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Characterization of prostanoid receptors mediating inhibition of histamine release from anti-IgE-activated rat peritoneal mast cells

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1 Prostanoid receptors mediating inhibition of anti-IgE induced histamine release from rat peritoneal mast cells have been characterized pharmacologically. PGD_2 and the specific DP receptor agonists BW 245C and ZK 118182 were the most potent inhibitors with half-maximal concentrations of 0.26, 0.06 and 0.02 μM respectively. The maximum inhibition attainable was 60–65% with 10^{-5} M BW 245C and ZK 118182.

2 Among several EP receptor agonists investigated, only PGE_2 and the EP_2/EP_3 receptor agonist misoprostol induced significant inhibition ($46.8 \pm 4.7\%$ at 10^{-4} M and $18.7 \pm 6.8\%$ at 10^{-5} M respectively). The IP receptor agonists cicaprost and iloprost were both less potent than the DP agonists in inhibiting histamine release ($45.2 \pm 3.3\%$ and $35.1 \pm 2.5\%$ inhibition respectively at 10^{-5} M), whereas $PGF_{2\alpha}$ and the TP receptor agonist U-46619 were only marginally effective.

3 The EP_4/TP receptor antagonist AH 23848 failed to affect the inhibitory actions of PGD_2 or PGE_2 even at 10^{-5} M, whereas the $DP/EP_1/EP_2$ receptor antagonist AH 6809 slightly enhanced the effect of PGD_2 at 10^{-6} M.

4 At concentrations of 3×10^{-6} to 10^{-5} M, the putative DP receptor antagonist ZK 138357 dose-dependently suppressed the inhibitory activities of the DP agonists, PGE_2 and cicaprost. The antagonism of ZK 138357 against the DP receptor agonists appeared to be competitive with pA_2 values of around six.

5 In conclusion, these data support our earlier proposal that an inhibitory DP receptor is the predominant prostanoid receptor in rat peritoneal mast cell. The properties of this receptor in relation to putative DP receptor subtypes reported in the literature are discussed.
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Keywords: BW 245C; cicaprost; DP receptor; mast cells; prostaglandin; histamine release

Abbreviations: BSA-FHB, full HEPES buffered tyrode supplemented with 1 mg/ml of bovine serum albumin; DSCG, disodium cromoglycate; FHB, full HEPES-buffered tyrode; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; IC_{30}, the prostanoid concentration which produced 30% inhibition; $-\log IC_{30}$, negative logarithm of the molar concentration which produced 30% inhibition

Introduction

Prostanoids, including prostaglandins and thromboxanes, are synthesized *via* the cyclo-oxygenase pathway from arachidonic acid released from phospholipids in the plasma membrane by phospholipase A_2 in response to a wide range of extracellular stimuli. Once synthesized, the prostanoids are quickly released and act as local hormones which modulate cellular functions in various physiological and pathological processes. Prostaglandins of the E and I subclasses make important contributions to the signs and symptoms of inflammatory diseases such as rheumatoid arthritis and asthma (Coleman *et al*., 1994b; Goodwin, 1991; Giles, 1990). The proinflammatory actions of prostanoids are mainly due to their direct dilating effects on the microvasculature and their synergism with other proinflammatory mediators to augment vascular permeability and sensitize pain receptors (Williams, 1979). However, a number of potential mechanisms for anti-inflammatory effects of prostaglandin, in particular PGEs have been identified, including the suppression of interleukin-1 production (Goodwin, 1991; Giles, 1990; Chouaib *et al*., 1985) and inhibition of neutrophil activation, superoxide release and leukotriene production (Fantone *et al*., 1983).

Although functional studies have demonstrated that various prostanoids can modulate mast cell function (Hogaboam *et al*., 1993; Peachell *et al*., 1988; Peters *et al*., 1982; 1992; Wescott & Kaliner, 1981) and previous radioligand binding studies have identified specific binding sites for prostaglandins E_2, I_2 and D_2 in mouse mastocytoma P-815 cells (Hashimoto *et al*., 1990; Negishi, *et al*., 1991a,b; Oka *et al*., 1994; Yoshimura *et al*., 1989) and human basophils (Virgolin *et al*., 1992), definitive pharmacological characterization of prostanoid receptors on mast cells isolated *in situ* has not been reported. Five main types of prostanoid receptors, coded DP, EP, FP, IP and TP, have now been identified and their pharmacology has been extensively reviewed by Coleman *et al*. (1994b). Our preliminary studies (Chan & Lau, 1998) demonstrated that prostanoids in general suppressed histamine release from rat peritoneal mast cells with DP receptor agonists being the most potent. In the present study, we have attempted to characterize in more detail the inhibitory prostanoid receptor(s) in rat peritoneal mast cells by comparing the effects of various prostanoid agonists and antagonists.

Methods

Isolation/incubation medium

Full HEPES-buffered tyrode (FHB) at pH 7.4 containing (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1, NaH_2PO_4 0.4, N-2-

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hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES), 10, glucose 5.6 and $MgCl_2$ 1.2 was used throughout the study.

Purification of rat peritoneal mast cells

Male Sprague-Dawley rats weighing 200–300 g were actively immunized by an intraperitoneal injection of 0.5 ml phosphate-buffered saline containing ovalbumin (0.5 mg), $Al(OH)_3$ (120 mg) and *Bordetella pertussis* (0.8 I.U.). Three to four weeks later, the sensitized animals were first anaesthetized with ether and then killed by decapitation. Mixed peritoneal cells were collected from each rat by peritoneal lavage with 20 ml of FHB supplemented with 1 mg ml^{-1} of bovine serum albumin (BSA-FHB). The cells were washed twice in ice-cold BSA-FHB by centrifugation ($190 \times g$, 4°C, 5 min), resuspended in 1 ml of ice-cold FHB, and then mixed with 4 ml of isotonic Percoll solution (SG = 1.017) in calcium-free FHB. Mast cells were purified by centrifugation ($190 \times g$, 4°C, 25 min) through the continuous density gradient generated by the Percoll and were pelleted at the bottom of the tube. Residual Percoll was eliminated by two washes in BSA-FHB and a final wash in FHB. The purified mast cells were finally resuspended in the required amount of FHB.

Cell incubation and histamine assay

Four hundred μl of purified mast cells, which had been equilibrated in FHB at 37°C for 10 min, were added to 50 μl of FHB with or without a prostanoid. Fifty μl of anti-rat-IgE was added either at the same time as the cells or after the cells had been incubated with the prostanoid for 5 min. When the effects of prostanoid receptor antagonists were investigated, mast cells were first incubated with ibuprofen (10^{-5} M) for 10 min. Ibuprofen-treated cells were then mixed with the antagonist, agonist and anti-IgE at the same time except in experiments which evaluated the effects of preincubation. The cells were further incubated at 37°C for 10 min after the addition of anti-IgE and the reaction was terminated by the addition of ice-cold FHB. The supernatant and cell pellet were then separated by centrifugation ($190 \times g$, 4°C, 5 min). The cell pellet was resuspended in buffer and heated to 80°C for 15 min to liberate the residual histamine. The histamine contents of the supernatant and the cell pellet were determined spectrofluorometrically using a Hitachi F-4010 fluorescence spectrophotometer by the method of Shore *et al*. (1959).

Drugs

The following prostanoid analogues are gifts from the sources indicated. Cicaprost, iloprost, sulprostone, ZK 118182 ((5*Z*, 13*E*)-(9*R*, 11*R*, 15*S*)-9-chloro-15-cyclohexyl-15-hydroxy-16, 17, 18, 19, 20-pentanor-3-oxa-5, 13-prostadienoic acid) and ZK 138357 ((5*Z*)-7-{(2*RS*,4*S*,5*S*)-2-(2-chlorophenyl)-5-[(1*E*)-(3*RS*)-3-hydroxy-3-cyclohexyl-1-propenyl]-1, 3-dioxolan-4-yl}-5-heptanoic acid) were from Schering AG, Germany. Butaprost was from Bayer, U.K. AH 23848 ([(±) 1α(Z), 2β, 5α,]-7-[5-[[(1, 1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid), AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid), BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin) were from Glaxo-Wellcome, U.K. Misoprostol, SC-46275 (methyl 7-[2-[6-(1-cyclopenten-1-yl)-4*R*-hydroxy-4-methyl-1E, 5E-hexadienyl]-3-hydroxy-5-oxo-1*R*, 1-cyclopentyl]-4Z-heptenoate) were from Searle Research and Development, Skokie, U.S.A. PGD_2, 13,14-dihydro-15-keto PGD_2, PGE_2, 9α,11β-PGF_2, $PGF_{2\alpha}$ and U-46619 were purchased from Cayman Chemicals. Anti-rat IgE was purchased from ICN Biomedicals. All other reagents were purchased from Sigma Chemicals, St. Louis, MO, U.S.A.

Dissolution and storage of drugs

With the exception of ZK 118182, which was dissolved in distilled water at concentration of 0.1 mM, all stock solutions of prostanoids were prepared at 10 mM or 100 mM (PGE_2) in ethanol and were freshly diluted to desired working concentration in buffer upon use. Since ethanol at concentrations >0.1% significantly suppresses both spontaneous and anti-IgE induced histamine release, the maximum concentrations of prostanoids tested were limited to 10^{-5} or 10^{-4} M (PGE_2).

Data analysis

In each experiment, the spontaneous histamine release in buffer alone was subtracted from all measurements. The percentage inhibition of anti-IgE induced histamine release (% Inhibition) was calculated from $\{(c - a)/c\} \times 100\%$, where c is the control anti-IgE induced histamine release in buffer and a is the anti-IgE induced histamine release in the presence of a prostanoid. All data are mean ± standard error of mean (s.e.mean) for n independent observations and statistical analyses were performed using the Student's t-test. Concentration-inhibition curves were analysed by least squares nonlinear iterative regression with the 'Prism' curve fitting programme (GraphPad software, San Diego, CA, U.S.A.) using a four-parameter logistic equation of the form:

$$Y = Y_{min} + \{(Y_{max} - Y_{min})/(1 + 10^{(LogEC_{50} - X) - HillSlope})\}$$

where Y is the observed % inhibition, Y_{max} and Y_{min} are the maximum and minimum % inhibition, X is the logarithmic value of the drug concentration. EC_{50} is the concentration that gives a response halfway between Y_{max} and Y_{min}. In general, Y_{min} was fixed at 0 while the remaining parameters were determined from each independently fitted concentration-inhibition curve to generate average parameter estimates for each group of curves. These average estimates were used for the fitting of the final curves illustrated in the figures.

For the computation of the antagonist affinity of ZK 138357 against the DP agonists, the control concentration-inhibition curve for the effect of the prostanoid agonist alone was first fitted with Y_{min} fixed at 0 to produce estimates for Y_{max}, EC_{50} and Hill slope in the absence of ZK 138357. Assuming that ZK138357 inhibited the effects of prostanoid agonists competitively, all the concentration-inhibition curves in the presence of increasing concentrations of ZK 138357 from the same experiment should share the same Y_{max} as the control curve and were fitted accordingly to obtain estimates for the corresponding EC_{50} and Hill slope parameters. The mean values of Y_{max}, EC_{50} and Hill slope were then used to construct the final curves in Figure 6. For the calculation of pA_2 values of ZK 138357 at single concentrations in an experiment, EC_{50} values estimated for the control and ZK 138357 affected curves from the same experiment were used in the equation:

$$pA_2 = log\{(EC_{50A}/EC_{50B}) - 1\} - log[ZK\ 138357]$$

where EC_{50A} and EC_{50B} are the agonist EC_{50} values in the presence of the antagonist and in buffer alone respectively, and $[ZK\ 138357]$ is the molar concentration of ZK 138357. Results from all the experiments were then used to calculate the mean values listed in Table 2. Since maximum inhibition was not achieved with cicaprost and PGE_2, best fitted concentration-

inhibition curves generated by the Prism programme using the averaged data were illustrated in Figure 7.

Results

Establishing conditions for immunological release of histamine

Not more than 15% of total cellular histamine was released spontaneously by rat peritoneal mast cells incubated in buffer alone and none of the prostanoid analogues tested affected this spontaneous release. The anti-IgE challenge to the cells was adjusted so that histamine release was approximately 30% of total cellular histamine above the spontaneous level. Since the inhibitory effects of prostanoids on anti-IgE induced histamine release were not affected by pretreating the cells with ibuprofen (as illustrated in Figure 1 for the DP agonists), mast cells were not preincubated with ibuprofen in experiments screening for agonist activities in order to reduce the total cell incubation period. Furthermore, we had previously reported that endogenously produced prostanoids did not affect spontaneous and anti-IgE induced histamine release from rat peritoneal mast cells (Lau & Stenton, 1998). However, in experiments studying the effects of prostanoid receptor antagonists, potential interaction between endogenously synthesized prostanoids and the antagonists was eliminated by preincubating the mast cells with 10^{-5} M of ibuprofen for 10 min.

Effects of DP receptor agonists and PGD_2 metabolites

PGD_2 and the two potent selective DP receptor agonists BW 245C and ZK 118182 caused concentration-dependent (10^{-9}–10^{-5} M) inhibition of anti-IgE induced histamine release from rat peritoneal mast cells with similar levels of inhibition (56.5±3.7, 65.9±4.8 and 60.6±3.7% respectively) at the

Table 1 Comparison of the inhibitory potencies of prostanoid analogues on anti-IgE induced histamine release from rat peritoneal mast cells

Prostanoid	n	*−log IC_{30}*	*Inhibition at 10^{-5}M (%)*
DP agonists			
PGD_2	6	6.58±0.15	56.5±3.7
BW 245C	6	7.20±0.14	65.9±4.8
ZK 118182	7	7.75±0.26	60.6±3.7
9α,11β–PGF_2	4	5.41±0.32	42.0±6.8
13,14–Dihydro–15–keto PGD_2	4	<5	13.4±5.8
EP agonists			
PGE_2	6	4.86±0.14	25.7±2.8
Misoprostol	5	<5	18.7±6.8
Butaprost	5	<5	0
SC–46275	5	<5	0
Sulprostone	5	<5	0
IP agonists			
Cicaprost	8	5.68+0.11	45.2±3.3
Iloprost	4	5.11±0.09	35.1±2.5
FP agonists			
$PGF_{2\alpha}$	4	<5	28.0±3.9
TP agonists			
U-46619	4	<5	3.8±0.8

The $-\log IC_{30}$ (negative logarithm of the molar concentration which produced 30% inhibition) values were extrapolated from individual concentration-inhibition curves which made up the averaged curves in Figure 2. Both the $\log IC_{30}$ values and the % inhibition values at 10^{-5} M are given as mean±s.e.mean for n experiments.

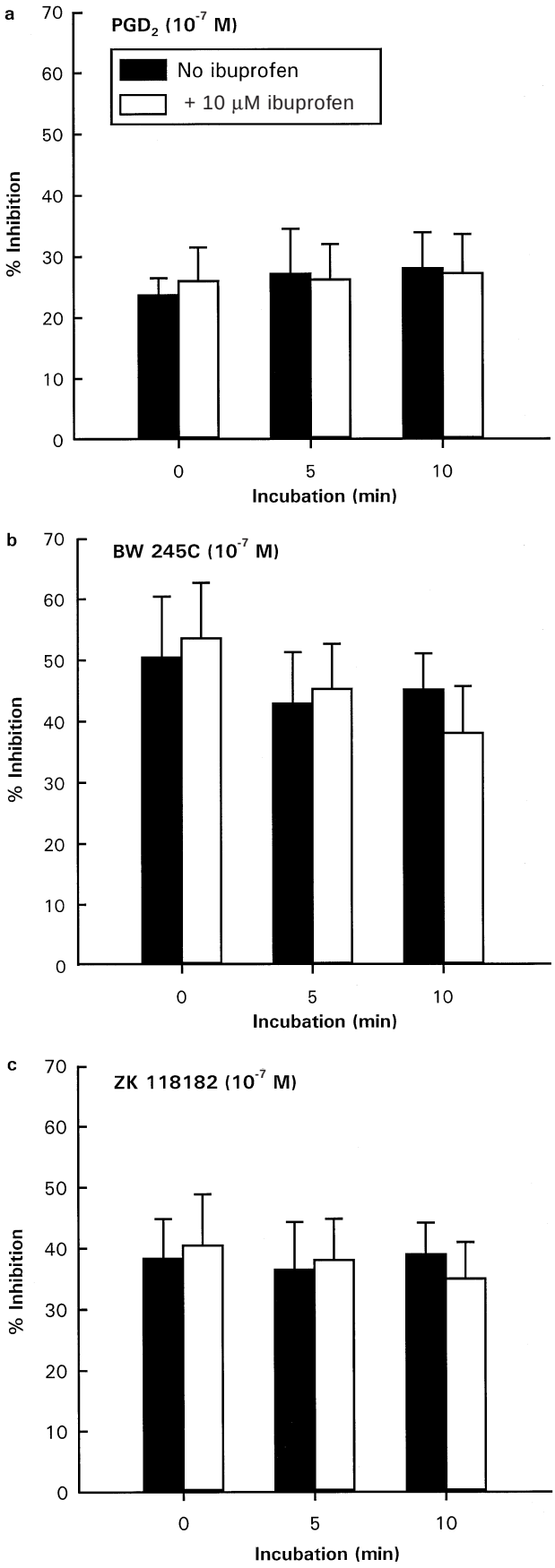

Figure 1 Effects of ibuprofen on (a) PGD_2, (b) BW 245C and (c) ZK 118182 induced inhibition of anti-IgE stimulated histamine release from rat peritoneal mast cells. Cells were preincubated with ibuprofen (10^{-5} M) for 0, 5 or 10 min prior to the addition of DP agonist and anti-IgE. Spontaneous histamine release was 11–15% and anti-IgE induced histamine release was 29–36% above spontaneous level. Results are mean±s.e.mean for $n=4$.

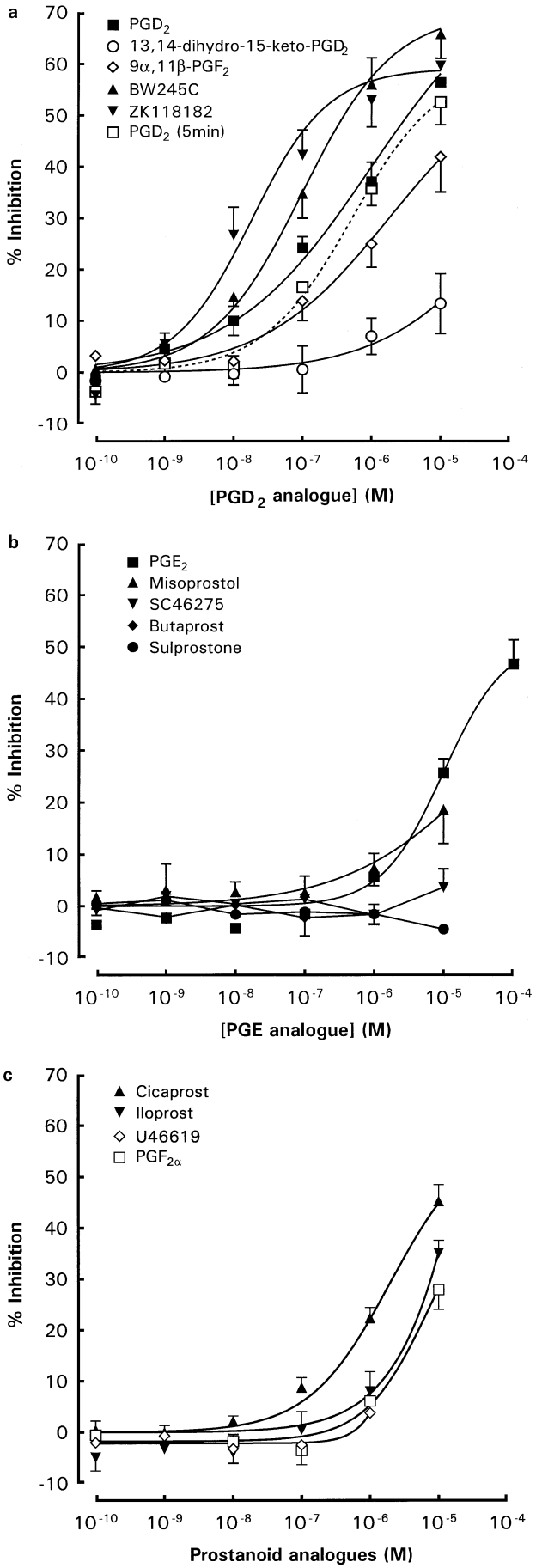

Figure 2 Concentration-inhibition curves for the effects of (a) PGD_2 analogues (b) PGE_2 analogues and (c) prostacyclin analogues, $PGF_{2\alpha}$ and U-46619 on anti-IgE-induced histamine release from rat peritoneal mast cells. Spontaneous release of histamine ranged from 8.0 to 13.0%, whereas anti-IgE induced histamine release ranged from 24.0 to 35.8%. All data are means$\pm$s.e.mean for *n* experiments as indicated in Table 1.

maximum concentration (10^{-5} M) tested (Figure 2a). When the concentrations which produced 30% inhibition (IC_{30}) were compared (Table 1), ZK 118182 and BW 245C were significantly more potent than PGD_2. Two metabolites of PGD_2, $9\alpha,11\beta$-PGF_2 and 13,14-dihydro-15-keto PGD_2, were significantly less potent than PGD_2 with maximum inhibitions of 42.0 ± 6.8 and 13.4 ± 5.8% respectively at 10^{-5} M. The inhibitory effects of PGD_2 were similar when it was added with anti-IgE and when it was added 5 min before anti-IgE challenge (Figure 2a); similar results were obtained for the other prostanoids (data not shown). Hence all subsequent studies were performed without prior preincubation of cells with prostanoids to minimize degradation of the natural prostanoids.

Effects of selective EP and IP agonists, $PGF_{2\alpha}$ and U-46619

Among the various EP receptor agonists tested, only PGE_2 and the EP_2/EP_3 receptor agonist, misoprostol caused significant inhibition of histamine release from anti-IgE activated rat peritoneal mast cells at concentrations higher than 10^{-7} M (Figure 2b). The EP_1/EP_3 agonist sulprostone and the selective EP_3 agonist SC-46275, as well as the EP_2 agonist butaprost, all had little effect even at 10^{-5} M. Dose-dependent inhibition of anti-IgE induced histamine release was also observed with the IP agonists cicaprost and iloprost at concentrations higher than 10^{-8} M (Figure 2c). Both $PGF_{2\alpha}$ and the TP receptor agonist U-46619 induced minimal inhibition of anti-IgE induced histamine release from rat peritoneal mast cells at concentrations up to 10^{-6} M. 10^{-5} M of $PGF_{2\alpha}$ produced 28.0 ± 3.9% inhibition, whereas higher concentrations of U-46619 were not tested.

Effects of prostanoid antagonists

The EP_4 antagonist AH 23848 at 10^{-5} M was without effect on the inhibitory activity of PGE_2 and PGD_2 (Figure 3). The DP/EP_1/EP_2 receptor antagonist AH 6809 at 10^{-7} M did not affect the inhibitory effect of PGD_2, whereas at 10^{-6} M the PGD_2 log concentration-response curve was shifted to the left by about one log unit (Figure 4). Increasing the preincubation period for 10^{-7} M of AH 6809 to 10 min before the addition of PGD_2 and anti-IgE did not significantly alter the effect of AH 6809 (Figure 5).

The putative DP receptor antagonist ZK 138357, at concentrations of 3×10^{-6}, 6×10^{-6} and 10^{-5} M caused progressive rightward shifts of dose-inhibition curves for PGD_2, ZK 118182, BW 245C, PGE_2 and cicaprost (Figures 6 and 7). As with AH 6809, increasing the preincubation period with cells did not significantly alter the antagonist potency of ZK 138357 against PGD_2 (Figure 5). Student *t*-test analysis comparing the Hill slopes of the dose-inhibition curves of the three DP agonists in the presence of increasing concentrations of ZK 138357 with that of the corresponding control curve showed no significant difference and there was little evidence of suppression of the maximum response. This profile is consistent with competitive antagonism. Use of the Schild equation gave comparable pA_2 values of about six for ZK 138357 versus the three DP agonists (Table 2). Full dose-inhibition curves in the

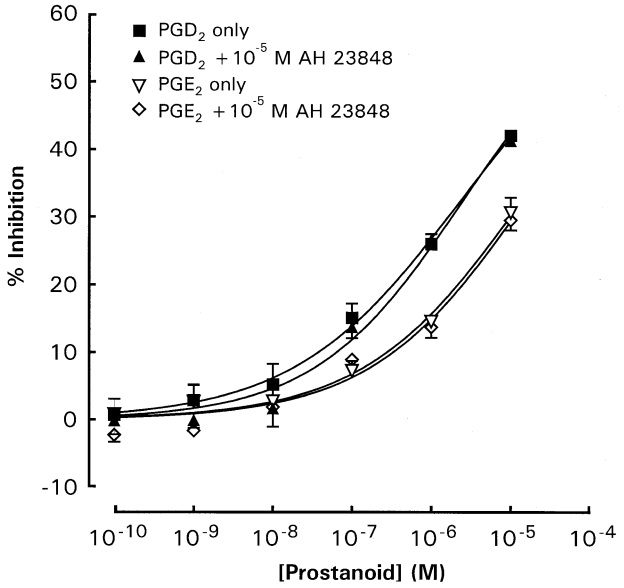

Figure 3 Effects of 10^{-5} M AH 23848 on the inhibitory actions of PGD_2 and PGE_2 on anti-IgE induced histamine release from rat peritoneal mast cells. Ibuprofen (10^{-5} M) -treated mast cells were exposed simultaneously to anti-IgE, the prostaglandin and AH 23848. Spontaneous histamine release was 12.2±0.9 and 11.2±0.9%, whereas anti-IgE induced histamine release were 29.0±2.3 and 30.4±2.6% for the PGD_2 and PGE_2 experiments respectively. Results are given as means±s.e.mean for $n=4$.

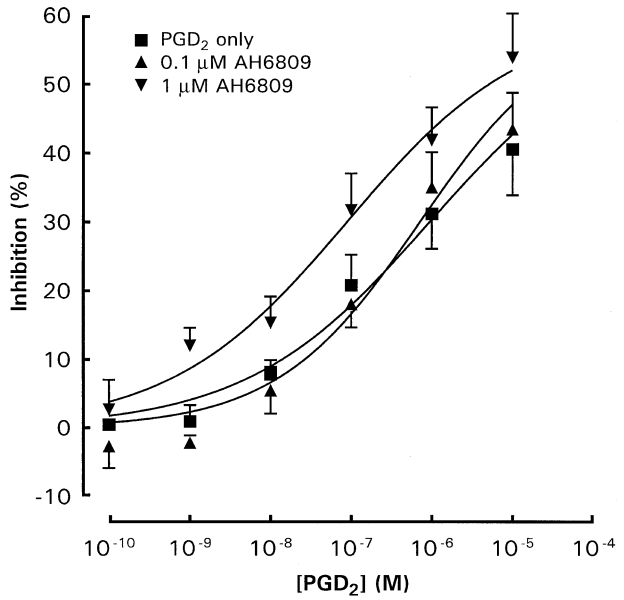

Figure 4 Effects of AH 6809 on the inhibitory actions of PGD_2 on anti-IgE induced histamine release from rat peritoneal mast cells. Ibuprofen (10^{-5} M) treated mast cells were exposed to anti-IgE and PGD_2 together with AH 6809. Spontaneous release of histamine was 13.9±1.1% whereas anti-IgE induced histamine release was 31.9±3.3%. Results are given as means±s.e.mean for $n=4$.

presence of ZK 138357 were not obtained for PGE_2 and cicaprost due to their lower potencies. However, the available portions of the inhibition curves appear to be parallel to the corresponding control curves (Figure 7), and the pA_2 values calculated from extrapolated EC_{50} values are similar to those obtained for the DP agonists (Table 2).

In order to examine the specificity of ZK 138357, the effect of the antagonist against inhibition of immunologically induced

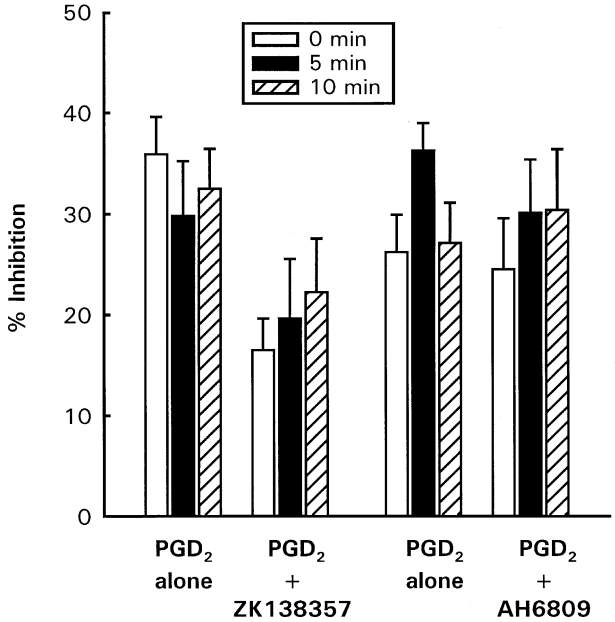

Figure 5 Effects of preincubation with ZK 138357 (10^{-5} M) and AH 6809 (10^{-7} M) on the inhibitory actions of PGD_2 (10^{-6} M) on anti-IgE induced histamine release from rat peritoneal mast cells. Ibuprofen (10^{-5} M) treated mast cells were exposed to anti-IgE and PGD_2 after 0, 5 or 10 min preincubation with the DP antagonists. For the ZK 138357 experiments, spontaneous histamine release was 9.8±1.0% (0 min), 11.5±1.1% (5 min) and 11.2±1.1% (10 min), whereas anti-IgE induced histamine release was 33.3±4.6% (0 min), 31.4±2.7% (5 min) and 31.6±3.1% (10 min). The corresponding values for the AH 6809 experiments were 13.9±1.1, 13.3±0.8, 13.9±1.5, 31.9±3.3, 31.7±3.0 and 31.8±4.4%. Results are given as means±s.e.mean for $n=4$.

Table 2 Calculated pA_2 values for ZK 138357 against DP receptor agonists, PGE_2 and cicaprost

		ZK 138357 concentration		
Agonists	n	3×10^{-6} M	6×10^{-6} M	10^{-5} M
PGD_2	6	5.65±0.29	5.86±0.16	5.90±0.27
BW 245C	6	5.83±0.23	6.02±0.17	6.24±0.24
ZK 118182	7	5.92±0.17	6.27±0.10	6.54±0.29
PGE_2	4	5.68±0.27	5.78±0.21	5.83±0.19
Cicaprost	7	5.49±0.36	6.33±0.24	6.82±0.55

pA_2 values from individual experiments making up the concentration inhibition curves in Figures 6 and 7 were determined using the Schild equation at the single concentrations of ZK 138357. Mean±s.e.mean values are reported here for n experiments.

histamine release by the two well documented mast cell stabilizers, disodium cromoglycate (DSCG) and theophylline was studied. As illustrated in Figure 8, 10^{-5} M of ZK 138357 did not affect the inhibitory effects of DSCG and theophylline.

Discussion

Our studies on the rat peritoneal mast cell have shown that prostanoid agonists covering the five prostanoid receptor types could inhibit histamine release, and no evidence for excitatory actions was obtained. Thus, none of the prostanoids increased the release of histamine in the resting state; this included PGE_2, $PGF_{2\alpha}$ and the TXA_2 mimetic U-46619, all of which are known to activate excitatory prostanoid receptors (EP_1, EP_3, FP and TP) in other systems (see Coleman, 1998; Coleman *et al.*, 1994b). These findings agree with earlier reports on rat and

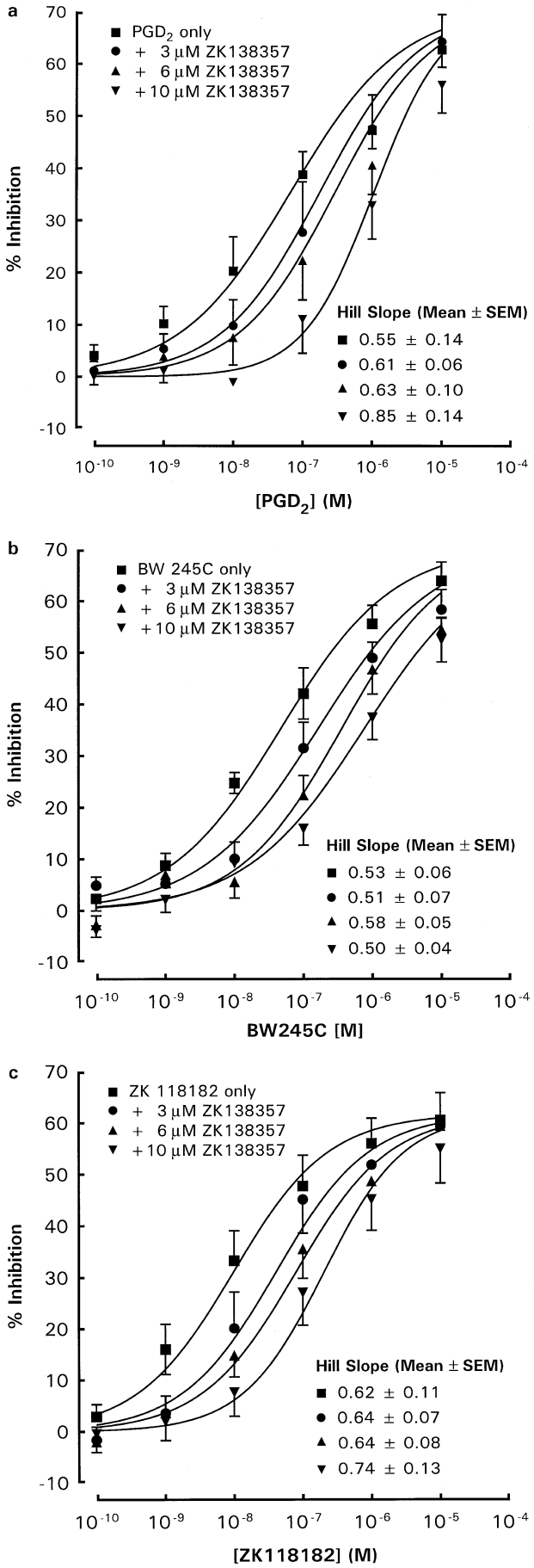

Figure 6 Effects of ZK 138357 on the inhibitory actions of (a) PGD_2, (b) BW 245C and (c) ZK 118182 on anti-IgE induced histamine release from rat peritoneal mast cells. Mast cells were exposed simultaneously to ZK 138357, anti-IgE and the DP agonist. Spontaneous histamine release was 9.3 ± 1.0, 10.8 ± 0.8 and $9.1 \pm 1.0\%$ whereas anti-IgE induced histamine release was 26.7 ± 3.3, 26.4 ± 3.3 and $28.5 \pm 4.0\%$ for the PGD_2 ($n=6$), BW 245C ($n=6$) and ZK 118182 ($n=7$) experiments respectively. Results are given as means $\pm$ s.e.mean for the number of experiments indicated.

human mast cells (Albro *et al*., 1972; Peters *et al*.,1982; Takei & Endo,1994; Wescott & Kaliner, 1981). In BNu-2cl3 cultured mouse mast cells however, there is the potential for excitatory actions through both EP_3 receptors and novel IP receptors linked to phosphoinositide metabolism *via* pertussis toxin-sensitive G proteins (Oka *et al*., 1993). The absence of excitatory EP receptors in rat peritoneal mast cells is further confirmed by our observation that the EP_1/EP_3 agonist,

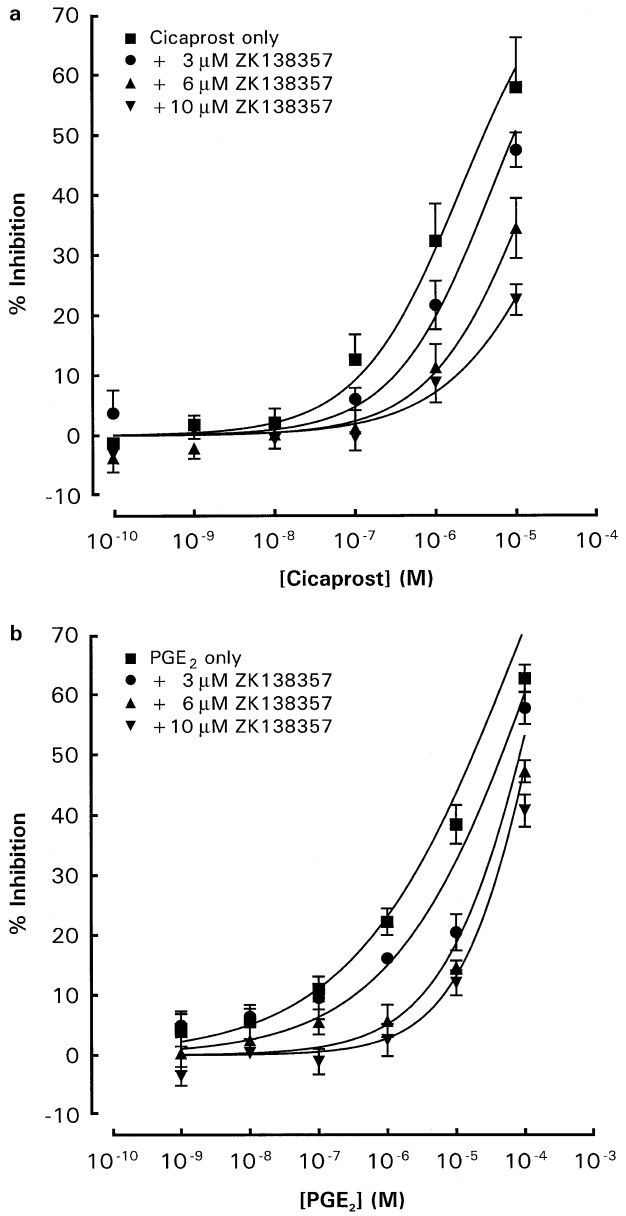

Figure 7 Effects of ZK 138357 on the inhibitory actions of (a) PGE_2 and (b) cicaprost on anti-IgE induced histamine release from rat peritoneal mast cells. Mast cells were exposed simultaneously to ZK 138357, anti-IgE and the prostanoid agonist. Spontaneous histamine release was 12.2 ± 0.9 and $11.2 \pm 0.9\%$ whereas anti-IgE induced histamine release was 29.0 ± 2.3 and $30.4 \pm 2.6\%$ for the PGE_2 ($n=4$) and cicaprost ($n=7$) experiments respectively. Results are given as means $\pm$ s.e.mean for the number of experiments indicated.

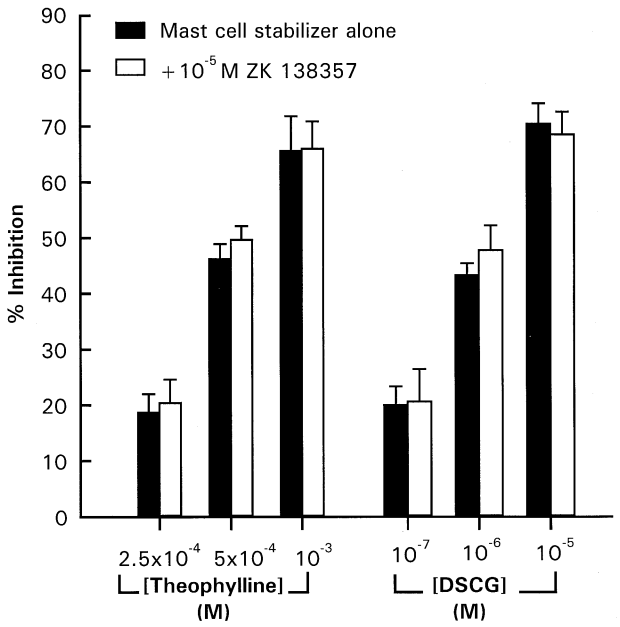

Figure 8 Effects of ZK 138357 on the inhibitory actions of theophylline and disodium cromoglycate (DSCG) on anti-IgE induced histamine release from rat peritoneal mast cells. Mast cells were exposed simultaneously to ZK 138357, anti-IgE and the mast cell inhibitor. Spontaneous histamine release was $9.9 \pm 0.2\%$ whereas anti-IgE induced histamine release was $36.3 \pm 1.6\%$. Results are given as means $\pm$ s.e.mean for $n = 5$.

sulprostone (Bunce *et al*., 1991) and the highly potent and selective EP_3 agonist SC-46275 (Savage *et al*., 1993) did not alter spontaneous or immunologically-induced histamine release.

The ranking of inhibitory potencies on the rat peritoneal mast cell indicates the presence of a DP-receptor (Table 1). First, PGD_2 is active at concentrations as low as 10 nM and is considerably more potent than $PGF_{2\alpha}$ and U-46619. Secondly, two purportedly selective DP-receptor agonists were at least five times more potent than PGD_2. BW 245C is an older agent previously shown to be slightly more potent than PGD_2 in activating adenylate cyclase in rat peritoneal mast cells (Narumiya & Toda, 1985). ZK 118182 is a newer prostanoid which is more potent then PGD_2 in raising cyclic AMP levels in human platelets (Darius *et al*., 1994) and suppressing superoxide production in human neutrophils (Pons *et al*., 1994). Thirdly, the DP-receptor antagonist ZK 138357 (Lydford *et al*., 1996) blocks the action of PGD_2, BW 245C and ZK 118182 to similar extents. The preferred DP antagonist in this type of study would be BW A868C because of its high affinity ($pA_2 = 8.5-9.3$) and specificity (Giles *et al*., 1989). Unfortunately this compound is not available for general use, and instead we have been obliged to use ZK 138357, which has some chemical resemblance to BW A868C but is a somewhat less potent DP blocker. ZK 138357 appeared to act specifically in our experiments, with the highest concentration used (10^{-5} M) having no effect on inhibition of histamine release due to either the anti-allergic compound disodium cromoglycate or the phosphodiesterase inhibitor theophylline. K.H. Thierauch and colleagues have reported that ZK 138357 has a high affinity for the DP receptor on human platelets ($pA_2 = 7.7$), but has no affinity for other prostanoid receptors (unpublished abstracts of 9th International Conference in Prostaglandins and Related Compounds, Florence, Italy, 1994).

Another DP antagonist AH 6809 ($pA_2 = 5.0$; Keery & Lumley, 1988) was tested in our system in the knowledge that it also blocks EP_1-receptors ($pA_2 = 7.1$; Coleman *et al*., 1985; Lawrence *et al*., 1992) and EP_2-receptors ($pA_2 = 6.5$; Regan *et al*., 1994). In our experiments, it had no effect on PGD_2-induced inhibition at 10^{-7} M and enhanced the inhibition at 10^{-6} M. The latter effect may be due to its ability to inhibit cyclic-AMP-phosphodiesterase, as found in rat lung mast cells (Keery & Lumley, 1988). Thus it was impossible to determine whether AH 6809 has significant DP blocking activity in the present study.

Since the concentration-inhibition curves of the prostanoid receptor agonists in general have Hill slopes of less than one, the observed inhibitory effects might be mediated by more than one receptor. Hence the question remains as to whether inhibitory EP or IP receptors are present on the rat peritoneal mast cell. Activation of EP_2 and/or EP_4 receptors (linked to adenylate cyclase) could account for the inhibitory activity of PGE_2, and in this context, binding sites for PGE_1 and PGE_2 have been identified in mouse mastocytoma P815 cells (Hashimoto *et al*., 1990; Nishigaki *et al*., 1995). Furthermore, both prostaglandin D_2 and BW245C were reported to produce a BW868C resistant phase of agonism in rabbit jugular vein which might be mediated through EP_2 receptors (Giles *et al*., 1989). However, we favour the alternative explanation that PGE_2 is a low potency DP agonist, based on two pieces of evidence. Firstly, ZK 138357 blocked the action of PGD_2 and PGE_2 to similar extent. Secondly, the selective EP_2 agonist butaprost did not inhibit histamine release, and the EP_4 antagonist AH 23848 (Coleman *et al*., 1994a) failed to affect the inhibitory activities of either PGD_2 or PGE_2. The relatively flat agonist concentration-inhibition curves may be due to the fact that we are measuring an indirect physiological consequence of receptor activation instead of measuring the immediate biochemical change induced by receptor activation. Actually, when we monitored the effects of PGD_2 on intracellular cyclic AMP level, a concentration-response curve with a Hill slope of near unity was observed (data not shown).

At present, the best chance of pharmacologically identifying an IP-receptor lies with the prostacyclin analogue cicaprost, due to its high potency and specificity (Dong *et al*., 1986; Lawrence *et al*., 1992). For example, it inhibits human platelet aggregation over the 0.1–1 nM concentration range (Armstrong *et al*., 1989) and relaxes vascular smooth muscle between 1 and 10 nM (see Jones *et al*., 1997). On other prostanoid systems we have come to expect cicaprost to be at least 100 times less potent than the appropriate standard agonist (e.g. PGE_2 on EP receptors). In the present study, cicaprost was a fairly weak agonist in absolute terms ($-\log IC_{30} = 5.68$), but was only less potent than PGD_2 by around 1 log unit. However, ZK 138357 blocked cicaprost with an affinity similar to that found with PGD_2 as agonist, suggesting that cicaprost is indeed a DP agonist on rat peritoneal mast cells.

Although there is no formal inclusion of DP-receptor subtypes in the current prostanoid receptor classification scheme (Coleman, 1998), there is some evidence in the literature for their existence. The first indication came from the work of Jones (1976; 1978) on the vasoconstrictor actions of PGD_2 in the sheep, rabbit, pig and dog. This receptor system differed from the classical (adenylate cyclase-linked) DP-receptor in the human platelet in that a 'natural' 15(S)-15-hydroxy substituent in the ω-chain was not essential to high biological potency. PGD_2, 15(*R*) PGD_2, 13,14-dihydro-15-keto PGD_2 and 15-O-methyl PGD_2 were all potent vasoconstrictors, and considerably more potent than the corresponding $PGF_{2\alpha}$ analogues. However, only PGD_2 showed inhibitory activity on human platelets. It is of interest that Rangachari &

Table 3 Comparison of the potencies of PGD_2, BW 245C and ZK 118182 in different isolated preparations

DP agonists	*Rat peritoneal mast cell (histamine release)*	**Rat peritoneal mast cell (↑cyclic AMP)*	***Rabbit isolated saphenous vein (relaxation)*	*†Human platelet (↑cyclic AMP)*	*Human non-pregnant myometrium (↓cloprostenol-induced activity)*	*††Human neutrophils (superoxide production)*
PGD_2	0.26	0.6	0.1	0.03	0.5	0.19
BW 245C	0.06	0.41	0.26	0.005	0.03	0.01
ZK 118182	0.02	Not done	0.43	0.06	Not done	0.06

The IC_{30} values reported in Table 1 were used as the EC_{50} (μM) equivalence in the first data column. Other data from:* Narumiya & Toda & Toda (1985); **Lydford *et al.* (1996); †Darius *et al.* (1994); ††Fernandes & Crankshaw (1995).

Betti (1993) were able to provisionally detect both DP-receptor subtypes in a single preparation, the dog isolated colonic epithelium. Short-circuit current (a measure of active Cl^- secretion) was usually increased by PGD_2 and always by 9α,11β PGF_2 (classical DP-receptor), whereas short-circuit current was decreased occasionally by PGD_2 and always by 13,14-dihydro-15-keto PGD_2 (non-classical DP-receptor). It is clear from our results on the rat peritoneal mast cell that 9α,11β PGF_2 is a moderately potent agonist and 13,14-dihydro-15-keto PGD_2 a very weak agonist, and this argues against the presence of the non-classical DP-receptor.

Narumiya & Toda (1985) have argued for the existence of three subtypes of DP-receptor. The first of these, in guinea-pig trachea and dog cerebral artery, was difficult to define as a DP-receptor, since the two most active excitatory agonists tested, PGD_2 and its 17-phenyl-ω-trinor analogue, were not particularly potent ($\geqslant$100 nM required for contraction). Furthermore, both preparations are known to be highly sensitive to the contractile actions of TP agonists and a TP antagonist was not used in the study. In addition, the guinea-pig trachea contains an excitatory EP_1 system, on which the corresponding 17-phenyl-ω-trinor derivative of PGE_2 is a highly potent agonist ($EC_{50} \sim 2$ nM) (Lawrence *et al.*, 1992). Activation of the second putative DP-receptor inhibited the growth of the mouse L-1210 leukaemia cell line and 9-deoxy-$\Delta^{9,12}$ PGD_2 was about three times more potent than PGD_2 (Fukushima *et al.*, 1982; Kikawa *et al.*, 1984). The problem here is that micromolar concentrations of PGD analogues are required, and there is the possibility of a non-prostanoid receptor mechanism whereby an enone unit in or generated from the PGD molecule covalently interacts with a component of the growth regulatory system. The remaining receptor, which was present in the tissue under study here, rat peritoneal mast cells, did indeed appear to be a DP receptor. The available, although limited, biological data for PGD_2, BW 245C and ZK 118182 acting on DP systems are shown in Table 3. The three human systems show reasonable agreement for agonist potencies, bearing in mind that the human platelet data are from a biochemical as opposed to a functional assay. On the rabbit and rat preparations, BW 245C appears to be somewhat less potent, but the differences are too small to conclude even tentatively the existence of DP receptor subtypes, and species variants would seem more likely.

The pA_2 value of around 6 for ZK 138357 estimated in the current study is intermediate between the two previously reported pA_2 values of 7.25 in human PMN leukocytes and 5.09 in the rabbit isolated saphenous vein for blocking the actions of BW 245C (Lydford *et al.*, 1996). Although these values were only estimated by using a single concentration of the antagonist and hence may represent extremes of estimation, the authors postulated that they may implicate the presence of DP receptor subtypes in the two systems. This is worthy of further investigation, although it is relevant to consider the work of Jones and co-workers on TP-receptors in smooth muscle and platelets (Tymkewycz *et al.*, 1991). Very good agreement was obtained between agonist potencies across both species and preparations, but the affinities of a range of structurally different antagonists varied considerably and no firm conclusions could be drawn about TP-receptor subtypes.

In conclusion, the current study has positively demonstrated that a PGD_2-specific receptor is the predominant prostanoid receptor in rat peritoneal mast cells. This receptor is potently activated by ZK 118182 and blocked, probably competitively, by the related compound ZK 138357. Since mast cell activation is an integral part of allergic reactions, development of highly specific agonists for the mast cell DP receptor may be useful in the management of allergic diseases such as asthma, providing that similar observations in human mast cells are obtained in future studies.

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