British Journal of Pharmacology (1997) 121, 77–82

Characterization of the prostanoid receptors mediating inhibition of PAF-induced aggregation of guinea-pig eosinophils

[1]Mauro M. Teixeira, Sabah Al-Rashed, [2]Adriano G. Rossi & Paul G. Hellewell

Applied Pharmacology, Imperial College of Medicine at the National Heart and Lung Institute, Dovehouse Street, London SW3 6LY

1 Prostanoids induce a wide range of biological actions which are mediated by specific membrane-bound receptors. We have recently shown that the E-type prostaglandins, PGE_1 and PGE_2, effectively inhibit eosinophil aggregation induced by platelet-activating factor (PAF). In an attempt to determine which prostanoid receptor(s) were involved, we investigated the effects of a range of selective prostanoid agonists and antagonists on eosinophil homotypic aggregation induced by PAF.

2 Both PGE_1 and PGE_2 (10^{-10} to 10^{-6} M) induced a concentration-related inhibition of the aggregation response induced by PAF. PGE_1 was more effective than PGE_2 but PGE_2 was slightly more potent than PGE_1 (approximate IC_{50} values for PGE_1 and PGE_2 of 1.5×10^{-8} M and 5×10^{-9} M, respectively).

3 The EP_2-selective agonists, 11-deoxy-PGE_1, butaprost and AH13205, and the EP_2/EP_3-selective agonist, misoprostol, also inhibited PAF-induced aggregation. The rank order of potency for EP_2-selective agonists was 11-deoxy-PGE_1 > misoprostol > butaprost = AH13205. The protein kinase A inhibitor, KT5720 (10^{-6} M), reversed the inhibitory effects of 11-deoxy-PGE_1 (10^{-6} M) and AH13205 (10^{-5} M).

4 The EP_1/EP_3-selective agonist, sulprostone, and the EP_1-selective agonist, 17-phenyl-ω-trinor PGE_2, had no significant inhibitory activity when tested at concentrations up to 10^{-6} M. The EP_4-receptor antagonist, AH23848B, had no effect on PAF-induced aggregation and did affect the inhibitory activity of PGE_1.

5 The IP-selective agonist, cicaprost (up to 10^{-6} M), and the IP/EP_1-receptor agonist, iloprost (up to 10^{-5} M), had no significant effect on PAF-induced eosinophil aggregation. However, iloprost significantly augmented the inhibitory effects of a maximally inhibitory concentration of PGE_2.

6 PGD_2 (10^{-5} M) had no effect on eosinophil aggregation and the inhibitory activity of PGE_1 on PAF-induced eosinophil aggregation was not altered by the DP-selective receptor antagonist, BWA868C.

7 The results presented here suggest that the inhibition of PAF-induced eosinophil aggregation by prostanoids is mediated by the occupation of EP_2-receptors. It is important to note that the effects of naturally occuring prostanoids, such as PGE_2, on eosinophil aggregation occur at low concentrations highlighting a potential role for EP_2 receptors in regulating eosinophil function *in vivo*.

Keywords: Eosinophils; prostanoid receptors; aggregation; cyclic AMP; prostaglandins

Introduction

Eosinophils are thought to play a major role in the pathophysiology of allergic diseases such as asthma and atopic dermatitis (Butterfield & Leiferman, 1993). For example, in asthma the number of eosinophils and eosinophil-derived secretory products (e.g. eosinophil major basic protein) are elevated in brochoalveolar lavage fluid and appear to correlate positively with the severity of the disease (Djukanovic *et al*., 1990; Gleich *et al*., 1993). Thus, it is possible that drugs which inhibit the activation of eosinophils may be useful in the treatment of allergic diseases.

Prostanoids induce a wide range of biological actions which are mediated by specific membrane-bound receptors (Coleman *et al*., 1994b). The classification of prostanoid receptor subtypes was initially based upon the activities of natural and synthetic agonists. More recently, this classification has been verified by the availability of cloning data and specific receptor antagonists (Coleman *et al*., 1994b; Pierce *et al*., 1995). These receptors have been categorized into 5 groups, namely the DP, EP, IP, FP and TP receptors, based on the binding characteristics of the five main naturally occurring prostanoids, prostaglandin (PG)D_2, PGE_2, PGI_2, $PGF_{2\alpha}$ and thromboxane A_2, respectively (Coleman *et al*., 1994b). In addition, the EP-receptors have been further divided into four subtypes; EP_1, EP_2, EP_3 and EP_4 receptors. Previous studies have shown that the occupation of EP_2, IP or DP prostanoid receptors inhibits certain functions of activated neutrophils, including the respiratory burst, homotypic aggregation and secretion (Rossi & O'Flaherty, 1989; Wheeldon & Vardey, 1993; Wise & Jones, 1994; Talpain *et al*., 1995). Interestingly, these three receptors are coupled to Gs and appear to mediate inhibition of neutrophil function via elevation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Coleman *et al*., 1994b).

We have previously shown that the E-type prostaglandins PGE_1 and PGE_2 effectively inhibited eosinophil aggregation induced by PAF and C5a (Teixeira *et al*., 1996a). In addition, we demonstrated that inhibition of eosinophil aggregation was mediated via the increase of cyclic AMP in eosinophils (Teixeira *et al*., 1996a). However, as yet, there have been few studies attempting to determine which type(s) of prostanoid receptor(s) are involved in the inhibition of eosinophil activity. Butchers & Vardey (1990) suggested that occupation of both DP and EP_2 receptors inhibited the secretion of eosinophil cationic protein by human eosinophils. However, these studies did not use purified eosinophil preparations leaving the possibility that the prostanoid agonists were acting indirectly to inhibit eosinophil activation. In this study, in an attempt to characterize the

[1] Author for correspondence.
[2]Present address: Respiratory Medicine Unit, Department of Medicine, Rayne Laboratory, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG.

prostanoid receptors mediating inhibition of eosinophil function *in vitro*, we investigated the effects of a range of selective prostanoid agonists and antagonists on eosinophil homotypic aggregation induced by PAF. Aggregation was assessed by measuring changes in light transmission after activation of eosinophils in suspension (Teixeira *et al*., 1995; 1996a).

Methods

Induction, harvesting and purification of guinea-pig eosinophils

Eosinophils were induced, harvested and purified as described previously (Teixeira *et al*., 1995; 1996a). Briefly, ex-breeder female guinea-pigs (Harlan, Oxon; 700–800 g) were treated with undiluted horse serum (1 ml i.p.) every other day for two to three weeks and the cells collected by peritoneal lavage with heparinized saline (10 iu ml^{-1}) 2 days after the last injection. The cells obtained were layered onto a discontinuous Percoll-HBSS (calcium- and magnesium-free) gradient followed by centrifugation (1500 *g*, 25 min at 20°C). Eosinophils were collected from the 1.090/1.095 and 1.095/1.100 g ml^{-1} density interfaces. Eosinophils were >95% pure as assessed by differential staining with DiffQuick (BDH, Dorset) and >98% viable as assessed by trypan blue exclusion. The cells were then washed twice in phosphate buffered saline (PBS, calcium- and magnesium-free, pH 7.4) to which $CaCl_2$ and $MgCl_2$ (final concentrations 1.0 mM and 0.7 mM, respectively) were added, and the cells kept on ice.

Eosinophil aggregation

Aggregation experiments were carried out as previously described (Teixeira *et al*., 1995; 1996a,b). Briefly, guinea-pig eosinophils were resuspended (5×10^6 cells ml^{-1}) in PBS and aliquots (300 μl) of cells were dispensed into siliconized cuvettes which were placed into a dual channel platelet aggregometer (Chronolog 440 VS) linked to a dual pen recorder (Chronolog 707). The cells were incubated for 5 min at 37°C with continuous stirring at 700 r.p.m. before addition of prostanoid agonists (PGE_1, PGE_2, PGD_2, 11-deoxy-PGE_1, butaprost, misoprostol, AH13205, sulprostone, 17-phenyl-ω-trinor PGE_2, iloprost and cicaprost). After two minutes incubation with the indicated agonist, eosinophils were stimulated with PAF (10^{-7} M). For the experiments with the protein kinase A inhibitor KT5720, eosinophils were pretreated with KT5720 (10^{-6} M) for 2 min before the addition of 11-deoxy-PGE_1, AH13205 or sulprostone. Similarly, eosinophils were incubated for 2 min with AH23848B (10^{-5} M) or BWA868C (10^{-5} M) before the addition of PGE_1 (10^{-7} or 10^{-8} M). The reference cuvette contained buffer alone. Responses were measured at the peak of aggregation and the results expressed as the percentage inhibition of the responses induced by 10^{-7} M PAF.

Reagents

Horse serum, phosphate-buffered saline (PBS, calcium- and magnesium-free, pH 7.4), and Hank's balanced salt solution (HBSS) were purchased from Life Technologies Ltd (Paisley). Percoll was purchased from Pharmacia (Milton Keynes). Misoprostol and C16 PAF were from Bachem (Saffron Walden). The following reagents were purchased from Sigma Chemical Company (Poole): bovine serum albumin (BSA), dimethyl sulphoxide (DMSO), D-glucose, prostaglandin (PGE_1), PGE_2, PGD_2. KT5720 ((8R*, 9S*, 11S*)-(−)-9-hydroxy-9-m-hexyl-8-methyl-2,3,9,10-tetrahydro-8, 11-epoxy-1H,8H,11H-2,7b,11a-triazadibenzo(a,g) cycloocta (cde)-frinden-1-one) was from Calbiochem (Nottingham). Cicaprost, sulprostone and iloprost were a gift from Dr F. McDonald (Schering AG, Germany). AH23848B ([1α(2),2β,5α]-(±)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-merpholinyl)-3-oxocyclopentyl]-5-heptenoic acid) and AH13205 (trans-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclopentaneheptanoic acid) were a gift from Dr R. Coleman (Glaxo, Ware) and Butaprost from Miles Inc (Slough). BWA868C ((±)-3-benzyl-5-(6-carboxyhexyl)- 1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin was a gift from Dr B.J.R. Whittle (Wellcome, Beckenham). 17-Phenyl-ω-trinor PGE_2 and 11-deoxy-PGE_2 were purchased from Cayman Chemicals (Ann Arbor, MI, U.S.A.). None of the vehicles used in this study significantly altered eosinophil aggregation induced by platelet-activating factor (PAF, data not shown). The chemical structures and receptor selectivity of the prostanoids given above are described in detail by Coleman *et al*., (1994b).

Statistical analysis of data

Data were analysed by Student's *t* test or analysis of variance where appropriate (*P* values assigned by Newman Keul's post test) with the statistical programme Instat (GraphPad Software V2.03). Results were considered significant when $P<0.05$ and data are shown as the mean±s.e.mean of *n* experiments.

Results

Effects of PGE_1 and PGE_2 on PAF-induced eosinophil aggregation

Both PGE_1 and PGE_2 (10^{-10} to 10^{-6} M) induced a concentration-related inhibition of the aggregation response induced by PAF (Figure 1). PGE_1 was more effective than PGE_2 producing a maximal inhibition of 100% and 66.2±4.5% ($n=4-7$, $P<0.01$), respectively, at a concentration of 10^{-6} M. However, the concentration-response curve for PGE_2 was steeper than PGE_1 up to 10^{-8} M such that PGE_2 was slightly more potent (approximate IC_{50} values for PGE_1 and PGE_2 were 1.5×10^{-8} M and 5×10^{-9} M, respectively, $P>0.05$).

Effects of EP-receptor agonists/antagonist on PAF-induced eosinophil aggregation

The EP_2-selective agonists, 11-deoxy-PGE_1, butaprost and AH13205 also inhibited PAF-induced aggregation by 65.8±6.3% ($n=4$), 55.5±5.6% ($n=5$) and 46.2±8.2% ($n=5$), respectively, at a concentration of 10^{-5} M (Figure 2). Whereas, 11-deoxy-PGE_1 (IC_{50} 8×10^{-8} M) was approximately 5 and 15 times less potent that PGE_1 and PGE_2, respectively (see

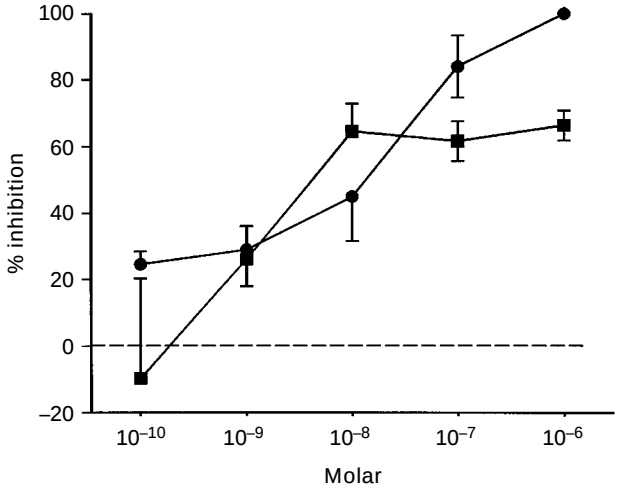

Figure 1 Effect of PGE_1 and PGE_2 on eosinophil aggregation induced by PAF. Eosinophils were incubated with PGE_1 (10^{-10} to 10^{-6} M, ●) or PGE_2 (10^{-10} to 10^{-6} M, ■) for 2 min and then activated with PAF (10^{-7} M). Results are expressed as the percentage inhibition of PAF-induced eosinophil aggregation and each point is the mean of 4–7 experiments; vertical lines show s.e.mean.

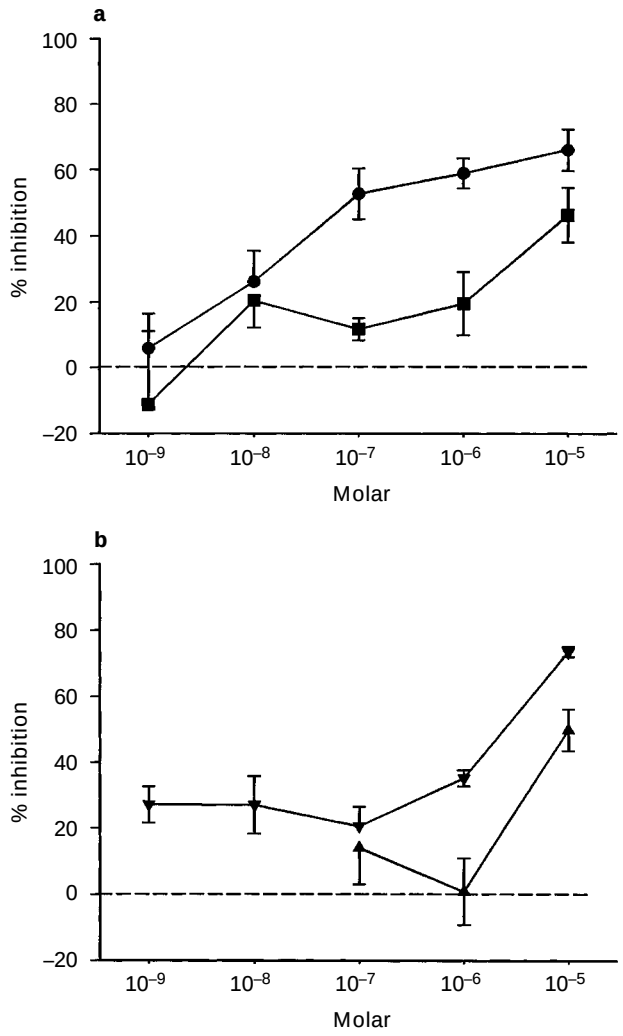

Figure 2 Effect of EP_2 receptor agonists on eosinophil aggregation induced by PAF. Eosinophils were incubated with (a) 11-deoxy-PGE_1 (10^{-9} to 10^{-5} M, ●) or AH13205 (10^{-9} to 10^{-5} M, ■) and (b) misoprostol (10^{-9} to 10^{-5} M, ▼) or butaprost (10^{-7} to 10^{-5} M, ▲) for 2 min and then activated with PAF (10^{-7} M). Results are expressed as the percentage inhibition of PAF-induced eosinophil aggregation and each point is the mean of 4–6 experiments; vertical lines show s.e.mean.

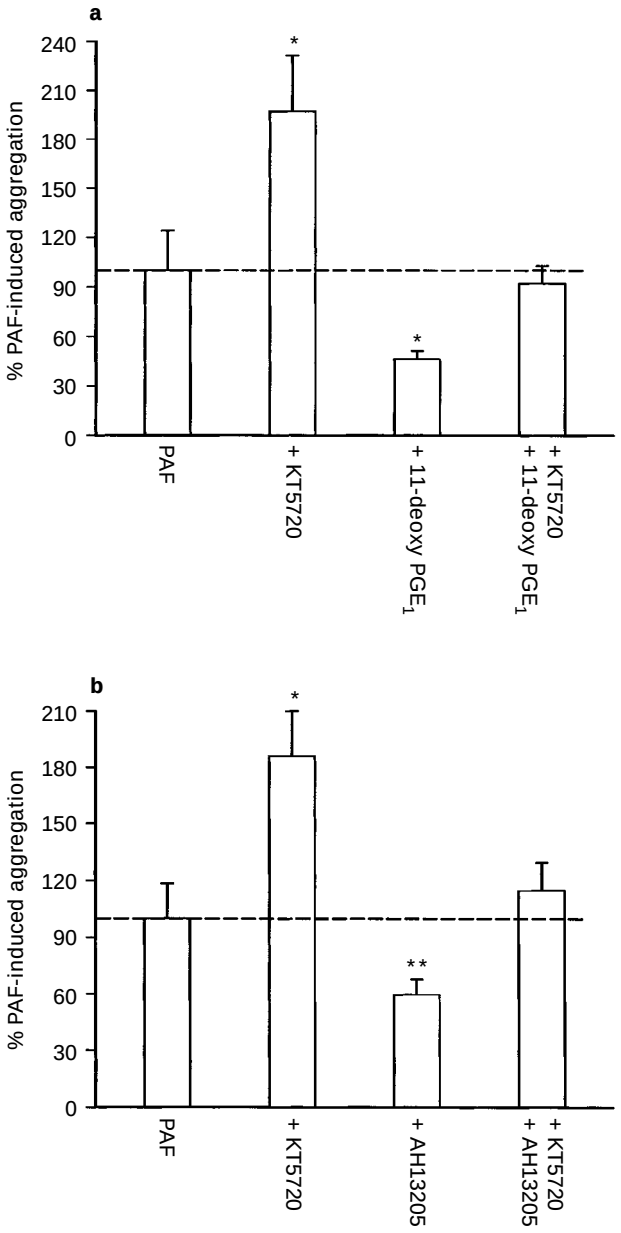

Figure 3 Effect of the protein kinase A inhibitor KT5720 on the inhibitory effects of (a) 11-deoxy-PGE_1 or (b) AH13205 on PAF-induced eosinophil aggregation. Eosinophils were incubated with KT5720 (10^{-6} M) or vehicle for 2 min, then 11-deoxy-PGE_1 (10^{-6} M), AH13205 (10^{-5} M) or vehicle were added for a further 2 min and the cells activated with PAF (10^{-7} M). Results are expressed as the percentage of PAF-induced eosinophil aggregation and each column is the mean ± s.e.mean of 4–5 experiments. $^*P<0.05$ and $^{**}P<0.01$, when compared to responses in the presence of PAF alone.

above), both AH13205 and butaprost significantly inhibited PAF-induced eosinophil aggregation at the highest concentration tested only (10^{-5} M). The EP_2/EP_3-receptor agonist misoprostol was an effective inhibitor of PAF-induced aggregation (73.5 ± 1.5%, $n=6$, at 10^{-6} M) but displayed low potency (IC_{50} 3×10^{-6} M). Thus the rank order of potency for EP_2-selective agonists was 11-deoxy-PGE_1 > misoprostol > butaprost = AH13205.

To assess the role of cyclic AMP in mediating the inhibitory effects of EP_2-selective agonists on PAF-induced eosinophil aggregation, we investigated the effect of the protein kinase A (PKA) inhibitor, KT5720 (10^{-6} M). At this concentration, KT5720 has been shown to have at least 30 fold specificity for PKA over other protein kinases (Irie *et al*., 1985). As shown in Figure 3, preincubation with KT5720 reversed the inhibitory effects of 11-deoxy-PGE_1 (10^{-5} M) and AH13205 (10^{-5} M) on eosinophil aggregation induced by PAF. This is in agreement with the reversal by KT5720 of the inhibitory effects of PGE_1 on PAF-induced eosinophil aggregation (Teixeira *et al*., 1996a). Similarly, as we have previously demonstrated (Teixeira *et al*., 1996a), preincubation of eosinophils with KT5720 alone significantly increased the aggregation response induced by PAF (Figure 3).

The EP_1/EP_3-selective agonist, sulprostone, had no significant inhibitory activity when tested at concentrations up to 3×10^{-6} M (Figure 4). Similarly, the EP_1-selective agonist, 17-phenyl-ω-trinor PGE_2, had no significant inhibitory activity on PAF-induced aggregation when tested at concentrations up to 10^{-6} M (Figure 4). However, 10^{-5} M 17-phenyl-ω-trinor PGE_2 significantly inhibited PAF-induced aggregation (52.8 ± 9.4%, $n=4$, $P<0.05$). The inhibitory effects of 10^{-5} M 17-phenyl-ω-trinor PGE_2 contrast to the lack of inhibitory effects of the EP_1/IP-selective agonist, iloprost, on PAF-induced aggregation (see below).

The EP_4-selective receptor antagonist, AH23848B, had no effect on eosinophil aggregation induced by PAF when used at concentrations up to 10^{-5} M (data not shown). This top con-

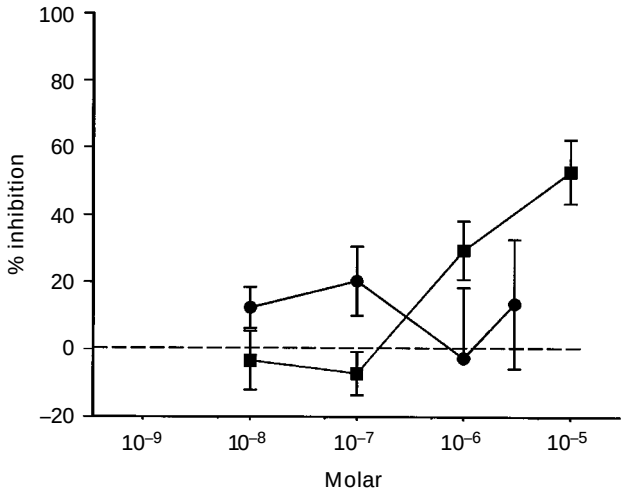

Figure 4 Effect of EP_1 and EP_3 receptor agonists on eosinophil aggregation induced by PAF. Eosinophils were incubated with sulprostone (10^{-8} to 3×10^{-6} M, ●) or 17-phenyl-ω-trinor PGE_2 (10^{-8} to 10^{-5} M, ■) for 2 min and then activated with PAF (10^{-7} M). Results are expressed as the percentage inhibition of PAF-induced eosinophil aggregation and each point is the mean of 4–5 experiments; vertical lines show s.e.mean.

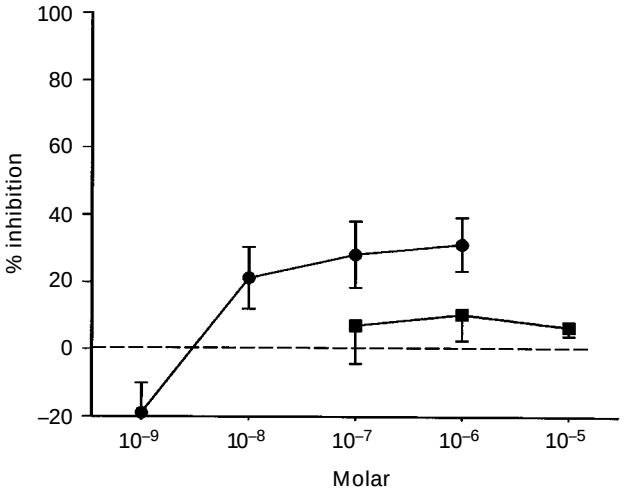

Figure 6 Effect of IP receptor agonists on eosinophil aggregation induced by PAF. Eosinophils were incubated with cicaprost (10^{-9} to 10^{-6} M, ●) or iloprost (10^{-7} to 10^{-5} M, ■) for 2 min and then activated with PAF (10^{-7} M). Results are expessed as the percentage inhibition of PAF-induced eosinophil aggregation and each point is the mean of 5 experiments; vertical lines show s.e.mean.

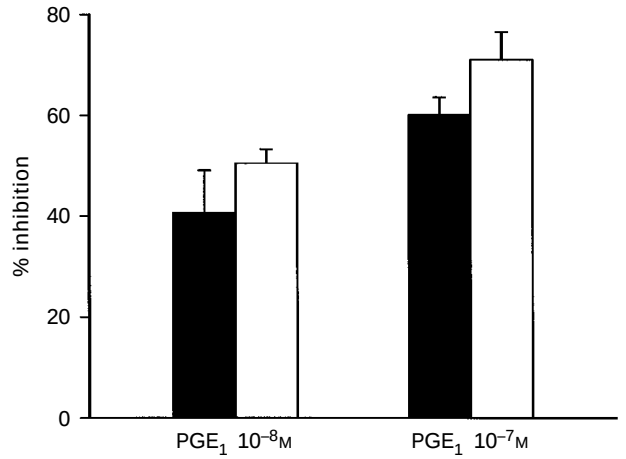

Figure 5 Effect of AH23848B on the inhibitory effects of PGE_1 on PAF-induced eosinophil aggregation. Eosinophils were incubated with AH23848B (10^{-5} M, open columns) or vehicle (solid columns) for 2 min, then PGE_1 (10^{-8} and 10^{-7} M) was added for a further 2 min and the cells activated with PAF (10^{-7} M). Results are expressed as the percentage of PAF-induced eosinophil aggregation and each column is the mean ± s.e.mean of 3 experiments.

centration of AH23848B was then used in further experiments. As shown in Figure 5, AH23848B had no significant effect on the inhibitory activity of PGE_1 against PAF-induced eosinophil aggregation.

Effects of IP-receptor agonists on PAF-induced eosinophil aggregation

The IP-selective agonist, cicaprost, had no significant effect on eosinophil aggregation induced by PAF (Figure 6). Maximal inhibition of PAF-induced response was obtained at 10^{-6} M ($31.3\pm7.9\%$, $n=5$) but this did not achieve statistical significance. Similarly, the IP/EP_1-receptor agonist, iloprost, had no effect on eosinophil aggregation induced by PAF when tested at concentrations up to 10^{-5} M (Figure 6). In contrast, pretreatment of eosinophils with 10^{-6} M iloprost significantly ($P<0.05$) augmented the effects of a maximally inhibitory concentration of PGE_2 (10^{-7} M) on PAF-induced aggregation (PAF, $49.6\pm7.0\%$ maximal aggregation; PAF + iloprost, $41.4\pm2.7\%$; PAF + PGE_2, $29.0\pm6.4\%$; PAF + PGE_2 + iloprost, $15.4\pm2.4\%$, $n=4$).

Effects of PGD_2 and a DP-receptor antagonist on PAF-induced eosinophil aggregation

PGD_2 had no effect on eosinophil aggregation induced by PAF when used at concentrations up to 10^{-5} M (Figure 7a). In addition and in contrast to data showing effects of PGD_2 on eosinophil chemotaxis (Butchers & Vardey, 1990), PGD_2 induced no measurable eosinophil aggregation (data not shown). Moreover, the DP-selective receptor antagonist, BWA868C, had no effect on eosinophil aggregation induced by PAF when used at concentrations up to 10^{-5} M (data not shown). This top concentration of BWA868C was then used in further experiments. As shown in Figure 7b, BWA868C had no significant effect on the inhibitory activity of PGE_1 against PAF-induced eosinophil aggregation.

Discussion

When activated *in vitro* with different inflammatory stimuli (e.g. PAF, C5a), guinea-pig eosinophils undergo a concentration-dependent aggregation response (Teixeira *et al.*, 1995). This eosinophil aggregation is also dependent on calcium and magnesium ions and on the cell adhesion molecules CD18 and L-selectin present on the eosinophil surface (Teixeira *et al.*, 1995; 1996b). *In vivo*, eosinophil aggregation around larvae of migrating parasites may represent an effective means of arresting parasite movement and facilitating parasite killing (McLaren, 1980). Eosinophil aggregation also occurs after intradermal injection of the β-chemokine RANTES in dog skin (Meurer *et al.*, 1993). In this investigation we have attempted to characterize the prostanoid receptors present on guinea-pig eosinophils which mediate inhibition of eosinophil function, by using a range of prostanoid agonists and antagonists. Eosinophil aggregation was used as a measure of eosinophil activation.

We have recently shown PGE_1 and other cyclic AMP-elevating agents (β-adrenoceptor agonists and phosphodiesterase type 4 inhibitors) to inhibit effectively eosinophil aggregation induced by PAF and C5a (Teixeira *et al.*, 1996a). The importance of cyclic AMP in mediating the inhibitory effects of

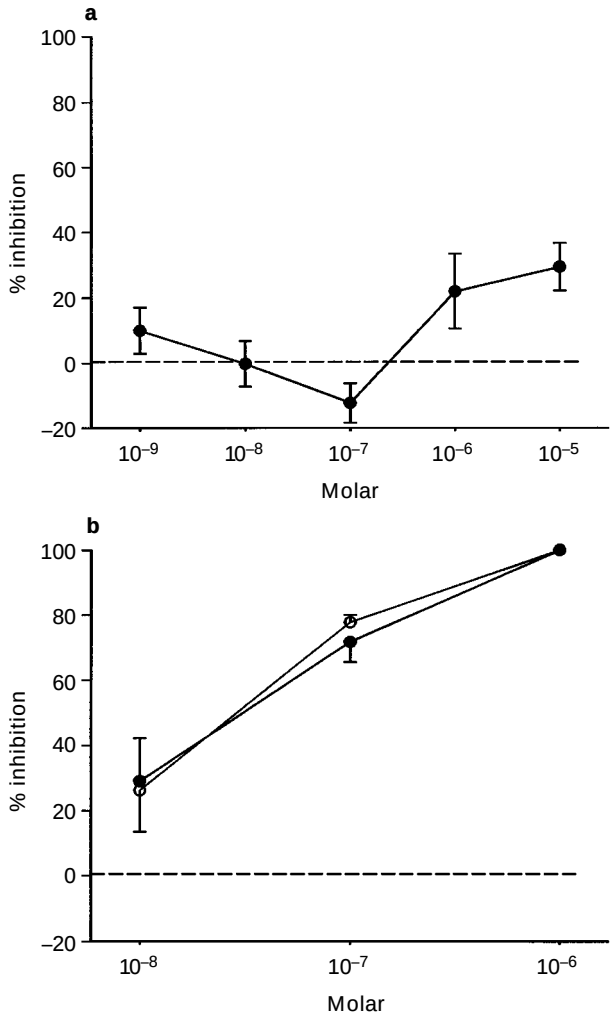

Figure 7 Effect of (a) PGD_2 on eosinophil aggregation induced by PAF and (b) the DP receptor antagonist BWA868C on the inhibitory effects of PGE_1 on PAF-induced eosinophil aggregation. In (a), eosinophils were incubated with PGD_2 (10^{-9} to 10^{-5} M) for 2 min and then activated with PAF (10^{-7} M). In (b), eosinophils were incubated with BWA868C (10^{-5} M, ○) or vehicle (●) for 2 min, then PGE_1 (10^{-8} to 10^{-6} M) was added for a further 2 min and the cells activated with PAF (10^{-7} M). Results are expressed as the percentage inhibition of PAF-induced eosinophil aggregation and each point is the mean of 3–5 experiments; vertical lines show s.e.mean.

these agents was based on two main findings: reversal of the inhibitory effects of PGE_1 by protein kinase A inhibitors (H-89 and KT5720) and the synergy of PGE_1 with a phosphodiesterase type 4 inhibitor, rolipram (Teixeira *et al*., 1996a). Since the effects of PGE_1 appear to be mediated via an increase in cyclic AMP, there are three adenylate cyclase-coupled prostanoid receptors (EP, DP and IP) which could be involved (Coleman *et al*., 1994b). Amongst the EP receptors, two subtypes are linked to and stimulate adenylate cyclase, EP_2 and EP_4 (Coleman *et al*., 1994b). In addition, an isoform of EP_3 ($EP_{3\gamma}$) can also couple to Gs and at a high agonist concentration stimulate adenylate cyclase (Irie *et al*., 1993).

The rank order of potency for prostanoids active on EP_2 receptors at inhibiting eosinophil aggregation was $PGE_2 \geqslant PGE_1 >$ 11-deoxy-PGE_1 > misoprostol > butaprost = AH 13205. Of particular interest was the relative lack of effect of the specific EP_2-selective receptor agonists butaprost and AH13205. Indeed, these two drugs were only effective at the highest concentration tested (10^{-5} M) at which non-specific effects may occur. This is in agreement with the relative lack of effects of butaprost and AH13205 in rat neutrophils (Wise & Jones, 1994) and may represent the low potency of these drugs on guinea-pig EP_2 receptors. Alternatively, this may reflect an action of the prostanoids on the newly described EP_4 receptor (see below). However, it is worth noting that despite its low potency, butaprost has very little activity on other prostanoid receptors even when used at high concentrations (reviewed in Coleman *et al*., 1994b). Together our data suggest that activation of EP_2 (or possibly EP_4) receptors in guinea-pig eosinophils is associated with inhibition of eosinophil aggregation induced by PAF. Inasmuch as the protein kinase A inhibitor KT5720 reversed the inhibitory effects of 11-deoxy-PGE_1 and AH13205, our data also suggest that activation of EP_2 receptors inhibits eosinophil aggregation via elevation of intracellular cyclic AMP.

More recently, a new EP receptor subtype, namely EP_4 has been identified which mediates PGE_2-induced relaxation of piglet saphenous vein (Coleman *et al*., 1994a). Although no selective agonist has been described, AH23848B blocks the effects of prostanoids on the piglet EP_4 receptor (Coleman *et al*., 1994a). When tested in our system, AH23848B failed to affect PAF-induced eosinophil aggregation and also failed to modulate the inhibitory effects of PGE_1. These results argue against a role for EP_4 receptors in mediating inhibition by prostanoids of PAF-induced eosinophil aggregation and suggest that prostanoid agonists act on EP_2 receptors to inhibit eosinophil aggregation. However, AH23848B has very low potency at the EP_4 receptor ($pA_2 = 5.4$) and has been described as both a weak agonist and an antagonist at the EP_4 receptor subtype (Coleman *et al*., 1994a). Clearly further studies will be necessary to exclude a role for EP_4 in mediating eosinophil aggregation when better reagents become available. Inasmuch as AH23848B is also a potent TP receptor antagonist, our results also argue against a role for TP receptors in the inhibitory effect of prostanoids. This is supported by the lack of inhibitory effects of the TP receptor agonist U46619 on PAF-induced eosinophil aggregation when used at concentrations up to 3 μM (data not shown).

The role of EP_1 receptors was investigated by use of sulprostone, 17-phenyl-ω-trinor PGE_2 and iloprost. Although 17-phenyl-ω-trinor PGE_2 inhibited aggregation when used at 10^{-5} M, this concentration is far greater than that necessary to activate EP_1 receptors in other preparations (reviewed in Coleman *et al*., 1994b) and similar non-specific effects of high concentrations of 17-phenyl-ω-trinor PGE_2 have been described in human neutrophils (Talpain *et al*., 1995). In addition, iloprost, which is more potent that PGE_1 at EP_1 receptors (Watabe *et al*., 1993), had no inhibitory effect at any of the concentrations tested. Together these results suggest there to be little role for EP_1 receptors in mediating inhibition of PAF-induced eosinophil aggregation by prostanoids and are in agreement with the restricted expression of this receptor in mouse tissues (Watabe *et al*., 1993). In addition, sulprostone which has been shown to be 3–20 times more potent than PGE_2 at activating EP_3 receptors (reviewed in Coleman *et al*., 1994b) failed to alter PAF-induced eosinophil aggregation. These results argue against a role for EP_3 receptors in mediating inhibition by prostanoids of this PAF-induced response.

Next we examined the effects of the two IP receptor agonists cicaprost and iloprost on eosinophil aggregation induced by PAF. Cicaprost inhibited PAF-induced responses by up to 30% but due to variability, this failed to reach statistical significance. In addition, iloprost was without any effect on eosinophil aggregation induced by PAF suggesting that there is a negligible role for IP receptors in mediating inhibition by prostanoids of PAF-induced eosinophil aggregation. However, it is worth noting that PGE_1 was significantly more effective than PGE_2 and other EP_2 receptor agonists. Since PGE_1 can activate IP receptors more potently and effectively than the other EP receptor agonists tested (Coleman *et al*., 1994b), it is possible that activation of IP receptors in addition to activation of EP_2 receptors may be necessary to achieve full inhibition of PAF-induced eosinophil aggregation. In this regard, combined treatment with iloprost and PGE_2 induced a signifi-

cantly greater inhibition of PAF-induced aggregation than when PGE_2 was used alone. The latter observation suggests that the IP receptor is present with low reserve in the system and needs activation of another receptor (ie. EP_2 receptor) before an inhibitory effect of IP receptor activation can be observed. However, aggregation was never fully inhibited by a combination of iloprost and PGE_2 and thus activation of the IP receptor in addition to the EP_2 receptor cannot fully explain the greater effectiveness of PGE_1 at inhibiting PAF-induced eosinophil aggregation.

In similar studies in human neutrophils (Rossi & O'Flaherty, 1989), PGD_2 significantly inhibited degranulation of human eosinophils upon stimulation with formyl-methionyl-leucyl-phenylalanine (Butchers & Vardey, 1990). However, in our study, PGD_2 failed to alter significantly eosinophil aggregation induced by PAF. In addition, when used in concentrations up to 10^{-5} M, PGD_2 failed to induce eosinophil aggregation by itself. This is in agreement with the lack of effect of high doses of PGD_2 (up to 10^{-7} mol per site) at inducing radiolabelled-eosinophil accumulation in guinea-pig skin (data not shown) but again contrasts with data showing significant effects of PGD_2 at inducing chemotaxis and elevation of intracellular calcium in human eosinophils (Butchers & Vardey, 1990; Raible *et al*., 1992). Furthermore, the DP receptor antagonist BWA868C had no significant effect on eosinophil aggregation induced by PAF and failed to alter the inhibitory effects of PGE_1. Although BW868C has been shown to be a partial agonist activity in murine DP receptors transfected into Chinese hamster ovary cells (Hirata *et al*., 1994), our results argue against a major role for DP receptors at activating guinea-pig eosinophils *in vitro* and *in vivo* and at mediating the inhibitory effects of prostanoids on PAF-induced eosinophil aggregation.

In conclusion, the results presented here suggest that the inhibition of PAF-induced aggregation by prostanoids is mediated by the occupation of EP_2-receptors on the surface of eosinophils. It is important to note that the effects of naturally occuring prostanoids, such as PGE_2, on eosinophil aggregation occur at low concentrations suggesting an important role for EP_2 receptors in mediating inhibition of eosinophil function *in vivo*

We are grateful to the National Asthma Campaign, Sandoz (Basle) and the Medical Research Council for financial support.

References

BUTCHERS, P.R. & VARDEY, C.J. (1990). The effect of prostanoids on the function of human eosinophils. *Agents Actions*, **S31,** 103–112.

BUTTERFIELD, J.H. & LEIFERMAN, K.M. (1993). Eosinophil-associated diseases. In *Immunopharmacology of Eosinophils*. ed. Smith, H. & Cook, R.M. pp. 152–192. London: Academic Press.

COLEMAN, R.A., GRIX, S.P., HEAD, S.A., LOUTTIT, J.B., MALLET, A. & SHELDRICK, R.L.G. (1994a). A novel inhibitory receptor in piglet saphenous vein. *Prostaglandins*, **47,** 151–168.

COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994b). VIII. International union of pharmacology classification of prostanoid receptors: properties, distribution and structure of the receptors and their subtypes. *Pharmacol. Rev*., **46,** 205–229.

DJUKANOVIC, R., ROCHE, W.R., WILSON, J.W., BEASLEY, C.R.W., TWENTYMAN, O.P., HOWARTH, P.H. & HOLGATE, S.T. (1990). Mucosal inflammation in asthma. *Am. Rev. Respir. Dis*., **142,** 434–457.

GLEICH, G.J. ADOLPHSON, C.R. & LEIFERMAN, K.M. (1993). The biology of the eosinophilic leukocyte. *Ann. Rev. Med.* **44,** 85–101.

HIRATA, M., KAKIZUKA, A., AIZAWA, M., USHIKUBI, S. & NARUMIYA, S. (1994). Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene. *Proc. Natl. Acad. Sci. U.S.A*., **91,** 11192–11196.

IRIE, A., SUGIMOTO, Y., NAMBA, T., HARAZONO, A., HONDA, A., WATABE, A., NEGISHI, M., NARUMIYA, S. & ICHIKAWA, A. (1993). Third isoform of the prostaglandin-E-receptor EP_3 subtype with different C-terminal tail coupling to both stimulation and inhibition of adenylate cyclase. *Eur. J. Biochem*., **217,** 313–318.

IRIE, K., TOKUDA, H., HAGIWARA, K., HAYASHI, H. MURAO, S. & ITO, Y. (1985). Structure-activity relationship in the induction of Epstein-Barr virus by teleocidin derivatives. *Int. J. Cancer*, **36,** 485–488.

MCLAREN, D.J. (1980). *Schistosoma mansoni: the Parasite Surface in Relation to Host Immunity*, Chichester, U.K.: Research Studies Press.

MEURER, R., VAN RIPER, G., FEENEY, W., CUNNINGHAM, P., HORA, D., SPRINGER, M.S., MACINTYRE, D.E. & ROSEN, H. (1993). Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1α, or human interleukin 8. *J. Exp. Med*., **178,** 1913–1921.

PIERCE, K.L., GIL, D.W., WOODWARD, D.F. & REGAN, J.W. (1995). Cloning of human prostanoid receptors. *Trends Pharmacol. Sci*., **16,** 153–256.

RAIBLE, D.G., SCHULMAN, E.S., DIMUZIO, J., CARDILLO, R. & POST, T.J. (1992). Mast cell mediators prostaglandin-D_2 and histamine activate human eosinophils. *J. Immunol*., **148,** 3536–3542.

ROSSI, A.G. & O'FLAHERTY, J.T. (1989). Prostaglandin binding sites in human polymorphonuclear neutrophils. *Prostaglandins*, **37,** 641–653.

TALPAIN, E., ARMSTRONG, R.A., COLEMAN, R.A. & VARDEY, C.J. (1995). Characterization of the PGE receptor subtype mediating inhibition of superoxide production in human neutrophils. *Br. J. Pharmacol*., **114,** 1459–1465.

TEIXEIRA, M.M., ROSSI, A.G., GIEMBYCZ, M.A. & HELLEWELL, P.G. (1996a). Effects of agents which elevate cyclic AMP on eosinophil homotypic aggregation. *Br. J. Pharmacol*., **118,** 2099–2106.

TEIXEIRA, M.M., ROSSI, A.G. & HELLEWELL, P.G. (1996b). Adhesion mechanisms involved in C5a-induced eosinophil homotypic aggregation. *J. Leukocyte Biol*., **59,** 389–396.

TEIXEIRA, M.M., WILLIAMS, T.J., AU, B-T., HELLEWELL, P.G. & ROSSI, A.G. (1995). Characterisation of eosinophil homotypic aggregation. *J. Leukocyte Biol*., **57,** 226–234.

WATABE, A., SUGIMOTO, Y., HONDA, A., IRIE, A., NAMBA, T., NEGISHI, M., ITO, S., NARUYAMA, S. & ICHIKAWA, A. (1993). Cloning and expression of cDNA for a mouse EP_1 subtype of prostaglandin E receptor. *J. Biol. Chem*. **268,** 20175–20178.

WHEELDON, A. & VARDEY, C.J. (1993). Characterization of the inhibitory prostanoid receptors on human neutrophils. *Br. J. Pharmacol*., **108,** 1051–1054.

WISE, H. & JONES, R.L. (1994). Characterization of prostanoid receptors on rat neutrophils. *Br. J. Pharmacol*., **113,** 581–587.

(Received September 23, 1996
Revised January 21, 1997
Accepted February 3, 1997)