E-type prostaglandins enhance local oedema formation and neutrophil accumulation but suppress eosinophil accumulation in guinea-pig skin

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1 Prostaglandins possess both pro- and anti-inflammatory actions depending on their route of administration and the experimental model used. In this study, we have investigated the effect of locally injected prostaglandins on oedema formation, neutrophil accumulation and eosinophil accumulation in inflammatory responses in guinea-pig skin.

2 Prostaglandin E₁ (PGE₁) significantly enhanced local oedema formation induced by zymosan-activated plasma (ZAP), bradykinin and in a passive cutaneous anaphylactic (PCA) reaction. The accumulation of ZAP-induced ¹¹¹In-labelled neutrophils was also significantly enhanced by PGE₁. In addition, the prostacyclin analogue, iloprost, enhanced ZAP-induced responses.

3 In contrast PGE₁ decreased the accumulation of ¹¹¹In-labelled eosinophils in skin sites. This was demonstrated on eosinophil accumulation and local oedema formation induced by PAF, compound 48/80 and in the PCA reaction. PGE₂ also suppressed eosinophil accumulation while iloprost had no detectable effect.

4 Isoprenaline inhibited eosinophil accumulation in a dose-dependent manner with no effect on local oedema formation, except in the case of responses to ZAP where suppression was observed.

5 The vasodilator neuropeptide, calcitonin gene-related peptide (CGRP), enhanced local oedema formation but had no detectable effect on eosinophil accumulation.

6 In conclusion, the magnitude of a given response to an inflammatory mediator *in vivo* depends on the net effect of stimulation of several cell types e.g. arteriolar smooth muscle cells, microvascular endothelial cells, mast cells and accumulating leukocytes. In this study, we have demonstrated that different components of the inflammatory response in guinea-pig skin can be differentially modulated by E-type prostaglandins and isoprenaline, suggesting that cyclic AMP has an important regulatory role.

Keywords: Prostaglandins; eosinophils; neutrophils, inflammation; isoprenaline; oedema

Introduction

Chemical signals are generated in tissues in response to an inflammatory stimulus and these mediators induce the features of the inflammatory process. One such group of mediators are the prostaglandins, which have been shown to mimic some of the features of inflammation when administered *in vivo* (Williams & Peck, 1977; Salmon & Higgs, 1987) and have been shown to be present in various experimental models of inflammation (Salmon & Higgs, 1987). The inhibition of prostaglandin synthesis by aspirin-like drugs is widely and effectively used for the treatment of some of the symptoms of inflammation, further substantiating the importance of prostaglandins as mediators of the inflammatory process (Vane, 1971; Insel, 1990).

Intradermal (i.d.) injection of prostaglandins potentiates the oedema-inducing activity of other mediators (Moncada *et al.*, 1973; Williams & Morley, 1973; Williams & Peck, 1977; Wedmore & Williams, 1981). This effect is thought to be related to the action of prostaglandins on the arterial side of the microcirculation where they cause vasodilatation and increase local blood flow. This leads to an elevated hydrostatic pressure in the post-capillary venules and it is from these vessels that the leakage of plasma protein occurs (Majno & Palade, 1961). In rabbit skin, i.d. injection of prostaglandin E₂ (PGE₂) also potentiates the rate of accumulation of ¹¹¹In-labelled neutrophils induced by N-formyl-methionyl-leucyl-phenylalanine or zymosan activated

plasma (ZAP) (Issekutz, 1981). This effect is also thought to be due to a local increase in blood flow which would enhance the delivery of cells available for accumulation at the site of inflammation (Issekutz, 1981).

Prostaglandins have also been shown to have anti-inflammatory effects both *in vivo* and *in vitro* (Morikawa *et al.*, 1992; Anastassiou *et al.*, 1992). For example, it has been shown that PGE₂ can inhibit eosinophil degranulation, an effect which is enhanced by co-incubation with a phosphodiesterase inhibitor (Kita *et al.*, 1991). Neutrophil function is also inhibited by prostaglandins of the E series (Takenawa *et al.*, 1986; Chopra & Webster, 1988). Interestingly, the systemic administration of a stable PGE₁ analogue was able to suppress inflammation in a model of cutaneous vasculitis and to other mediators (Kunkel *et al.*, 1979; Fantone *et al.*, 1980). These inhibitory effects were related to the capacity of a PGE₁ analogue to inhibit neutrophil activation. In a model of inflammation in rabbit skin, Rampart & Williams (1986) have shown that prostaglandins can either enhance (when given locally) or inhibit (when given systemically) neutrophil-dependent oedema. Thus, it seems that prostaglandins can possess either inflammatory or anti-inflammatory properties depending on the route of administration.

In this study, we have investigated the effect of locally injected prostaglandins on oedema formation and leukocyte (neutrophils and eosinophils) accumulation in inflammatory responses in guinea-pig skin. We have found that locally-injected prostaglandins of the E series, whilst enhancing oedema formation and neutrophil accumulation induced by different mediators, unexpectedly suppress the accumulation of eosinophils in response to the same mediators.

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Methods

Preparation of zymosan-activated plasma

Zymosan-activated plasma (ZAP) was used as a source of guinea-pig C5a des-Arg. ZAP was prepared by incubating guinea-pig heparinized (10 u ml^{-1}) plasma with zymosan (5 mg ml^{-1}) for 30 min at 37°C . Zymosan was removed by centrifugation ($2 \times 10 \text{ min}$ at 3000 g). The activated plasma was desalted on a PD-10 sephadex G-25M column and stored in aliquots at -20°C .

Preparation of passive cutaneous anaphylaxis sera and reactions

Details of the preparation of IgG₁-rich sera are described elsewhere (Weg *et al.*, 1991). Briefly, male guinea-pigs (Harlan Porcellus, 350–400 g) were immunized with bovine gamma-globulin (BGG) in Freund's complete adjuvant ($0.2 \text{ mg BGG } 0.2 \text{ ml}^{-1}$ of adjuvant s.c.). These animals received a boost of antigen in Freund's incomplete adjuvant on day 21 and the serum was prepared on day 30. Recipient animals received an injection of $50 \mu\text{l}$ of a 1/50 dilution of the anti-sera i.d., followed 16–20 h later by the i.d. injection of antigen (BGG).

Induction, purification and radiolabelling of guinea-pig eosinophils

The method is described in detail elsewhere (Faccioli *et al.*, 1991). Briefly, horse serum (1 ml) was injected i.p. in ex-breeder female guinea-pigs (Harlan Porcellus, Oxon; 700–800 g) every other day for two weeks and a boost was given the day prior to collection of the cells. The animals were killed by exposure to CO_2 and their peritoneal cavities washed with heparinized saline (10 u ml^{-1}). These cells were layered onto a discontinuous percoll-HBSS (calcium and magnesium-free) gradient prepared on the day of the procedure and the eosinophils collected from the 1.085/1.090 and 1.090/1.095 interfaces. The purity of the cells used was always greater than 95%. The main contaminating cells were mononuclear cells and the presence of neutrophils ($>1\%$ contamination) was a major exclusion criterion for the use of the preparation. Viability, assessed by trypan blue exclusion, was greater than 98%. The purified eosinophils ($2\text{--}3 \times 10^7$ cells) were incubated with $^{111}\text{Indium}$ ($100 \mu\text{Ci}$ in $10 \mu\text{l}$) chelated to 2-mercaptopyridine-*N*-oxide ($40 \mu\text{g}$ in 0.1 ml of 50 mM PBS, pH 7.4) for 15 min at room temperature. The cells were then washed twice in HBSS (Ca^{2+} and Mg^{2+} -free) containing 10% guinea-pig platelet poor plasma and resuspended at a final concentration of 10^7 cells ml^{-1} prior to injection.

Induction, purification and radiolabelling of guinea-pig neutrophils

Neutrophils were elicited in the peritoneal cavity of naive ex-breeder guinea-pigs by the i.p. injection of 20 ml of 0.2% (w/v) glycogen. After 8–12 h, the animals were killed and the peritoneal cavity washed with heparinized saline (10 u ml^{-1}). The rest of the procedure was as described for the eosinophils. The cells were also collected from the 1.085/1.090 and 1.090/1.095 interfaces and were used only if over 95% pure. The major contaminants were eosinophils (3–4%) and mononuclear cells (2–3%). Viability tested by trypan blue exclusion was greater than 98%.

Measurements of local oedema formation and leukocyte accumulation in guinea-pig skin

Radiolabelled leukocyte infiltration and oedema formation were measured simultaneously. ^{125}I -human serum albumin ($\sim 5 \mu\text{Ci}$) was added to the ^{111}In -labelled eosinophils or neutrophils and these were injected intravenously (5×10^6 cells per animal) into recipient guinea-pigs (350–400 g) anaesthetized with Hypnorm (0.15 ml , i.m.). After 5 min, inflammatory mediators or antigen were injected i.d. into the dorsal skin of shaved animals with or without prostaglandins, calcitonin-gene related peptide or isoprenaline. All drugs were mixed before the injection. Each animal received a duplicate of each treatment following a randomized injection plan and the inflammatory response (^{111}In -labelled cell accumulation and oedema formation) was assessed after 2 h. This time point was chosen based on previous experiments showing that most of the ^{111}In -labelled leukocyte accumulation occurred during the first 2 h (Faccioli *et al.*, 1991; Teixeira *et al.*, unpublished observations). At this time, a blood sample was obtained by cardiac puncture, the animals were killed by an overdose of sodium pentobarbitone, the dorsal skin was removed, cleaned free of excess blood and the skin sites punched out with a 17 mm punch. The samples were counted in an automatic 5-head gamma-counter (Canberra Packard Ltd, Pangbourne, Berks) and the counts were cross-channel corrected for the two isotopes.

Leukocyte numbers are expressed as the number of ^{111}In -labelled cells per skin site and oedema formation as the ratio of ^{125}I counts of the skin sample divided by the ^{125}I counts in $1 \mu\text{l}$ of plasma.

Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): bradykinin, glycogen, bovine gamma globulin, compounds 48/80, (–)-isoprenaline, zymosan. Hanks solutions, HEPES and horse serum were purchased from Gibco Limited (Paisley, Renfrewshire). Percoll was purchased from Pharmacia (Milton Keynes, Bucks), PAF and calcitonin-gene related peptide (CGRP) from Bachem (Saffron Walden, Essex) and prostaglandins E_1 and E_2 from Janssen Pharmaceuticals (Belgium). Iloprost was a gift from Dr F. McDonald (Schering AG, Berlin, Germany).

Statistics

Data were analysed by Student's paired *t* test. Multiple comparisons were evaluated by two-way analysis of variance on normally distributed data. Values of $P < 0.05$ were considered significant.

Results

The percentages of ^{111}In -labelled neutrophils and eosinophils circulating 2 h after i.v. injection were 5.8 ± 1.9 ($n = 9$) and $7.8 \pm 0.7\%$ ($n = 31$), respectively. At 2 h, over 95% of the total plasma ^{111}In was bound to the infused circulating leukocytes. Previous studies have shown that the radiolabelled leukocytes accumulated extravascularly at sites of cutaneous inflammation (Faccioli *et al.*, 1991; Weg *et al.*, unpublished observations). Each recipient animal received an i.v. injection of 5×10^6 ^{111}In -eosinophils or ^{111}In -neutrophils. Thus, approximately 60% of the total circulating eosinophil population was radiolabelled after the i.v. injection while the ^{111}In -neutrophils represented about 6% of the total.

Effect of PGE_1 on neutrophil accumulation

The coinjection of PGE_1 (3×10^{-11} and $3 \times 10^{-10} \text{ mol}$ per site) significantly enhanced the oedema formation induced by 10% ZAP and bradykinin ($\text{BK } 10^{-10} \text{ mol}$ per site) (Figure 1b). These doses of PGE_1 , which have been previously shown to potentiate oedema formation in guinea-pig skin (Williams & Morley, 1973), had no effect when injected alone (Figure 1b). The accumulation of ^{111}In -labelled neutrophils measured in the same sites was slightly, but significantly, enhanced by PGE_1 (Figure 1a). BK did not induce a significant accumula-

tion of neutrophils above saline background (Figure 1a). In the same experiments, PGE₁ had no effect on PCA (1 µg of BGG per site)-induced neutrophil accumulation, but significantly enhanced PCA-induced oedema formation (data not shown). The prostacyclin analogue, iloprost (10⁻⁹ mol per site), also enhanced the accumulation of neutrophils induced by ZAP by 55.7 ± 14.2% (*n* = 6, *P* < 0.01). Oedema responses in the same sites were enhanced by 254 ± 92% (*n* = 6, *P* < 0.01). Iloprost did not alter the accumulation of neutrophils induced by PCA (BGG, 1 µg per site, 4321 ± 1644 ¹¹¹In-neutrophils; BGG + iloprost, 4472 ± 1847 ¹¹¹In-neutrophils, *n* = 6).

Effect of PGE₁ on eosinophil accumulation

Surprisingly, while PGE₁ (3 × 10⁻¹⁰ mol per site) enhanced ZAP-induced oedema formation, it significantly inhibited in the same sites ZAP-induced eosinophil accumulation (Figure 2). Further analysis of the effects of PGE₁ (10⁻¹² to 3 × 10⁻⁸ mol per site) on ZAP-induced responses is shown in Figure 3. PGE₁ dose-dependently inhibited ZAP-induced eosinophil accumulation whilst potentiating oedema formation in the same sites. The ED₅₀ values for PGE₁-mediated inhibition of eosinophil accumulation and potentiation of oedema formation were approximately 3 × 10⁻¹¹ mol per site and 2 × 10⁻¹¹ mol per site, respectively.

PGE₁ (3 × 10⁻¹⁰ mol per site) also potentiated oedema formation while suppressing eosinophil accumulation induced by other mediators of inflammatory stimuli (Table 1). For example, PGE₁ suppressed eosinophil accumulation induced in the PCA reaction (1 µg of BGG per site) and PAF (10⁻⁹ mol per site) by 35% and 48%, while oedema forma-

tion in the same sites was potentiated by 49% and 64%, respectively (Table 1). In addition, PGE₁ significantly inhibited eosinophil accumulation induced by compound 48/80 (3–30 µg per site) while potentiating oedema formation in the same sites (Table 1).

Effect of PGE₂ and iloprost on eosinophil accumulation and oedema formation

Another E-type prostaglandin, PGE₂ (3 × 10⁻¹⁰ mol per site) had similar effects to PGE₁. Coinjection of PGE₂ with ZAP, enhanced oedema formation (29.4 ± 4.7 µl without PGE₂ and 36.6 ± 4.3 µl with PGE₂, *n* = 6, *P* < 0.05) and suppressed eosinophil accumulation (2507 ± 218 ¹¹¹In-eosinophils without PGE₂ and 1312 ± 147 ¹¹¹In-eosinophils with PGE₂, *n* = 6, *P* < 0.01). Nevertheless, it was without effect against PCA (1 µg of BGG per site)-induced eosinophil accumulation or oedema formation (data not shown).

The stable prostacyclin analogue, iloprost (10⁻⁹ mol per site), was capable of potentiating oedema formation induced by ZAP and BK (10⁻¹⁰ mol per site) but it had no effect on ZAP-induced eosinophil accumulation (Figure 4). PCA-induced oedema formation appeared to be enhanced by iloprost, but this did not reach significance (*P* = 0.06) (Figure 4b). Iloprost had no effect on PCA-induced eosinophil accumulation (Figure 4a).

Effect of isoprenaline on eosinophil accumulation and oedema formation

Since some of the effects of E-series prostaglandins are thought to occur through a G-protein linked receptor leading

Figure 1 Effect of prostaglandin E₁ (PGE₁) on the accumulation of ¹¹¹In-labelled neutrophils (a) and local oedema formation (b) induced by zymosan-activated plasma (ZAP, 10% in saline) and bradykinin (BK, 10⁻¹⁰ mol per site). ¹¹¹In-neutrophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean ± s.e.mean of 4–8 animals in control sites (open columns), sites injected with PGE₁ 3 × 10⁻¹¹ mol per site (solid columns) or PGE₁ 3 × 10⁻¹⁰ mol per site (cross-hatched columns). The dashed lines represent the background values in sites injected with saline alone. **P* < 0.05.

Figure 2 Effect of prostaglandin E₁ (PGE₁) on the accumulation of ¹¹¹In-labelled eosinophils (a) and oedema formation (b) induced by zymosan-activated plasma (ZAP, 10% in saline). ¹¹¹In-eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean ± s.e.mean of 9 animals in control sites (open columns) or sites injected with PGE₁ 3 × 10⁻¹⁰ mol per site (solid columns). The dashed lines represent the background values in sites injected with saline alone. **P* < 0.05.

to an increase in the adenosine 3':5'-cyclic monophosphate (cyclic AMP) content of cells (Coleman & Humphrey, 1993), we used the β -agonist isoprenaline which also increases intracellular cyclic AMP (Goldie *et al.*, 1991). Isoprenaline (10^{-10} to 10^{-8} mol per site) caused a dose-dependent inhibition of ZAP-, PCA- and PAF-induced eosinophil accumulation, but also inhibited ZAP-induced oedema formation (Table 2); PCA- and PAF-induced oedema formation were not significantly affected. ZAP-induced ^{111}In -neutrophil accumulation was not significantly altered by isoprenaline (ZAP alone, 6386 ± 2532 ^{111}In -neutrophils; ZAP + 10^{-9} mol isoprenaline per site, 6729 ± 2511 ; ZAP + 10^{-8} mol isoprenaline per site, 7133 ± 2693 , $n = 6$).

Effect of calcitonin gene-related peptide (CGRP) on eosinophil accumulation and oedema formation

Neuropeptides have been shown to be potent vasodilators in animal and human skin (Brain *et al.*, 1985). Since these peptides may also act through an increase in cyclic AMP in different cell types (Goldie *et al.*, 1991), we tested the effect of CGRP on ZAP- and PCA-induced eosinophil accumulation and oedema formation. CGRP caused a dose-dependent increase in oedema formation in these reactions (Figure 5b), while eosinophil accumulation was unaltered (Figure 5a). CGRP also effectively potentiated oedema formation induced by BK (Figure 5b).

Figure 3 Dose-dependent effect of prostaglandin E_1 (PGE_1) on the eosinophil accumulation (a) and oedema formation (b) induced by zymosan-activated plasma (ZAP 10% in saline). ^{111}In -eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as a percentage of the control response to ZAP. Responses in control sites were 2840 ± 223 ^{111}In -labelled neutrophils per site and 28.2 ± 3.5 μl of plasma per site. Values are means $\pm$ s.e.mean of 4–6 animals.

Figure 4 Effect of iloprost on the accumulation of ^{111}In -labelled eosinophils (a) and oedema formation (b) induced by zymosan-activated plasma (ZAP, 10% in saline), the passive cutaneous anaphylaxis (PCA) reaction (0.1 μg of BGG per site) and bradykinin (BK, 10^{-10} mol per site). ^{111}In -eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean $\pm$ s.e.mean of 4–7 animals in control sites (open columns) or sites injected with iloprost 10^{-9} mol per site (solid columns). The dashed lines represent the background values in sites injected with saline alone. * $P < 0.05$.

Table 1 Effect of prostaglandin E_1 (PGE_1 , 3×10^{-10} mol per site) on the eosinophil accumulation and oedema formation induced by the passive cutaneous anaphylaxis reaction (PCA), platelet-activating factor (PAF) and compound 48/80 (C48/80) in guinea-pig skin

	^{111}In -eosinophils Control	+ PGE_1	Oedema (μl of plasma) Control	+ PGE_1
PCA 0.1 μg	2049 ± 170	$1334 \pm 427^*$	21.1 ± 1.0	25.0 ± 2.9
1.0 μg	4353 ± 712	2969 ± 512	26.1 ± 2.3	$33.9 \pm 2.9^*$
PAF 10^{-10} moles	1535 ± 239	$866 \pm 189^*$	35.6 ± 3.8	$64.1 \pm 5.3^*$
3×10^{-10} moles	2498 ± 670	$1655 \pm 471^*$	45.8 ± 4.7	$72.1 \pm 5.8^*$
10^{-9} moles	3590 ± 583	$2071 \pm 241^*$	53.1 ± 4.4	$82.2 \pm 7.6^*$
C48/80 3 μg	3797 ± 920	$1648 \pm 866^*$	42.7 ± 3.6	$49.4 \pm 4.2^*$
10 μg	5693 ± 810	$2933 \pm 722^*$	50.6 ± 2.6	$64.7 \pm 3.5^*$
30 μg	8589 ± 1505	$5486 \pm 835^*$	55.2 ± 2.6	$72.3 \pm 3.6^*$
Saline	441 ± 65	418 ± 55	13.1 ± 1.4	14.6 ± 1.3

The PCA reaction was induced by 0.1 or 1.0 μg BGG per site. ^{111}In -eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean $\pm$ s.e.mean, $n = 5$ –7 experiments.

* $P < 0.05$.

Table 2 Effect of isoprenaline (10^{-10} to 10^{-8} mol per site) on the eosinophil accumulation and oedema formation induced by zymosan-activated plasma (ZAP), passive cutaneous anaphylaxis reaction (PCA) and platelet-activating factor (PAF) in guinea-pig skin

	¹¹¹ In-eosinophils per site				Oedema (μ l of plasma per site)			
	Control	Isoprenaline (mol per site)			Control	Isoprenaline (mol per site)		
		10^{-10}	10^{-9}	10^{-8}		10^{-10}	10^{-9}	10^{-8}
ZAP	2006 $\pm$ 248	1781 $\pm$ 528	1597 $\pm$ 211*	1221 $\pm$ 142*	30.5 $\pm$ 4.6	23.1 $\pm$ 2.5*	25.2 $\pm$ 5.3*	24.9 $\pm$ 2.0*
PCA	2309 $\pm$ 313	2452 $\pm$ 445	1701 $\pm$ 212*	1548 $\pm$ 346*	27.1 $\pm$ 2.7	22.8 $\pm$ 3.5	28.1 $\pm$ 3.3	28.3 $\pm$ 3.8
PAF	1395 $\pm$ 120	884 $\pm$ 164*	740 $\pm$ 92*	692 $\pm$ 30*	38.9 $\pm$ 11.1	31.1 $\pm$ 5.2	30.4 $\pm$ 5.8	31.0 $\pm$ 5.9
Saline	496 $\pm$ 47	461 $\pm$ 21	412 $\pm$ 64	440 $\pm$ 38	15.5 $\pm$ 1.5	12.8 $\pm$ 3.0	16.3 $\pm$ 0.92	15.6 $\pm$ 1.7

ZAP was diluted 10% in saline, the PCA reaction induced by 1 μ g BGG per site and PAF used at 10^{-9} mol per site. ¹¹¹In-eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean $\pm$ s.e.mean, $n = 4-6$ experiments.

* $P < 0.05$.

Figure 5 Effect of calcitonin gene-related peptide (CGRP) on the accumulation of ¹¹¹In-labelled eosinophils (a) and oedema formation (b) induced by zymosan-activated plasma (ZAP 10% in saline), passive cutaneous anaphylaxis (PCA) reaction (0.1 μ g of BGG per site) and bradykinin (BK, 10^{-10} mol per site). ¹¹¹In-eosinophil accumulation and oedema formation were assessed over a 2 h period. Results are expressed as the mean $\pm$ s.e.mean of 4 animals in control sites (open columns) and sites injected with CGRP 10^{-11} mol per site (solid columns). The dashed lines represent the background values in sites injected with saline alone. * $P < 0.05$.

Discussion

The capacity of different vasodilator prostaglandins to potentiate cutaneous oedema formation induced by different mediators of inflammation has been demonstrated extensively *in vivo* (Williams & Morley, 1973; Williams & Peck, 1977). This synergism is explained by a local increase in blood flow which is thought to enhance hydrostatic pressure in post-capillary venules, thus facilitating the extravasation of plasma proteins leading to oedema formation. Neutrophil accumulation at local sites of inflammation in skin is also enhanced by intradermal administration of vasodilators such as PGE₂ and CGRP and this has been attributed to increased delivery of

inflammatory cells to the lesion (Issekutz, 1981; Issekutz & Movat, 1982; Buckley *et al.*, 1991).

In the present study, local administration of PGE₁ with various stimuli (ZAP, PCA, compound 48/80 and PAF) potentiated oedema formation and neutrophil accumulation in guinea-pig skin. However, eosinophil accumulation was suppressed by PGE₁ in sites at which local oedema formation was enhanced. The capacity of PGE₁ to inhibit eosinophil accumulation was partially mimicked by PGE₂. The lower efficacy of PGE₂ at inhibiting the PCA-induced eosinophil accumulation may be at least partially explained by its lesser potency *in vivo* to potentiate oedema formation in guinea-pig skin (Williams & Morley, 1973). PGE₂ has also been shown to be less effective than PGE₁ as an inhibitor of leukocyte function (Zurier, 1982). In contrast to the effects of PGE₁, the prostacyclin analogue, iloprost, while potentiating oedema formation and neutrophil accumulation, did not inhibit eosinophil accumulation. Since some of the biological effects of prostaglandin E₁ and E₂ are thought to be mediated by an increase of cyclic AMP within the target cell (Coleman & Humphrey, 1993), we used a β -adrenoceptor agonist, which also increases cyclic AMP within cells through a G-protein linked receptor (Goldie *et al.*, 1991), to test if this pathway was important. Interestingly, the β -agonist, isoprenaline, dose-dependently inhibited the accumulation of eosinophils induced by ZAP, PAF and in the PCA reaction. ZAP-induced oedema formation was also partially reduced by isoprenaline. The inhibition of oedema by isoprenaline has been previously shown, and is thought to be related to its action on endothelial cells (Beets & Paul, 1980). Indeed, isoprenaline acts on cultured endothelial cells to increase the levels of intracellular cyclic AMP (McEwan *et al.*, 1990). Prostaglandins of the E-series also increase cyclic AMP levels in endothelial cells (Hopkins & Gorman, 1981; Allison *et al.*, 1986), however, their effect on vascular smooth muscle cell cyclic AMP may be greater and the net effect is to increase local blood flow. We have also found that certain phosphodiesterase inhibitors suppress eosinophil accumulation in the guinea-pig skin, providing additional support for a role of cyclic AMP in the inhibitory effect of prostaglandins and isoprenaline (Teixeira *et al.*, unpublished observations). If elevation of cyclic AMP does have a role in the inhibitory effects of PGE₁, the receptor most likely to be involved is the EP₂ receptor (Coleman & Humphrey, 1993). Nevertheless, further studies are necessary to define unequivocally the prostanoïd receptor(s) involved.

There are several possible cell targets on which prostaglandins and isoprenaline may be acting to inhibit the accumulation of eosinophils *in vivo*. Eosinophil function has been shown to be directly inhibited *in vitro* by prostaglandins (Giembycz *et al.*, 1990; Kita *et al.*, 1991), an effect which appears to be mediated by an increase in cyclic AMP, and this may explain the inhibitory effects that we observe *in vivo*. However, the fact that neutrophil functions (including chemotaxis) are also inhibited by different prostaglandins or

prostaglandin analogues *in vitro* (Takenawa *et al.*, 1986; Chopra & Webster, 1988) argues against a direct effect of prostaglandins on either the eosinophil or the neutrophil in our experimental model. It has been reported that, compared with other leukocytes, neutrophils are less responsive to agonists that activate adenylate cyclase (Plaut *et al.*, 1980) but whether this is true and relevant *in vivo* for the accumulation of guinea-pig neutrophils is not known. The mast cell is another possible cellular target particularly if one considers the inhibition of compound 48/80- and PCA-induced eosinophil accumulation by PGE₁. However, one would expect an inhibition of both eosinophil accumulation and oedema formation if the mast cell were a target for the inhibitory effects of PGE₁ since, at least for the PCA and compound 48/80, both functions are thought to be related to the activation of the mast cell.

Platelets have been shown to release eosinophil chemoattractants (Kameyoshi *et al.*, 1992; Burgers *et al.*, 1993) and they may also have a role in allergic states in guinea-pig lung (Lellouch-Tubiana *et al.*, 1988). If platelets are important in eosinophil accumulation in our skin model, then inhibition of platelet secretion by prostaglandins may explain the inhibitory effect on eosinophil accumulation.

Leukocytes express different adhesion molecules which are thought to be important for regulating their influx into the tissue (Williams & Hellewell, 1992). The integrin VLA-4 is expressed on the cell surface of the eosinophil, but not the neutrophil, and its presence appears to be important for eosinophil migration into sites of cutaneous inflammation (Weg *et al.*, 1993). VCAM-1, one of the ligands for VLA-4, is expressed after several hours on the surface of activated endothelial cells *in vitro* (Williams & Hellewell, 1992). Interestingly, the expression of endothelial cell VCAM-1 *in vitro* is inhibited by agents which elevate cyclic AMP (Pober *et al.*, 1992). However, it is not known whether VCAM-1 is expressed on venular endothelial cells in skin under our acute experimental procedure, or if prostaglandins of the E series and isoprenaline can inhibit this expression through an increase in cyclic AMP.

CGRP, a neuropeptide which has been shown to be a potent vasodilator in the microcirculation (Brain *et al.*, 1985), significantly enhanced oedema formation induced by ZAP, the PCA reaction and bradykinin. Nevertheless, it had no

effect on ZAP- or PCA-induced eosinophil accumulation. Since CGRP may ultimately lead to an increase of cyclic AMP within various cells (Goltzman & Mitchell, 1985; McEwan *et al.*, 1991), it was surprising that it did not inhibit the accumulation of eosinophils. Neuropeptides, such as CGRP and substance P, have been recently shown to modulate chemotaxis of eosinophils from allergic subjects (Numao & Agrawal, 1992). Nevertheless the mechanism of modulation was not investigated in that study. It is possible that the receptor for CGRP may be lacking on the cells responsible for the inhibitory effects on eosinophil accumulation, eg. venular endothelial cells.

Human airways have been shown to release eicosanoids both *in vitro* (Schulman *et al.*, 1982) and *in vivo* (Wenzel *et al.*, 1989). It is suggested that asthmatic subjects produce relatively few 'bronchoprotective' eicosanoids as opposed to bronchoconstrictors and that the generation of prostaglandins may help preserve patency of the airways (Wenzel *et al.*, 1989). In animal models, a decrease in the production of PGE₂ has been suggested to be responsible for airways hyper-reactivity (Folkert *et al.*, 1989; Gray *et al.*, 1992). PGE₂ is also partially responsible for the beneficial effects of frusemide against exercise-induced asthma (Pavord *et al.*, 1992). These data support a role for prostaglandin synthesis and protection in asthma. Eosinophils have been identified as important effector cells in allergic diseases such as asthma (Djukanovic *et al.*, 1990). If prostaglandins also inhibit the accumulation of eosinophils in the lungs of asthmatic subjects, this may prove to be another possible protective mechanism exerted by prostaglandins. It also implies that drugs which act similarly, such as the phosphodiesterase inhibitors, may have a role as anti-inflammatory drugs in asthma (Kuehl *et al.*, 1987; Torphy & Undem, 1991).

In summary, we have shown that prostaglandins of the E series enhance oedema formation and neutrophil accumulation, but significantly inhibit eosinophil accumulation into inflammatory sites. Isoprenaline partially mimics these effects suggesting that the inhibitory actions of both β -agonist and prostaglandins may be through an increase of cyclic AMP within the target tissue.

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