density of 0.1×10^6 mast cells per ml in six-well plates with, usually, 0.5×10^6 mast cells per condition with or without a prostanoid. After completion of the incubations, the cells were washed three times with PBS and reconstituted in the same buffer for mediator release experiments. Incubations of mast cells with prostanoids had no effect on either the total number of mast cells recovered, the total histamine content or the spontaneous histamine release compared to mast cells incubated in buffer.

Assays for cAMP

Total cell cAMP levels were monitored according to methods that have been described elsewhere (Chong *et al*., 1998). Purified cells were incubated (10 min) without or with PGE_2 and the reaction terminated by the addition of ice-cold acidified ethanol and snap-freezing of samples in liquid nitrogen. After thawing, samples were pelleted by centrifugation, supernatants saved and the ethanol evaporated using a rotary evaporator. Samples were reconstituted in assay buffer and cAMP levels were determined by radioimmunoassay.

Materials

The following were purchased from the sources indicated; AH6809, AH23848, butaprost methyl ester, sulprostone, PGE_2, anti-human IgE, Bu_2-cAMP, collagenase, DNase, forskolin, HSA, IBMX, isoprenaline, Percoll (all Sigma, Poole, U.K.); U-46619 and 17-phenyl-trinor-PGE_2 (Biomol, Plymouth Meeting, PA, U.S.A.); iloprost (Cayman Chemical, Ann Arbor, MI, U.S.A.); formoterol (Yamanouchi, Ibaraki, Japan) was kindly provided as a gift.

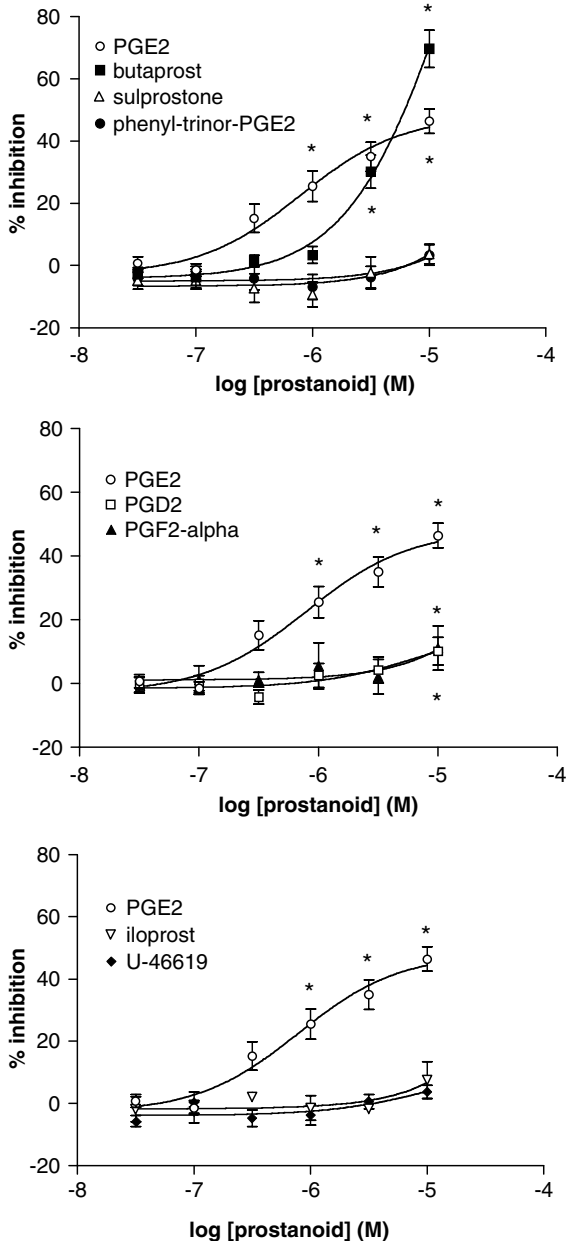

Figure 1 Effects of prostanoids on mast cells. Cells were incubated for 10 min with a given prostanoid before challenge with anti-IgE (1 : 300) for 25 min for histamine release. Values are expressed as the percentage inhibition of the control histamine release which was $23 \pm 4\%$. Values are means $\pm$ s.e.m. for eight (PGE_2, PGD_2, $PGF_{2\alpha}$, butaprost, sulprostone) or four (17-phenyl-trinor-PGE_2, iloprost, U-46619) experiments. Asterisks denote statistically significant ($P<0.05$ at least) effects. Split panels are provided to aid clarity.

Data analysis

Maximal responses (E_{max}) and potencies (pEC_{50}) were determined by nonlinear regression analysis (GraphPad Prism, version 3.0a). Antagonist affinity was estimated using the following formula: $pK_B = \log(\text{dose ratio} - 1) - \log(\text{antagonist concentration})$, where pK_B is the negative logarithm of the apparent dissociation constant and the dose ratio is the ratio of EC_{50} values in the presence and absence of antagonist. Statistical significance was assessed utilizing either repeated measures ANOVA or Student's *t*-test.

Results

Studies with agonists

PGE_2 (3×10^{-8}–10^{-5} M) inhibited IgE-mediated histamine release from human lung mast cells in a concentration-dependent manner (Figure 1). Alternative EP receptor ligands (3×10^{-8}–10^{-5} M) were also studied. Of these only the EP_2-selective agonist, butaprost, inhibited histamine release from mast cells whereas neither sulprostone (EP_1/EP_3 ligand) nor 17-phenyl-trinor-PGE_2 (EP_1 agonist) was effective (Figure 1a). Neither the DP receptor agonist PGD_2 nor the FP receptor agonist $PGF_{2\alpha}$ was an effective inhibitor of IgE-dependent histamine release (Figure 1b). Moreover, neither the IP receptor agonist iloprost nor the TP receptor agonist U-46619 had any effect on the IgE-mediated release of histamine from mast cells (Figure 1c).

In these initial experiments, close to maximal inhibitory effects were obtained with PGE_2, but not with butaprost, over the concentration range used. Therefore, in additional experiments, higher concentrations of butaprost (and PGE_2) were studied in order to achieve maximal inhibition and, thereby, more reliable values for agonist potency (Table 1). PGE_2 was about fivefold more potent than butaprost as an inhibitor of IgE-mediated histamine release but butaprost was the more efficacious agonist.

PGE_2 caused a concentration-dependent increase in total cell cAMP levels in human lung mast cells (Figure 2). In a total of nine experiments, PGE_2 (3×10^{-5} M) caused a $176 \pm 52\%$ ($P<0.05$) enhancement in cAMP levels in mast cells (purity, 74–91%; mean, $81 \pm 2\%$) over basal.

Studies with antagonists

The effect of AH6809, an antagonist at EP_1 and EP_2 receptors, on the PGE_2 inhibition of histamine release was investigated (Figure 3). AH6809 (10^{-5} M) caused an approximately fivefold

Table 1 E_{max} and pEC_{50} values for PGE_2 and butaprost

	PGE_2	*Butaprost*
E_{max} (%)	58.1 ± 3.3	87.2 ± 4.4
pEC_{50}	5.81 ± 0.11	5.15 ± 0.16

Mast cells were incubated for 10 min with or without either PGE_2 (10^{-7}–3×10^{-5} M) or butaprost (10^{-7}–3×10^{-5} M) before challenge with anti-IgE (1 : 300) for 25 min. Concentration–response curves for the inhibition of histamine release by the prostanoids were constructed and E_{max} and pEC_{50} values determined for each experiment. Values are means $\pm$ s.e.m. of 23 (PGE_2) and six (butaprost) experiments.

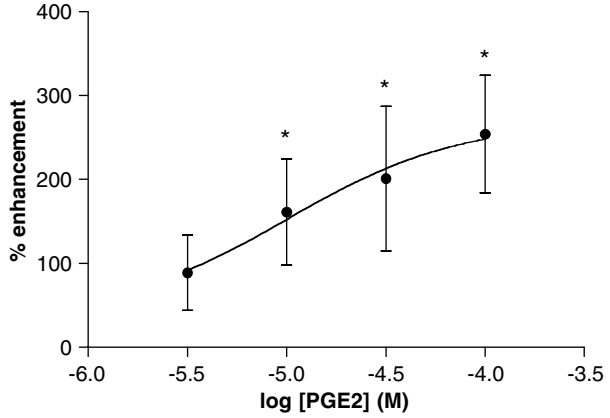

Figure 2 Effect of PGE_2 on cAMP levels in mast cells. Mast cells were incubated without or with increasing concentrations of PGE_2 for 10 min. After this incubation, the cells were snap frozen, solubilized and total cell cAMP levels measured. Results are expressed as the percentage enhancement in cAMP levels over basal. Mast cell purities ranged from 74 to 87% (mean, 80±3%). Values are means±s.e.m. for five experiments. Asterisks denote statistically significant ($P<0.05$ at least) effects.

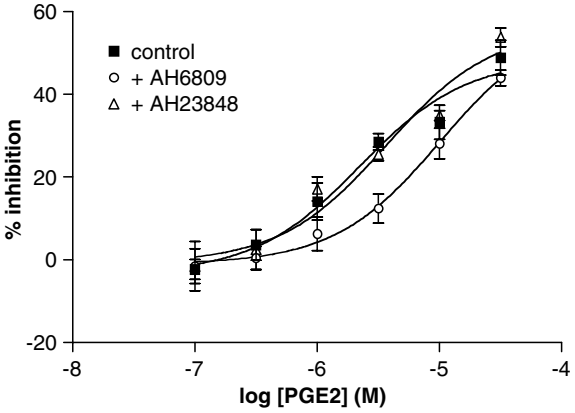

Figure 3 Effect of prostanoid receptor antagonists on the PGE_2 inhibition. Mast cells were incubated without or with either AH6809 (10^{-5} M) or AH23848 (3×10^{-5} M) and without or with PGE_2 for 10 min before challenge with anti-IgE (1 : 300) for 25 min. Values are expressed as the percentage inhibition of control histamine release which were 36±2 (control), 35±4 (AH6809) and 30±5% (AH23848). Values are means±s.e.m. for five experiments.

rightward shift in the PGE_2 concentration response curve. Reliable EC_{50} values for PGE_2, in the presence of antagonist, could only be determined in three of the five experiments performed. Based on data from these three experiments a pK_B value of 5.6±0.1 was calculated for AH6809. By contrast, AH23848 (3×10^{-5} M), which is known to act at EP_4 receptors but not EP_2 receptors, failed to antagonize the inhibitory effects of PGE_2 (Figure 3).

Desensitization studies

In order to gain further insight into the EP receptor that mediates the effects of PGE_2 in mast cells, cross-desensitization experiments were performed. Long-term exposure (24 h) of mast cells to either PGE_2 (10^{-5} M) or butaprost (10^{-5} M), but not sulprostone (10^{-5} M), attenuated the subsequent effectiveness of PGE_2 (10^{-7}–3×10^{-5} M) to inhibit IgE-mediated histamine release (Figure 4). Long-term treatment (24 h) of mast cells with PGE_2 (10^{-5} M) did not affect the ability of the β-adrenoceptor agonists, isoprenaline and formoterol, to inhibit IgE-mediated histamine release (Figure 5, Table 2). Moreover, long-term incubation (24 h) of mast cells with PGE_2 did not influence the inhibitory activity of either forskolin, a direct activator of adenylyl cyclase, IBMX, a non-selective phosphodiesterase inhibitor or, Bu_2-cAMP, a direct activator of protein kinase A (Table 2).

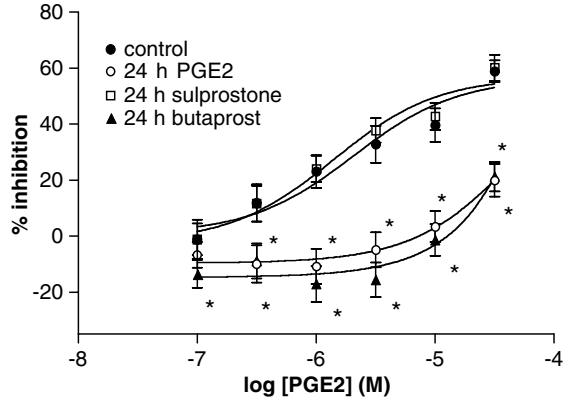

Figure 4 Desensitization of PGE_2-mediated responses in mast cells. Cells were incubated (24 h) without (control) or with PGE_2, sulprostone or butaprost (all at 10^{-5} M) and then washed extensively. Cells were then incubated without or with increasing concentrations of PGE_2 for 10 min before challenge with anti-IgE (1 : 300) for 25 min for histamine release. Values are expressed as the percentage inhibition of the unblocked histamine releases which ranged from 27±7 to 33±8% after 24 h treatments with buffer or agonists. Values are means±s.e.m. for six experiments. Asterisks denote statistically significant ($P<0.05$ at least) reductions in inhibition following long-term treatments with agonists.

Discussion

In the present study, we have attempted to characterize the receptor that mediates the effects of PGE_2 on human lung mast cells. In accord with previous studies, PGE_2 was found to inhibit IgE-mediated histamine release from human lung mast cells in a concentration-dependent manner suggesting that mast cells express EP receptors (Drury *et al*., 1998). Indeed it seems probable that, of the main classes of prostanoid receptor, mast cells express EP receptors alone because agonists directed at DP (PGD_2), FP ($PGF_{2\alpha}$), IP (iloprost) and TP (U-46619) receptors were ineffective in mast cells. The very modest effects of PGD_2 and $PGF_{2\alpha}$ in mast cells (Figure 1b) probably represent interactions of these agonists with EP receptors.

A variety of EP receptor agonists were also studied and of these only butaprost (EP_2-selective agonist) was effective, whereas neither sulprostone (EP_1/EP_3 ligand) nor 17-phenyl-trinor-PGE_2 (EP_1 agonist) displayed any activity. These data suggest that mast cells express EP_2 receptors as responses to butaprost are considered diagnostic for EP_2 receptors (Kiriyama *et al*., 1997). However, it should be noted that, in this study, the commercially available methyl ester form of butaprost, rather than the free acid, was employed. The free acid and methyl ester forms of butaprost have been

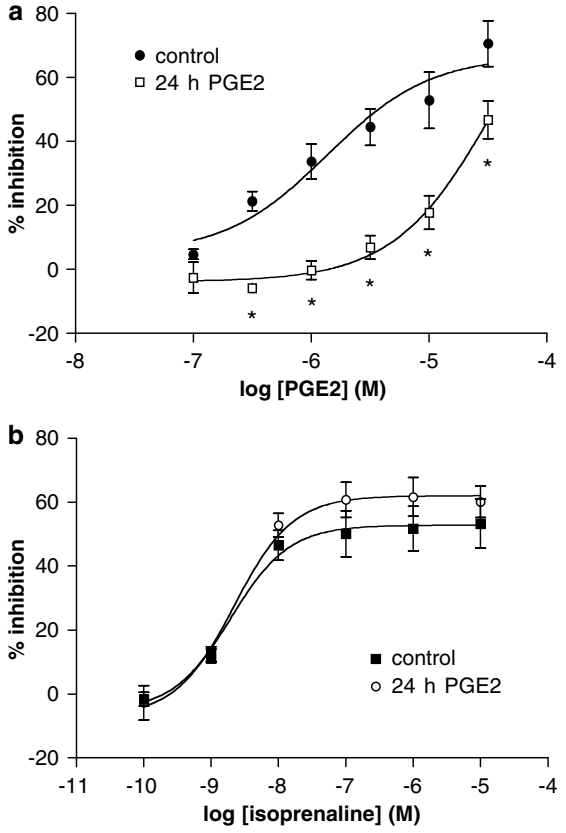

Figure 5 Effect of PGE_2 treatment on β-adrenoceptor-mediated responses in mast cells. Cells were incubated (24 h) without (control) or with PGE_2 (10^{-5} M) and then washed extensively. Cells were then incubated without or with increasing concentrations of either (a) PGE_2 or (b) isoprenaline for 10 min before challenge with anti-IgE for 25 min for histamine release. Values are expressed as the percentage inhibition of the control histamine releases and these were 36 ± 4 (control) and $28\pm5\%$ (PGE_2-treated). Values are means$\pm$s.e.m. for five experiments. Asterisks denote statistically significant ($P<0.05$ at least) reductions in inhibition following long-term PGE_2 treatment.

Table 2 Effect of long-term PGE_2 treatment on the inhibitory activities of cAMP-elevating compounds

	% Inhibition	
	Control	*PGE_2-treated*
PGE_2 (10^{-5} M)	45 ± 6	21 ± 5*
Formoterol (10^{-6} M)	42 ± 5	41 ± 7
Forskolin (10^{-5} M)	80 ± 6	85 ± 3
IBMX (3×10^{-4} M)	59 ± 8	67 ± 4
Bu_2-cAMP (10^{-3} M)	48 ± 5	44 ± 7

Mast cells were incubated (24 h) without (control) or with PGE_2 (10^{-5} M) and then washed extensively. The cells were then incubated with a cAMP-elevating compound for 10 min before challenge with anti-IgE (1 : 300) for a further 25 min for histamine release. Values are expressed as the percentage inhibition of the control histamine releases and these were 39 ± 7 (control) and $34\pm8\%$ (PGE_2-treated). Values are means$\pm$s.e.m. for five experiments. Asterisk denotes a significantly different ($P<0.01$) level of inhibition following PGE_2 treatment compared to control.

shown to be, respectively, about two- and 30-fold less potent than PGE_2 at EP_2 receptors (Wilson *et al*., 2004). That butaprost methyl ester was only fivefold less potent than PGE_2 as an inhibitor of histamine release indicates that the methyl ester was more potent than might have been anticipated. This increase in relative potency could be due, at least in part, to the conversion of the methyl ester, by cellular esterases, to the free acid (Abramovitz *et al*., 2000; Wilson *et al*., 2004). However, that butaprost was more efficacious than PGE_2 in mast cells, is less readily explained by a mechanism involving significant conversion of the methyl ester to the free acid as butaprost free acid has been shown to be a partial agonist relative to PGE_2 in a recombinant cell system (Wilson *et al*., 2004). However, differences in the responses of a given receptor to ligands, when expressed in different systems, might be anticipated. Moreover, the type of functional output used to monitor receptor behaviour may also influence the relative activity of ligands. Taking these considerations as a whole, that mast cells respond to butaprost methyl ester suggests that mast cells express EP_2 receptors. However, these studies indicate that care needs to be exercised in the interpretation of data when the methyl ester form of butaprost is employed as a probe (Wilson *et al*., 2004).

That PGE_2 induced increases in intracellular levels of cAMP in mast cells also provides support for the expression of EP_2 receptors (Stillman *et al*., 1999; Wilson *et al*., 2004) but these experiments do not exclude the possibility that mast cells express EP_4 receptors as these also are coupled to adenylyl cyclase (Wilson *et al*., 2004). In addition, certain EP_3 receptor splice variants have been shown to be coupled to adenylyl cyclase (Irie *et al*., 1993) but as sulprostone, an agonist at EP_3 receptors, was ineffective it seems unlikely that EP_3 receptors mediate the effects of PGE_2 in mast cells.

The finding that AH6809 antagonized the effects of PGE_2 in mast cells provides further evidence that mast cells express EP_2 receptors. Moreover, the affinity of AH6809 (pK_B, 5.6 ± 0.1) as an antagonist of the effects of PGE_2 in mast cells is consistent with the findings of others investigating effects at EP_2 receptors (Norel *et al*., 1999; Clarke *et al*., 2004). It should be noted that AH6809 is not EP_2-selective having activity at EP_1, DP and TP receptors (Kiriyama *et al*., 1997; Abramovitz *et al*., 2000). However, the failure of the agonists 17-phenyl-trinor-PGE_2, iloprost and U-46619 to affect mediator release from mast cells argues against the expression of these receptors in this system. An alternative antagonist, AH23848, failed to antagonize the inhibitory effects of PGE_2 in mast cells. As AH23848 has activity at EP_4 receptors (and TP receptors) but is essentially ineffective at EP_2 receptors (Abramovitz *et al*., 2000) these data suggest that PGE_2 does not mediate effects through EP_4 receptors in mast cells.

In order to gain further insight into the EP receptor that mediates the effects of PGE_2 in mast cells, cross-desensitization experiments were performed. This was determined by considering the effects of long-term exposure of mast cells to PGE_2 and other agonists on the subsequent ability of PGE_2 to inhibit mediator release from mast cells. Long-term treatment of mast cells with PGE_2 substantially suppressed the subsequent ability of PGE_2 to stabilize mast cell responses. The desensitizing treatment selectively affected PGE_2 as the inhibitory responses of isoprenaline and formoterol (β-adrenoceptor agonists), forskolin (direct activator of adenylyl cyclase), IBMX (a phosphodiesterase inhibitor) and Bu_2-cAMP (activator of protein kinase A) were not affected by the treatment. On the basis of these data, it is likely that long-term treatment of mast cells with PGE_2 leads to the selective desensitization of EP

receptors (Nishigaki *et al*., 1996; Bastepe & Ashby, 1997). In further studies, it was established that long-term treatment of mast cells with the EP_2-selective ligand, butaprost, also led to a substantial reduction in the subsequent inhibitory effects of PGE_2. By contrast, long-term treatment of mast cells with sulprostone, which acts at EP_1 and EP_3 receptors, had no effect on cell responses to PGE_2. That butaprost mimicked the desensitizing capability of PGE_2 provides additional support for the expression of EP_2 receptors by mast cells.

It is of interest that mast cells isolated from different species differ in the manner by which they respond to PGE_2. For example, PGE_2 inhibits the release of mediators from rat peritoneal mast cells but this is likely to be mediated by an inhibitory DP receptor as little experimental evidence for the expression of EP receptors exists in these cells (Chan *et al*., 2000). By contrast, PGE_2 enhances the antigen-driven release of mediators from mouse bone marrow-derived mast cells, an effect that is mediated by EP_3 receptors (Nguyen *et al*., 2002). These differences in the response of rodent and human mast cells to PGE_2 highlight the recognized functional heterogeneity that exists among mast cells isolated from different species (Pearce, 1983; Lowman *et al*., 1988).

As well as differences among species, there appear to be quite marked differences in the response to PGE_2 of human mast cells derived from different sites. In human cord blood-derived mast cells, PGE_2 alone has been shown to stimulate the release and generation of vascular endothelial growth factor by an EP_2 receptor-mediated mechanism without affecting the release of the granule-associated mediator, β-hexosimanidase (Abdel-Majid & Marshall, 2004). These findings in human cord blood-derived mast cells are in direct contrast to findings in human lung mast cells in which the EP_2 receptor has been shown, in the present study, to be inhibitory to mast cell function. These highly discordant findings could perhaps be explained by the fact that mast cells from human lung are predominantly tryptase-containing (MC_T mast cells) whereas cord blood-derived mast cells contain significantly greater numbers of mast cells containing tryptase and chymase (MC_{TC} mast cells) (Shichijo *et al*., 1999; Ahn *et al*., 2000; Oskeritzian *et al*., 2005). As MC_T and MC_{TC} mast cells are known to be functionally heterogeneous (Oskeritzian *et al*., 2005) this could provide a potential explanation for the differences in response to PGE_2 in human mast cells. This potential explanation breaks down in light of data showing that agents that elevate cAMP inhibit the IgE-dependent release of histamine and the *de novo* generation of eicosanoids and cytokines from both cord blood-derived mast cells and human lung mast cells (Weston & Peachell, 1998; Shichijo *et al*., 1999). However, it is possible that cord blood-derived mast cells represent a highly diverse subset of mast cells whose phenotype may be heavily influenced by the culture conditions different groups employ (Shichijo *et al*., 1999; Ahn *et al*., 2000; Abdel-Majid & Marshall, 2004). By extension, this could determine the way in which cord blood-derived mast cells respond to agents such as PGE_2.

A growing appreciation that PGE_2 exerts an important homeostatic role in the lung has evolved more recently (Vancheri *et al*., 2004). For example, PGE_2 has been shown to relax human bronchi *via* an EP_2-mediated mechanism and activation of EP_2 receptors on airway smooth muscle cells attenuates cytokine generation and proliferation (Norel *et al*., 1999; Clarke *et al*., 2004; Vancheri *et al*., 2004). Indeed, the EP_2 receptor has been found to inhibit the activity of a wide variety of inflammatory cells (Tilley *et al*., 2001; Harris *et al*., 2002). In general agreement with these studies, the present work has shown that EP_2 receptors can stabilize human lung mast cell activity. Collectively, these data suggest that targeting EP_2 receptors in the lung could prevent airway smooth muscle contraction and attenuate pulmonary inflammation.

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