

Short communication

Prostaglandin E₂ potentiates the immunologically stimulated histamine release from human peripheral blood-derived mast cells through EP₁/EP₃ receptors

Background: Mast cells cultured from human peripheral blood have been widely used to study human mast cell function. Prostanoids are the important regulators of mast cell activity, however, there were no reports about the class of prostanoid receptors expressed on such cultured cells.

Aims: The present study was to characterize pharmacologically the prostanoid receptors by investigating the effects of prostanoid receptor agonists on the immunoglobulin E (IgE)-mediated histamine release from the cultured mast cells.

Methods: Mast cells cultured from human progenitor cells in peripheral blood were sensitized with human myeloma IgE, and then challenged with anti-human IgE following pretreatment with diverse prostanoid receptor agonists. The histamine content in supernatants and cell pellets were measured by histamine auto-analyzer.

Results: Of the prostanoid receptor agonists tested, the prostaglandin E₂ (PGE₂) receptor (EP receptor) agonist PGE₂ (10^{-7} to 10^{-11} M) produced concentration-related potentiation of IgE-mediated histamine release from the cultured mast cells. Sulprostone, an EP₁/EP₃ agonist, SC-46275, a selective EP₃ agonist, and 11-deoxy-PGE₁, a selective EP₂/EP₃/EP₄ agonist also caused a significant increase in histamine release induced by anti-IgE. BW245C, fluprostone, cicaprost and U46619 for the prostaglandin D₂, F_{2α}, I₂, and thromboxane A₂ receptors respectively, and the EP₂/EP₄ receptor agonist butaprost had little effect on anti-IgE stimulated histamine release from mast cells.

Conclusions: The present results suggest that PGE₂ potentiates the IgE-mediated histamine release from the cultured mast cell via EP₃ and/or EP₁ receptors.

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Prostanoids consisting of prostaglandin (PG) D₂, PGE₂, PGF_{2α}, PGI₂ and thromboxane A₂ (TXA₂) are the primary products of cyclooxygenase metabolism of arachidonic acid (1). In addition to a wide range of physiological actions, these prostanoids also play a vital role in a number of pathological processes, especially in the inflammatory disorders (2, 3). Prostanoids have shown to be an important modulator of inflammatory cell activity during inflammatory responses (3).

Mast cells are known for their critical roles in asthma, allergic reactions and other inflammatory diseases attributed to their potent capability to produce multiple proinflammatory mediators after activation (4). A series of studies have demonstrated that prostanoids, particularly the PGD₂ and PGE₂, were able to regulate the synthesis and release of mast cell products. For instance, PGD₂ effectively inhibited immunologically stimulated histamine release from rat mast cells (5). Prostaglandin E₂

inhibited IgE-mediated histamine and leukotriene C₄ release from human lung mast cells (6), but enhanced anti-IgE induced production of IL-6 by the rat peritoneal mast cells and murine bone marrow-derived mast cells (7).

Like other local hormones, prostanoids exert their actions through binding the five major specific receptors for PGD₂ (DP receptor), PGE₂ (EP receptor), PGF_{2α} (FP receptor), PGI₂ (IP receptor) and TXA₂ (TP receptor), which are linked to different transduction cascades in targeted cells (2, 3). It is, therefore, critical to identify the prostanoid receptor types to understand the modification of prostanoids on mast cell function. Although mast cells cultured from human peripheral blood have been widely used in different research fields relevant to mast cells, there were no reports about the characterization of prostanoid receptors expressed on such cultured cells. The present study was to characterize

pharmacologically the prostanoid receptors involved in the regulation of IgE-mediated histamine release from the cultured mast cells.

Materials and methods

Materials

Prostaglandin E₂, BW 245C (Cayman Chemicals, Ann Arbor, MI, USA) and U-46619 (Sigma Chemicals, St Louis, MO, USA) were dissolved in absolute ethanol. Butaprost, 11-deoxy-PGE₁ (Cayman Chemicals), cicaprost, sulprostone (Schering AG, Berlin, Germany), SC-46275 (Searle, Skokie, IL, USA) and fluprostone (Sigma Chemicals) were dissolved in 0.9% sodium chloride. Solutions were stored at -20°C as stocks at 100 mM until use and diluted with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) buffer (137 mM NaCl, 5.6 mM glucose, 10 mM HEPES, 2.7 mM KCl, 0.4 mM NaH₂PO₄, and 1 mM CaCl₂, pH 7.4) to the desired working concentrations with HEPES buffer containing 0.03% human serum albumin (HAS, Fraction V; Sigma). All drugs were tested to be harmless to mast cells in the present experiments. Rabbit anti-human IgE (anti-IgE) was obtained from Dako (Glostrup, Denmark). Recombinant human stem cell factor (SCF), IL-3, and IL-6 were all purchased from PreproTech EC (London, UK).

Mast cell culture

Mast cells were cultured from the peripheral blood (PB) of healthy volunteer according to the methods described by Iida et al. (8). Briefly, mononuclear cells were separated from PB by density-gradient centrifugation with lymphocyte separation medium (AXIS-SHIELD PoC AS, Oslo, Norway) and incubated with the mixture of magnetic microbead-conjugated monoclonal mouse anti-human CD4, CD8, CD11b, CD14b, and CD19 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) for 30 min at 4°C. Mast cell progenitors were then purified from the mononuclear cells using SuperMACS II system (Miltenyi Biotec) and cultured in serum-free methylcellulose medium (SFBIT H4236; Stem Cell Technologies Inc., Vancouver, Canada) containing 200 ng/ml SCF, 50 ng/ml IL-6, and 5 ng/ml IL-3. After 1 week of culture, 0.3 ml of fresh methylcellulose medium containing 100 ng/ml SCF and 50 ng/ml IL-6 was layered over the methylcellulose cultures every 10–14 days. The cells cultured for 6 weeks were collected and maintained in complete Iscove's modified Dulbecco's medium (IMDM) supplemented with 100 ng/ml SCF, 50 ng/ml IL-6, and 10% fetal calf serum (Gibco BRL, Grand Island, NY, USA). Immunostaining with mouse anti-human mast cell tryptase and chymase monoclonal antibodies (Chemicon Int. Inc., Temecula, CA, USA) respectively, showed that more than 90% of cultured cells recovered after 12 weeks or later were positively stained for tryptase, but not for chymase (9).

Histamine release experiment

Mast cells cultured for 12 weeks with a viability of 100%, as determined by trypan blue exclusion, were sensitized with 0.1 µg/ml of human myeloma IgE (GIBCO) for 1 h at 37°C. The sensitized mast cells were washed with HEPES buffer and resuspended in HEPES buffer containing 0.03% of HAS. To study the effects of prostanoid receptor agonists on mast cells, 200 µl of the prewarmed mast cell suspension ($0.5\text{--}3.0 \times 10^4$ cells) was added to tubes each containing 200 µl of different agonist solution or control buffer

alone and further incubated for 10 min at 37°C and then challenged with 1 µg/ml of anti-IgE at 37°C for 30 min. Reactions were stopped by addition of ice-cold buffer and centrifugation (430 g) for 5 min at 4°C. The cell pellets and supernatants were collected separately for histamine assay. Cells incubated in buffer alone served as a measure of spontaneous mediator release.

Histamine release assays

Histamine in cell pellets and supernatants was measured by using a Bran-Luebbe AutoAnalyzer 2 (Bran-Luebbe GmbH, Norderstedt, Germany). Histamine content was expressed as a percentage of total histamine release and calculated as the following formula: histamine in supernatant/(histamine in supernatant + histamine in cell pellets) × 100%.

Data analysis

The percentage potentiation of the anti-IgE induced mediator release was calculated as follows: % potentiation = [(mediator release in the presence of drug – mediator release in the absence of drug)]/[mediator release in the absence of drug] × 100%. Here, mediator release was the values subtracted from the spontaneous release. The EC₂₅ is the concentration of agonists that causes 25% potentiation of anti-IgE induced histamine release and was extrapolated from the individual dose-response curves of enhancement of histamine release induced by agonists with the computer-aided curve-fitting program GraphPad Prism 3.0 software (San Diego, CA, USA). Results are expressed as the mean ± SE for *n* experiments and statistical analysis were performed by the Student's *t*-test for paired data and *P* < 0.05 was considered statistically significant.

Results

In the present study, BW245C, fluprostone, cicaprost, U46619 or PGE₂ alone did not produce any effect on unstimulated mast cells (data not shown). After incubation of mast cells with agonists at concentrations between 10⁻⁸ and 10⁻⁶ M for 10 min, BW245C, fluprostone, cicaprost and U-46619 were all shown to have no effects on anti-IgE-induced histamine release from mast cells (Table 1). In contrast, PGE₂ (10⁻⁹ to 10⁻⁷ M) significantly enhanced histamine release from anti-IgE activated mast cells in a dose-dependent manner with a maximal

Table 1. Effects of prostanoid receptor agonists on anti-immunoglobulin (IgE) stimulated histamine release (%) from cultured mast cells

Group	<i>n</i>	Anti-IgE (%)	Anti-IgE + agonists (%)		
			10 ⁻⁸ M	10 ⁻⁷ M	10 ⁻⁶ M
Fluprostone	5	30.34 ± 3.88	33.42 ± 4.90	33.10 ± 4.34	33.07 ± 3.47
BW245C	4	26.29 ± 3.65	30.04 ± 3.03	26.19 ± 3.12	27.46 ± 6.33
Cicaprost	4	26.29 ± 3.65	28.44 ± 5.69	28.92 ± 5.86	26.90 ± 6.23
U-46619	5	28.45 ± 3.65	28.30 ± 3.94	29.34 ± 4.20	29.33 ± 4.25

Histamine release was expressed as a percentage of total histamine release and corrected for spontaneous histamine release, which were 9.48 ± 1.73% (fluprostone), 9.25 ± 1.91% (BW245C), 9.25 ± 1.91% (cicaprost) and 8.15 ± 1.89% (U-46619). Results are given as mean ± SE for *n* tests in each group.

effect of $32.37 \pm 4.58\%$ at the highest tested concentrations of 10^{-7} M (Fig. 1).

Prostaglandin E₂ exerts its effects by activating four different EP receptor subtypes: EP₁, EP₂, EP₃ and EP₄ receptor. To further evaluate which EP receptor subtypes mediated the action of PGE₂ in cultured mast cells, we investigated the effects of synthetic agonists with high affinity to one or more EP receptor subtypes on these mast cells. All tested EP receptor agonists by themselves did not produce any significant effects on mast cells without stimulation (data not shown). The highly selective EP₃ agonist SC-46275 (10^{-10} to 10^{-6} M), the selective EP₁/EP₃ agonist sulprostone (10^{-8} to 10^{-6} M) and the

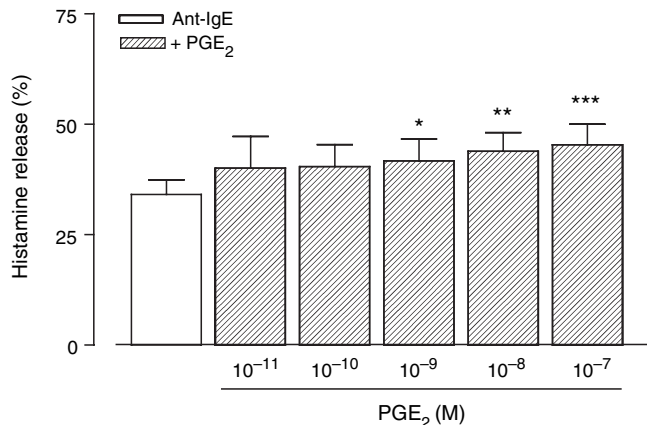


Figure 1. Effect of prostaglandin (PG) E₂ on the anti-immunoglobulin E (IgE)-induced histamine release from cultured mast cells. Sensitized mast cells were pretreated with PGE₂ for 10 min and then challenged with anti-IgE for 30 min. Results were corrected for spontaneous histamine release, which was $8.71 \pm 1.57\%$ ($n = 8$). Values are expressed as mean $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ when compared with anti-IgE alone induced histamine release.

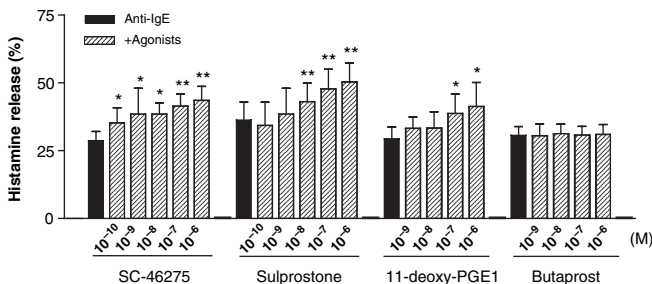


Figure 2. Effect of EP receptor agonists on the anti-immunoglobulin (IgE) induced histamine release from cultured mast cells. Sensitized mast cells were pretreated with agonists for 10 min and then challenged with anti-IgE for 30 min. Results were corrected for spontaneous histamine release, which was $9.02 \pm 1.53\%$ (SC-46275, $n = 5$), $8.15 \pm 1.62\%$ (sulprostone, $n = 5$), $8.68 \pm 1.97\%$ (11-deoxy-PGE₁, $n = 4$) and $8.26 \pm 2.57\%$ (butaprost, $n = 4$). Values are given as mean $\pm$ SE. * $P < 0.05$, ** $P < 0.01$ when compared with anti-IgE alone induced histamine release.

Table 2. The enhancing effects of EP receptor agonists on anti-immunoglobulin E (IgE)-induced histamine release from cultured mast cells

	<i>n</i>	EC ₂₅ ($\times 10^{-8}$ M)	Potential at 10^{-6} M (%)
PGE ₂	8	5.87 ± 1.89	ND
SC-46275	5	0.34 ± 0.17	51.71 ± 7.82
Sulprostone	5	2.35 ± 1.83	45.30 ± 2.13
11-deoxy-PGE ₁	4	4.25 ± 1.65	36.35 ± 16.61

Results are given as mean $\pm$ SE for *n* tests per agonist.
ND, not determined.

selective EP₂/EP₃/EP₄ agonist 11-deoxy-PGE₁ (10^{-7} and 10^{-6} M) all enhanced significantly the anti-IgE-stimulated histamine release from cultured mast cells in a dose dependent manner (Fig. 2). The maximal enhancement of different agonists at 10^{-6} M concentration and EC₂₅ value were evaluated as seen in Table 2. SC-46275 showed more potent enhancing effects on mast cells than other agonists. Butaprost had no significant effect on histamine release from anti-IgE-activated mast cells (Fig. 2).

Discussion

In the present study, the selective and potent DP receptor agonist BW245C, FP receptor agonist fluprostone, IP receptor agonist cicaprost and TP receptor agonist U46619, did not produce any effect on histamine release from immunologically activated mast cells, suggesting that those prostanoid receptors are unlikely to be present in the cultured mast cells. On the contrary, the result that PGE₂ caused a dose-dependent enhancement of anti-IgE-induced histamine release shows that the EP receptors are predominant in these cells.

Prostaglandin E₂ exerts its biological action by interacting with four subtypes of EP receptors, EP₁, EP₂, EP₃ and EP₄ receptors on target cells. EP₂ and EP₄ receptors activated adenylate cyclase leading to elevation of intracellular cAMP concentration and hence, mediated PGE₂-induced inhibitory effects in different cells (10, 11). EP₁ receptors activated phospholipase C and phosphatidyl inositol turnover and stimulated the release of intracellular Ca²⁺. EP₃ receptors principally inhibited adenylate cyclase through a G_i-protein and also stimulated calcium release (2, 3). Contrary to the inhibitory actions of EP₂ and EP₄ receptors, EP₁ and EP₃ receptors mediated promoting effects induced by PGE₂ in various mast cells (7, 12). The present observation that PGE₂ produced a potentiating effect on anti-IgE-induced histamine release suggests that the excitatory EP₁/EP₃ subtypes, and not the inhibitory EP₂/EP₄ receptor subtypes, are present in the cultured mast cells.

SC-46275 was an highly selective and potent EP₃ agonist in guinea pig and mouse *in vivo* (13). It has been shown to be about 10^6 -fold more potent at EP₃ receptor than at EP₁ receptor in isolated guinea pig ileal smooth

muscle and to activate EP₁ receptors only at concentrations of up to 3×10^{-5} M (13). Although sulprostone was considered as a selective EP₁/EP₃ agonist, it has been shown to have higher affinity to the EP₁ receptor than the EP₃ receptor in Chinese hamster ovary cells (14). In isolated guinea pig ileum, in contrast to the weak activity of SC46275 at the EP₁ receptor, sulprostone at 2×10^{-8} M was able to activate EP₁ receptors (13). In the present study that both SC-46275 and sulprostone enhanced histamine release from anti-IgE activated mast cells with a similar efficacy, suggesting that EP₁ and EP₃ receptors both are very likely to exist on cultured mast cells. 11-deoxy-PGE₁ showed affinities to EP₂, EP₃, and EP₄ receptors with a specificity of EP₂ > EP₄ > EP₃ > EP₁ whereas butaprost showed affinity only to the EP₂ receptor (2, 14). The present findings that anti-IgE-induced histamine release was enhanced by 11-deoxy-PGE₁, but not affected by butaprost demonstrate the absence of EP₂/EP₄ receptors, and support the existence of EP₃ receptors in cultured mast cells. Therefore, these data suggest the involvement of EP₁ and/or EP₃ receptors

in PGE₂-directed potentiation of histamine release from immunologically activated mast cells.

In our culture system, more than 90% of cultured mast cells expressed tryptase and only about 10% of cells contained both tryptase and chymase. These cultured mast cells therefore were the same phenotype as the mast cells dispersed from human lung tissues according to their protease contents (15), however, our results were opposite to the findings reported with human lung mast cells in which it has been reported that PGE₂ inhibited immunologically stimulated histamine release (6). It shows that mast cells obtained under the artificial conditions are different from the normal human lung mast cells in some ways.

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