Br. J. Pharmac (1987), **92**, 635–638

Prostaglandin D_2 release from human skin mast cells in response to ionophore A23187

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1 Cells were dispersed from human foreskin by proteolytic digestion and enriched or depleted in mast cell content by density gradient flotation on discontinuous gradients of Percoll.

2 Cells were harvested at six interfaces on the density gradient. Mast cell purity ranged from 0.6–85.0%, compared to 5.5% in the unfractionated cells.

3 Challenge of the cells with the calcium ionophore A23187 resulted in release of both histamine and prostaglandin D_2 (PGD_2). In fractions depleted of mast cells, histamine release and net PGD_2 generation were low, but increasing amounts of these mediators were released as mast cell purity was increased up to 59%.

4 Overall, there was a significant correlation between the net generation of PGD_2 and histamine ($r = 0.9234$, $P < 0.001$) and also between PGD_2 release and mast cell number ($r = 0.7475$, $P < 0.001$).

5 These data provide the first direct evidence of the capacity of the human cutaneous mast cell to synthesize and release PGD_2.

Introduction

Release of mediators from human skin mast cells has been implicated in the pathogenesis of many skin disorders including urticaria, psoriasis and adverse drug reactions. These cells contain a similar amount of histamine to human lung mast cells, but differ from lung mast cells in their ability to secrete histamine in response to substance P, morphine and compound 48/80 (Benyon *et al.*, 1987). Lung mast cells generate prostaglandin D_2 (PGD_2) *in vitro* following calcium ionophore A23187- and IgE-dependent activation (Holgate *et al.*, 1984). Evidence of PGD_2 generation by human skin derives from experiments *in vivo* in which local provocation of cold or heat urticaria and anaphylaxis is accompanied by a rise in venous plasma histamine or PGD_2 concentrations (Heavey *et al.*, 1986; Koro *et al.*, 1986; Barr *et al.*, 1986). The precise function of released PGD_2 in the skin is not known, although recent studies have shown it to be a weak vasodilator in the human cutaneous vasculature (Beasley *et al.*, 1987) and it is also a weak chemokinetic agent in human leukocytes (Goetzl *et al.*, 1976) causing neutrophil accumulation when injected intradermally (Soter *et al.*, 1983). It is also an inhibitor of human platelet aggregation (Smith *et al.*, 1974). Using purified cell preparations, we now provide evidence that human skin mast cells synthesize and release PGD_2 in response to calcium-dependent activation.

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Methods

Mast cells were dispersed from human skin as previously described (Benyon *et al.*, 1987). Briefly, fresh foreskin (patient age 1–9 years) obtained at circumcision was chopped finely and the fragments digested in 10 volumes of Eagle's minimum essential medium (Gibco, Paisley, Scotland) supplemented with 1.5% foetal calf serum (MEM/FCS) and which contained 1.5 mg ml^{-1} collagenase and 0.5 mg ml^{-1} hyaluronidase (Sigma, Poole, Dorset). After washing, the dispersed cells were resuspended in MEM/FCS and layered onto discontinuous gradients of 40–80% isotonic Percoll (density 1.051–1.1 g ml^{-1}) which were centrifuged at 500 *g* and 22°C for 10 min. Cells from each interface were harvested, washed twice and resuspended in N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid (HEPES) buffered-balanced salt

solution at pH 7.2 (composition, mM: NaCl 137, HEPES 10, D-glucose 5, KCl 2.7, NaH_2PO_4 0.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8). Cell numbers were determined by light microscopy after staining with toluidine blue.

For mediator release experiments, 270 μl aliquots of cells were warmed at 37°C for 10 min and stimulated with 30 μl of ionophore A23187 to give a final concentration of 1 μM. Release reactions were terminated after 30 min by centrifugation at 10,000 *g* for 30 s (Beckman Microfuge B). Supernatant and cell-associated histamine was measured spectrophotofluorimetrically with an autoanalyser. Radioimmunoassay of PGD_2 was performed by a modification of the method of Holgate *et al.* (1984). Assay of thromboxane B_2 (TXB_2) was performed using an antibody which was a generous gift of Dr L. Levine, Brandeis University, M.A., U.S.A. The cross reactivity of this antibody has been described elsewhere (Gaudet *et al.*, 1980). The PGD_2 metabolite 9α,11β-PGF_2 was measured using a rabbit antibody raised in our laboratory. Cross reaction with heterologous ligands at 50% maximum binding was: $PGF_{2\alpha}$ < 0.0006%, PGD_2 0.0025%, TXB_2 0.2%, PGE_2 0.036%, 6-keto-$PGF_{1\alpha}$ 0.001%, PGJ_2 0.02%. The tritiated 9α,11β-PGF_2 tracer used in this assay was prepared from tritiated PGD_2 using a guinea-pig liver cell-free preparation rich in PGD_2 11-keto reductase activity (Bacon *et al.*, 1987). Purification was achieved by reversed phase high performance liquid chromatography using a mobile phase comprising 0.017 M orthophosphoric acid: acetronitrile (67.2: 32.8 v:v) and a 25 × 0.46 cm μBondapak C_{18} column. The purified product was extracted from the mobile phase into ethyl acetate, dried with magnesium sulphate and stored at −20°C until use. Final specific activity was 158 Ci $mmol^{-1}$.

Statistical analysis

Results are shown as mean ± s.e.mean of *n* experiments as indicated. The significance between spontaneous and ionophore stimulated mediator release was evaluated with Student's *t* test for paired data in the case of net release calculations. Student's *t* test for unpaired data was used for other comparisons. Correlation coefficients were calculated by unweighted least squares linear regression.

Materials

Percoll was obtained from Pharmacia, Uppsala, Sweden. TXB_2 was a generous gift of the Upjohn Co., Kalamazoo, M.I., U.S.A. Prostaglandins were obtained from Salford Ultrafine Chemicals and Research Manchester. Ionophore A23187 was purchased from Sigma. [5,6,8,9,12,14,15-(n)-^{3}H]-PGD, and [5,6,8,9,12,14,15-(n)-^{3}H]-TXB_2 were purchased from Amersham International plc, Aylesbury, Bucks.

Results

The results are summarized in Table 1. In five experiments, unfractionated cells, which contained 5.5 ± 0.7% mast cells, responded to ionophore activation with a net release of 3.09 ± 0.93 ng PGD_2 per 10^6 nucleated cells (NC) ($P < 0.05$ in comparison with 0.42 ± 0.31 ng in unstimulated cells). Fraction 1, equivalent to a Percoll concentration of <40% and which was depleted in mast cells, released significantly ($P < 0.05$) less PGD_2 and histamine compared with unfractionated cells. As mast cell purity increased there was a statistically significant increase in the net release of PGD_2 to a maximum of 39.1 ± 13.2 ng per 10^6 NC in cells of fraction 4 from the 60–70% Percoll interface ($P < 0.05$ with respect to unfractionated cells). This was accompanied by an increase in the release of histamine to 769.5 ± 262.0 μg per 10^6 NC. Spontaneous release of PGD_2 and histamine was 0.49 ± 0.33 and 236.2 ± 110.1 per 10^6 NC respectively in this fraction. Paradoxically, there was a parallel reduction in the release of both mediators when mast cell purity was increased further. In gradient fractions overall, release of PGD_2 was significantly correlated with that of histamine ($r = 0.9234$, $n = 32$, $P < 0.001$). Furthermore, there was a significant correlation between PGD_2 release and mast cell number ($r = 0.7475$, $n = 33$, $P < 0.001$), but not between net release of PGD_2 and the number of nucleated cells ($r = 0.1613$, $n = 33$, $P > 0.1$).

Small amounts of 9α,11β-PGF_2 and TXB_2 were detected in the supernatant of both unstimulated cells and in cells stimulated by ionophore A23187 although the amounts after A23187 challenge were not significantly greater. In gradient fractions overall, there was no significant correlation between either 9α,11β-PGF_2 or TXB_2 generation and either histamine release or mast cell numbers.

Discussion

This study provides a direct demonstration that human cutaneous mast cells have the capacity to synthesize and release PGD_2. This evidence is based on cell purification experiments in which significant correlations were established between PGD_2 generation, histamine release and the numbers of mast cells, but not of nucleated cells. Our data suggest that the cutaneous mast cell is one likely source of the PGD_2 detected during local urticaria and cutaneous anaphylaxis *in vivo*.

An interesting feature of the density gradient experiments was the parallel decrease in the net release of both histamine and PGD_2 in cells harvested from 70–80% and >80% Percoll interfaces (fractions 5 and 6). We have observed a similar phenomenon in

Table 1 Ionophore-induced histamine and prostanoid release from human dispersed skin cells

Fraction number (% Percoll)	*Mast cell purity (%)*	*Net histamine release (%)*	*Histamine*	*Net release* (ng per 10^6 NC) PGD_2	$9\alpha,11\beta\text{-}PGF_2$	TXB_2
Unfractionated cells	5.5 ± 0.7	27.0 ± 5.9	51.9 ± 15.1	3.09 ± 0.93	0.29 ± 0.12	0.19 ± 0.10
1 (< 40)	0.6 ± 0.2	16.6 ± 3.0	3.7 ± 0.4	0.23 ± 0.03	0.15 ± 0.06	0.31 ± 0.18
2 (40/50)	2.0 ± 0.5	23.3 ± 7.0	14.5 ± 3.2	1.00 ± 0.16	0.11 ± 0.05	0.44 ± 0.18
3 (50/60)	18.8 ± 5.5	25.7 ± 4.3	166.3 ± 60.5	13.30 ± 5.50	0.22 ± 0.08	0.49 ± 0.27
4 (60/70)	59.0 ± 6.5	32.4 ± 8.7	769.5 ± 262.0	39.14 ± 13.20	0.35 ± 0.15	0.42 ± 0.38
5 (70/80)	79.0 ± 4.3	24.1 ± 8.1	549.7 ± 263.8	27.50 ± 10.59	1.14 ± 1.09	0.33 ± 0.33
6 (> 80)	85.2 ± 6.3	22.9 ± 5.3	453.3 ± 76.0	17.10 ± 4.43	1.50 ± 1.34	0.01 ± 0.01

Each result is the mean ± s.e.mean for 5 experiments in which net mediator release induced by 1 μM calcium ionophore A23187 has been corrected for spontaneous release.

Fraction number indicates the interface between the specified Percoll densities. Isotonic Percoll concentrations of 40%, 50%, 60%, 70%, 80% are equivalent to densities of 1.051, 1.063, 1.076, 1.090 and 1.100 g ml^{-1}.

human lung cells separated by density flotation on continuous gradients of Percoll (Holgate *et al.*, 1984). In mast cells obtained by bronchoalveolar lavage of rhesus monkeys and purified by sedimentation on Metrizamide gradients, the densest cells had high histamine content but were less sensitive to immunological activation (Patterson *et al.*, 1980). One possible explanation for these findings is that high densities of Percoll or Metrizamide inhibit mediator release from mast cells. An alternative view is that the phenomenon is due to the differential ability of mast cells of different sizes to respond to cellular activation. For example, large mast cells purified from human lung parenchyma by countercurrent elutriation release 2–5 times more histamine and PGD_2 in response to IgE-dependent activation than do small mast cells (Schulman *et al.*, 1983). Further experiments will be necessary to differentiate these possibilities.

Prostaglandin D_2 has been reported to be released from human pulmonary mast cells (Holgate *et al.*, 1984) but not basophils (MacGlashan *et al.*, 1983). The amount of PGD_2 per unit amount of histamine released from skin cells by calcium ionophore, 52 pg per ng histamine, is similar to that obtained from human lung mast cells, 32 pg per ng histamine (Holgate *et al.*, 1984) suggesting that these cells have a similar capacity to generate this prostanoid. The lack of correlation between the amounts of $9\alpha,11\beta\text{-}PGF_2$ and TXB_2 with either mast cell numbers or histamine release suggests that the mast cell is not the direct source of the small amounts of these prostanoids detected. Furthermore, it was of interest that A23187-dependent activation failed to elicit a net increase in the amount of either compound. In the case of $9\alpha,11\beta\text{-}PGF_2$, which is known to be an 11-ketoreductase dependent metabolite of PGD_2 (Bacon *et al.*, 1987), these observations suggest that if PGD_2 is converted to $9\alpha,11\beta\text{-}PGF_2$ in this system, it may occur in cells other than mast cells. At this stage we cannot formally exclude the possibility that $9\alpha,11\beta\text{-}PGF_2$ may arise by facile reduction of prostaglandin endoperoxides with an inversion of the α stereochemistry at C11. Experiments are in progress to define the generation of other lipid mediators from human skin mast cells in response to other secretagogues.

We thank the MRC for financial support. Ms Dianne Wilson provided excellent secretarial assistance.

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(Received May 8, 1987.
Revised June 17, 1987
Accepted June 19, 1987.)