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Effects of phosphodiesterase isoenzyme inhibitors on cutaneous inflammation in the guinea-pig

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1 Inflammation is central to the pathophysiology of asthma. The recent findings that different inflammatory cells may express different phosphodiesterase (PDE) isoenzymes have centred attention on inhibitors of these isoenzymes as new drugs for the treatment of asthma. In this study, we investigated the effect of different PDE isoenzyme inhibitors on the accumulation of ^{111}In-labelled eosinophils and local oedema formation at sites of allergic- and mediator-induced inflammation in guinea-pig skin.

2 Systemic treatment with SK&F 94120, a type III PDE inhibitor, or zaprinast, a type V PDE inhibitor, had no effect on the ^{111}In-eosinophil accumulation and oedema formation induced by i.d. injection of zymosan-activated plasma (ZAP), PAF, histamine or in a passive cutaneous anaphylaxis (PCA) reaction.

3 Systemic treatment with rolipram, a type IV PDE inhibitor, effectively inhibited ^{111}In-eosinophil accumulation induced by ZAP, PAF, histamine and in a PCA reaction. However, oedema formation measured in the same sites was not affected. Systemic administration of higher doses of theophylline produced similar results. In contrast, ^{111}In-neutrophil accumulation induced by ZAP or in a PCA reaction was not altered by systemic treatment with rolipram.

4 Locally-injected rolipram had little effect on ^{111}In-eosinophil accumulation and oedema formation induced by histamine, PAF and in a PCA reaction.

5 These data show that systemic, but not local, treatment with rolipram effectively inhibits allergic- and mediator-induced ^{111}In-eosinophil accumulation but not oedema formation or ^{111}In-neutrophil accumulation. This, taken together with the potent inhibitory effects of PDE type IV inhibitors on eosinophil function *in vitro*, suggest that this class of drugs may be beneficial in disease states such as asthma where eosinophils are thought to play a major pathophysiological role.

Keywords: Eosinophil; phosphodiesterase inhibitors; rolipram; inflammation; allergy; passive cutaneous anaphylaxis reaction

Introduction

Eosinophils are inflammatory cells thought to have important effector function in allergic diseases such as asthma (Venge, 1990), rhinitis (Klementsson, 1992), dermatitis (Bruijnzeel-Koomen *et al.*, 1992) and conjunctivitis (Foster *et al.*, 1991). These cells are capable of secreting several lipid and protein mediators which, in the airways, can alter bronchial smooth muscle tonus, cause oedema formation and affect the function of other cells (Djukanovic *et al.*, 1990; Venge, 1990). Eosinophils possess cationic proteins in their granules (e.g. major basic protein and eosinophil-derived neurotoxin) which can be released upon activation and inflict damage to epithelial cells (Wardlaw *et al.*, 1988; Montefort *et al.*, 1992) which, in the lung, may be an important pathological mechanism in allergic diseases like asthma. For example, accumulation of eosinophils and their activation appears to correlate with disease severity (Bentley *et al.*, 1992) and eosinophil secretory products, for example major basic protein, reproduce some of the asthmatic symptoms in experimental animals (Djukanovic *et al.*, 1990; Gundel *et al.*, 1991).

Despite the new findings in the understanding of asthma pathophysiology, there is evidence suggesting an increase in the prevalence and severity of the disease (Lebowitz & Spinaci, 1993). This increase has led to the search for new drug treatments for asthma. One strategy to develop drugs has been based on the use of theophylline which has bronchodilator and anti-inflammatory activities and may therefore be useful in the treatment of asthma (Persson, 1986). Thus, theophylline has been shown to inhibit polymorphonuclear function at therapeutic levels (Nielson *et al.*, 1988) and late airway responses to allergen challenge, even at sub-therapeutic doses (Ward *et al.*, 1993). Interestingly, some chronic steroid-dependent asthmatic subjects may find an improvement in their symptoms when using theophylline (Nassif *et al.*, 1981). However, the narrow therapeutic index, potential toxicity and need for plasma monitoring of theophylline make this drug difficult to use (Johnston, 1990).

The mechanism of action of theophylline is as yet unclear, but inhibition of phosphodiesterase (PDE) enzymes is a possibility which has gained strong support (Kuehl *et al.*, 1987). PDEs are enzymes responsible for the breakdown of cyclic nucleotides (adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and guanosine 3′:5′-cyclic monophosphate (cyclic GMP)) within cells (Beavo & Reifsnyder, 1990; Nicholson *et al.*, 1991). Five families of PDE isoenzymes (PDE I–V) have been identified and these are differentially distributed in cells (Beavo & Reifsnyder, 1990; Nicholson *et al.*, 1991). Inflammatory cells contain mainly a PDE type IV isoenzyme (cyclic AMP-specific) which accounts for most of the metabolism of cyclic AMP in these cells (Torphy & Undem, 1991; Giembycz & Dent, 1992). Inhibition of this isoenzyme (PDE IV) in both neutrophils and eosinophils leads to an effective inhibition of cell function (Nielson *et al.*, 1990; Dent *et al.*, 1991). This is consistent with the widespread anti-inflammatory effects induced by elevating intracellular levels of cyclic nucleotides in various cell types (Torphy & Undem, 1991). Also relevant to the treatment of asthma is the observation that inhibition of PDE types III and IV effectively suppress contraction of human bronchial smooth muscle *in vitro* and bronchoconstriction induced by different stimuli or antigen in sensitized animals (Torphy & Undem, 1991; de Boer *et al.*, 1992; Howell *et al.*, 1993).

In guinea-pig skin, intradermal injection of different known mediators of inflammation leads to a dose-dependent accumulation of radiolabelled eosinophils and local oedema formation (Faccioli *et al.*, 1991; Teixeira *et al.*, 1993a). The

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injection of antigen in sites previously sensitized with an antigen-specific (BGG) IgG_1-rich anti-serum (passive cutaneous anaphylaxis (PCA) reaction) also leads to the accumulation of radiolabelled eosinophils and oedema formation (Weg *et al.*, 1992; Teixeira *et al.*, 1993a). In this PCA reaction, oedema formation is dependent on the release of histamine and newly formed lipid mediators, PAF and leukotriene D_4 (LTD_4) (Weg *et al.*, 1991). The mediators responsible for cell accumulation have not been fully characterized, but a 5-lipoxygenase product, probably LTB_4, appears to play an important role (Teixeira & Hellewell, 1994). The mechanism by which radiolabelled eosinophils accumulate in guinea-pig skin has been recently demonstrated to be dependent on both CD18 and VLA-4 integrin adhesion molecules on the eosinophil (Weg *et al.*, 1993; Teixeira *et al.*, 1994). The aim of the present study was to assess the effects of selective inhibitors of PDE types III, IV and V isoenzymes on the accumulation of ^{111}In-eosinophils and oedema formation in response to various known mediators of inflammation and in a type I allergic (PCA) reaction in guinea-pig skin. The effect of a type IV PDE inhibitor on ^{111}In-neutrophil accumulation was also studied. The following selective PDE isoenzyme inhibitors were used: SK&F 94120 (type III inhibitor), rolipram (type IV inhibitor) and zaprinast (type V inhibitor) (Beavo & Reifsnyder, 1990; Dent *et al.*, 1991). The findings with these PDE inhibitors in this model of eosinophil accumulation may have a bearing on not only asthma but also on other allergic diseases such as rhinitis (Klementsson, 1992), dermatitis (Bruijnzeel-Koomen *et al.*, 1992) and allergic conjunctivitis (Foster *et al.*, 1991) where eosinophils are thought to play a role.

Methods

Preparation of zymosan-activated plasma

Zymosan-activated plasma (ZAP) was used as a source of guinea-pig C5a des Arg. Guinea-pig heparinized (10 iu ml^{-1}) plasma was incubated with zymosan (5 mg ml^{-1}) at 37°C. After 30 min, zymosan was removed by centrifugation (2 × 10 min at 3000 *g*). ZAP was then 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 sera and doses of antigen are described elsewhere (Weg *et al.*, 1991). Briefly, male guinea-pigs (Harlan Porcellus, Oxon; 350–400 g) were immunized with bovine gamma-globulin (BGG) in Freund's complete adjuvant followed by a boost on day 21 and serum collected on day 30. Recipient animals received an i.d. injection of 50 µl of a 1/50 dilution of the anti-serum followed, 16–20 h later, by the injection of antigen (BGG, 0.01–1 µg per site). Most of the tissue fixing antibody was of the IgG_1 isotype (Weg *et al.*, 1991).

Induction, purification and radiolabelling of guinea-pig eosinophils

The method is described in detail elsewhere (Faccioli *et al.*, 1991; Teixeira *et al.*, 1993a). Briefly, ex-breeder female guinea-pigs (Harlan Porcellus; 700–800 g) were treated with neat horse serum (1 ml, i.p.) every other day for two 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 (> 95% pure, > 98% viable) were collected from the 1.090/1.095 and 1.095/1.100 g ml^{-1} density interfaces. The purified eosinophils were radiolabelled by incubation with $^{111}InCl_3$ (100 µCi in 10 µl) chelated to 2-mercaptopyridine-N-oxide (Merc, 40 µg 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 (calcium- and magnesium-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 the naive ex-breeder guinea-pigs by the i.p. injection of 15 ml of a 5% (w/v) solution of casein as previously described (Teixeira *et al.*, 1993b). After 12 h, the animals were killed and the peritoneal cavity washed with heparinized saline (10 iu ml^{-1}). The rest of the procedure was as described for the eosinophils. The cells were also collected from the 1.090/1.095 and 1.095/1.100 g ml^{-1} interfaces. The purity of the preparation was greater than 98% and the rare contaminants were eosinophils and occasional mononuclear cells. Viability, tested by trypan blue exclusion, was greater than 98%. Neutrophils were also radiolabelled with ^{111}In-Merc and resuspended at a concentration of 10^7 cells ml^{-1}.

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

Radiolabelled leukocyte infiltration and oedema formation were measured simultaneously in the skin. ^{125}I-labelled human serum albumin, ([^{125}I]-HSA, 5 µCi) was added to the ^{111}In-labelled eosinophils or neutrophils prior to i.v. injection (2.5×10^6 cells per animal), into recipient guinea-pigs (Harlan Porcellus; 350–400 g) anaesthetized with Hypnorm (0.2 ml, i.m.). PDE inhibitors were given either systemically or locally. For systemic treatment, drugs were given at the dose of 5 mg kg^{-1} i.p. 30 min and 0.5 mg kg^{-1} i.v. 10 min prior to the injection of radiolabelled cells and [^{125}I]-HSA. Similar doses of rolipram have been shown to inhibit antigen- or mediator-induced bronchoconstriction effectively in the guinea-pig (Howell *et al.*, 1993; Underwood *et al.*, 1993). Experiments were conducted in pairs and control animals received vehicle in the same volume as treated animals. For the local treatment, rolipram (0.1 to 10.0 µg per site) was mixed with the mediators or antigen prior to the i.d. injections. Five minutes after injection of cells, inflammatory mediators or antigen were injected i.d. in 0.1 ml volumes into the dorsal skin of the shaved animals. 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. At this time, blood was obtained by cardiac puncture and the animals were killed by an overdose of sodium pentobarbitone. The dorsal skin was removed, cleaned free of excess of 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.

The number of leukocytes accumulating in each site is expressed as ^{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 µl of plasma.

Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): bradykinin, dimethylsulphoxide (DMSO), histamine, casein, bovine gamma globulin (BGG), theophylline and zymosan. Hanks solutions, HEPES and horse serum were purchased from Life Technologies Limited (Paisley, Scotland). Percoll was from Pharmacia (Milton Keynes, Bucks) and C16 PAF from Bachem (Saffron Walden, Essex). [^{125}I]-human serum albumin ([^{125}I]-HSA) and $^{111}InCl_3$ were purchased from Amersham International plc,

Amersham. The following selective PDE isoenzyme inhibitors were used: SK&F 94120 (type III inhibitor), rolipram (type IV inhibitor) and zaprinast (type V inhibitor) (see Beavo & Reifsnyder, 1990, for review on PDE inhibitors). SK&F 94120 and zaprinast were dissolved in saline with 0.01 M sodium hydroxide while rolipram was dissolved in DMSO and diluted further in saline. These drugs were a gift from Sandoz, Basle, Switzerland.

Statistical analysis

Comparisons between control and untreated groups were carried out using Student's paired t test. For the local treatment, two-way analysis of variance (ANOVA) was used. Percentage inhibition was calculated after subtracting background (saline) values. Results were presented as the mean ± s.e.mean for the number of animals given and were considered significant when $P<0.05$.

Results

Two h after i.v. injection of the radiolabelled cells, 11.5 ± 1.9% ($n = 14$) and 4.7 ± 1.3% ($n = 5$) of infused ^{111}In-eosinophils and ^{111}In-neutrophils, respectively, were circulating. None of the drug treatments used in this study significantly altered the number of circulating radiolabelled cells (data not shown). Our previous experiments have shown that the majority of radiolabelled cell accumulation and oedema formation induced by known mediators of inflammation or in a PCA reaction occurs over the first 2 h (Weg *et al.*, 1992).

Effects of SK&F 94120 and zaprinast on ^{111}In-eosinophil accumulation and oedema formation

At the dose used (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.), SK&F 94120, a type III PDE inhibitor (Beavo & Reifsnyder, 1990), had no effect on the ^{111}In-eosinophil accumulation or oedema formation in a PCA reaction (0.1 to 1.0 μg of BGG per site, Figure 1). Similarly, SK&F 94120 had no effect on the ^{111}In-eosinophil accumulation (Table 1) and oedema formation (data not shown) in response to i.d. ZAP (10 to 100% in saline) and PAF (10^{-10} and 10^{-9} mol per site). Responses elicited by i.d. histamine (2.5×10^{-9} and 2.5×10^{-8} mol per site) or bradykinin (10^{-10} and 10^{-9} mol per site) were also unaffected (data not shown). The same doses of inflammatory stimuli were used in all subsequent experiments unless stated otherwise.

Zaprinast (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.), a type V PDE inhibitor (Beavo & Reifsnyder, 1990), also had no significant effect on the ^{111}In-eosinophil accumulation and oedema formation in a PCA reaction (Figure 2) or in response to i.d. injection of ZAP, PAF, bradykinin or histamine (Table 1 and data not shown).

Preliminary experiments using theophylline at similar doses (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) showed that ^{111}In-eosinophil accumulation or oedema formation induced by the same stimuli were unaltered (data not shown). However, higher doses of theophylline (50 mg kg^{-1}, i.p. and 5 mg kg^{-1}, i.v.) effectively inhibited ^{111}In-eosinophil accumulation

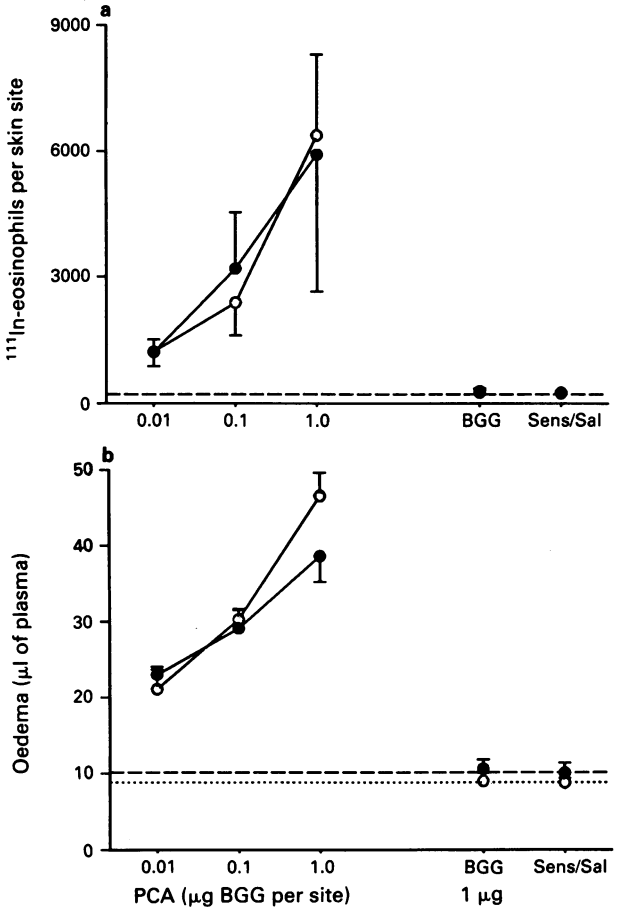

Figure 1 Effect of systemic treatment with SK&F 94120 (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) on ^{111}In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylaxis (PCA) reaction in guinea-pig skin. Control animals are shown by (●) and treated animals by (○). Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine gamma-globulin, BGG) in saline-treated sites (shown as BGG) or sites previously sensitized with an IgG_1-rich anti-sera (shown as PCA). The lines across the graphs represent the background values in sensitized skin sites injected with saline (Sens/Sal) in control (dashed line) and SK&F 94120-treated (dotted line) animals. Results are mean ± s.e.mean of 5 pairs of animals.

Table 1 Effect of systemic treatment with zaprinast or SK&F 94120 on the ^{111}In-eosinophil accumulation induced by PAF and zymosan activated plasma in guinea-pig skin

		^{111}*In-eosinophils per site*			
		SK&F 94120		*Zaprinast*	
		Control	*Treated*	*Control*	*Treated*
Saline		202 ± 38	189 ± 8	202 ± 39	214 ± 36
ZAP	10%	2939 ± 859	2963 ± 771	2986 ± 907	2324 ± 584
	30%	7351 ± 2303	6148 ± 1173	8319 ± 2412	5677 ± 1386
	100%	12615 ± 3148	12379 ± 2732	14089 ± 3215	10026 ± 2339
PAF	10^{-10}	1423 ± 666	1634 ± 413	1434 ± 275	1259 ± 268
	10^{-9}	1939 ± 642	2898 ± 848	2997 ± 919	2341 ± 413

Zaprinast or SK&F 94120 were given at a dose of 5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v. 30 and 10 min, respectively, before the i.v. injection of ^{111}In-eosinophils. PAF (10^{-10} amd 10^{-9} mol per site) and zymosan-activated plasma (ZAP, 10%, 30% and 100% in saline) were injected i.d. and ^{111}In-eosinophil accumulation assessed 2 h later. Results are mean ± s.e.mean of 5 pairs of animals.

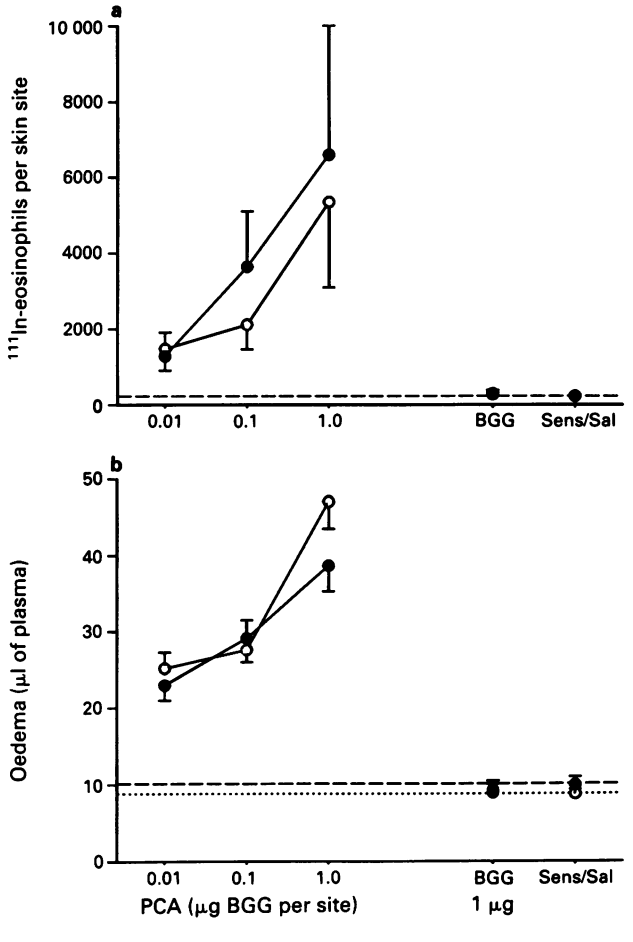

Figure 2 Effect of systemic treatment with zaprinast (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) on ^{111}In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylaxis (PCA) reaction in guinea-pig skin. Control animals are shown by (●) and treated animals by (○). Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine gamma-globulin, BGG) in saline-treated sites (shown as BGG) or sites previously sensitized with an IgG_1-rich anti-sera (shown as PCA). The lines across the graphs represent the background values in sensitized skin sites injected with saline (Sens/Sal) in control (dashed line) and zaprinast-treated (dotted line) animals. Results are mean ± s.e.mean of 5 pairs of animals.

Table 2 Effects of systemic treatment with theophylline on ^{111}In-eosinophil accumulation induced by PAF, zymosan-activated plasma and in a PCA reaction in guinea-pig skin

		^{111}*In-eosinophils per site*	
		Control	*Theophylline*
Saline		134 ± 5	166 ± 24
ZAP	10%	1510 ± 563	506 ± 63**
	30%	3154 ± 375	1476 ± 117*
	100%	9582 ± 1327	4793 ± 826*
PAF	10^{-10}	1263 ± 253	407 ± 40**
	10^{-9}	2025 ± 186	394 ± 56**
PCA	0.01	811 ± 152	342 ± 97**
	0.1	2428 ± 180	506 ± 169**
	1.0	4430 ± 1154	654 ± 180**

Theophylline was given at the dose of 50 mg kg^{-1}, i.p. and 5.0 mg kg^{-1}, i.v. 30 and 10 min respectively, before the i.v. injection of ^{111}In-eosinophils. Zymosan-activated plasma (ZAP, 10%, 30% and 100% in saline), PAF (10^{-10} and 10^{-9} mol per site) and antigen (BGG, 0.01 to 1.0 μg per site) in sites pre-sensitized with IgG_1-rich sera were injected i.d. and ^{111}In-eosinophil accumulation assessed 2 h later. Results are mean ± s.e.mean of 3 pairs of animals. *$P<0.05$ and **$P<0.01$.

induced by different meadiators or in a PCA reaction (Table 2). For example, ^{111}In-eosinophil accumulation in the PCA reaction (0.1 μg of BGG per site) was inhibited by 85% ($n = 3$, $P<0.01$). Oedema formation measured in the same sites was not significantly affected (data not shown).

Effects of systemic rolipram on ^{111}In-eosinophil accumulation and oedema formation

The type IV PDE inhibitor, rolipram (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) virtually abolished the ^{111}In-eosinopil accumulation in the PCA reaction, but did not affect oedema formation measured in the same sites (Figure 3). For example, rolipram inhibited by 97% the ^{111}In-eosinophil accumulation in sites injected with 0.1 μg of BGG. Similarly, ZAP-induced ^{111}In-eosinophil accumulation, but not oedema formation, was inhibited by up to 89% by systemic rolipram (Figure 4). Table 3 depicts the effects of rolipram on the ^{111}In-eosinophil accumulation and oedema formation induced by i.d. injection of PAF, histamine and bradykinin. Histamine induced small, but significant, ^{111}In-eosinophil accumulation. Both histamine- and PAF-induced ^{111}In-eosinophil accumulation, but not oedema formation, were

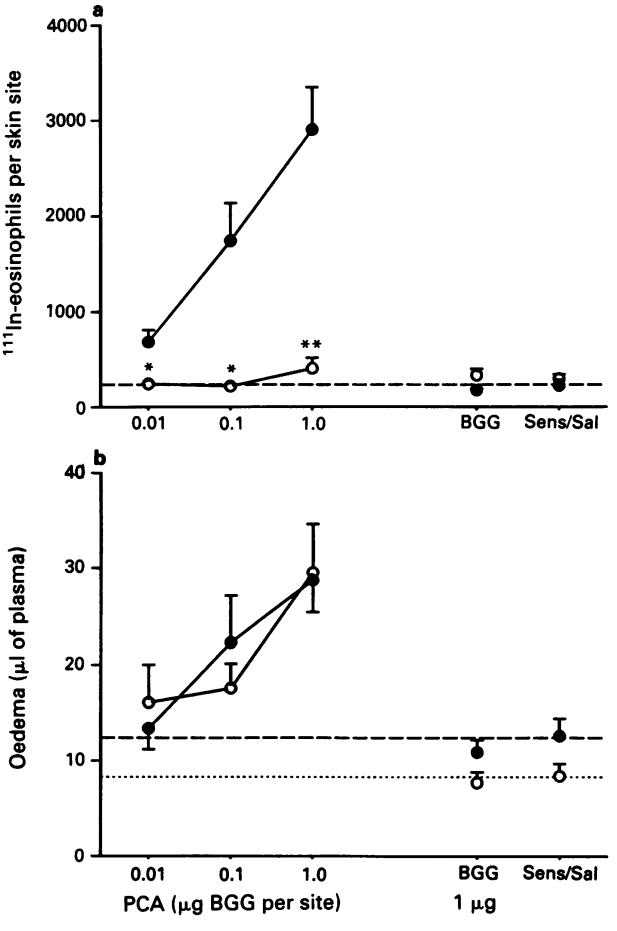

Figure 3 Effects of systemic treatment with rolipram (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) on ^{111}In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylaxis (PCA) reaction in guinea-pig skin. Control animals are shown by (●) and treated animals by (○). Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine gamma-globulin, BGG) in saline-treated sites (shown as BGG) or sites previously sensitized with an IgG_1-rich anti-sera (shown as PCA). The lines across the graphs represent the background values in sensitized skin sites injected with saline (Sens/Sal) in control (dashed line) and rolipram-treated (dotted line) animals. Results are mean ± s.e.mean of 5 pairs of animals where *$P<0.05$ and **$P<0.01$, respectively, when compared to control animals.

significantly inhibited by systemic rolipram (Table 3). Bradykinin did not induce significant influx of ^{111}In-eosinophils when compared to saline (Table 3).

Effects of systemic rolipram on ^{111}In-neutrophil accumulation

Both neutrophils and eosinophils have been shown to possess a PDE type IV isoenzyme which seems to account for most of the cyclic AMP hydrolytic activity in these cells (Nielson *et al.*, 1990; Dent *et al.*, 1991). Inhibitors of this isoenzyme (such as rolipram) have been shown to inhibit both eosinophil and neutrophil function effectively *in vitro* (Nielson *et al.*, 1990; Dent *et al.*, 1991; Souness *et al.*, 1991). Since

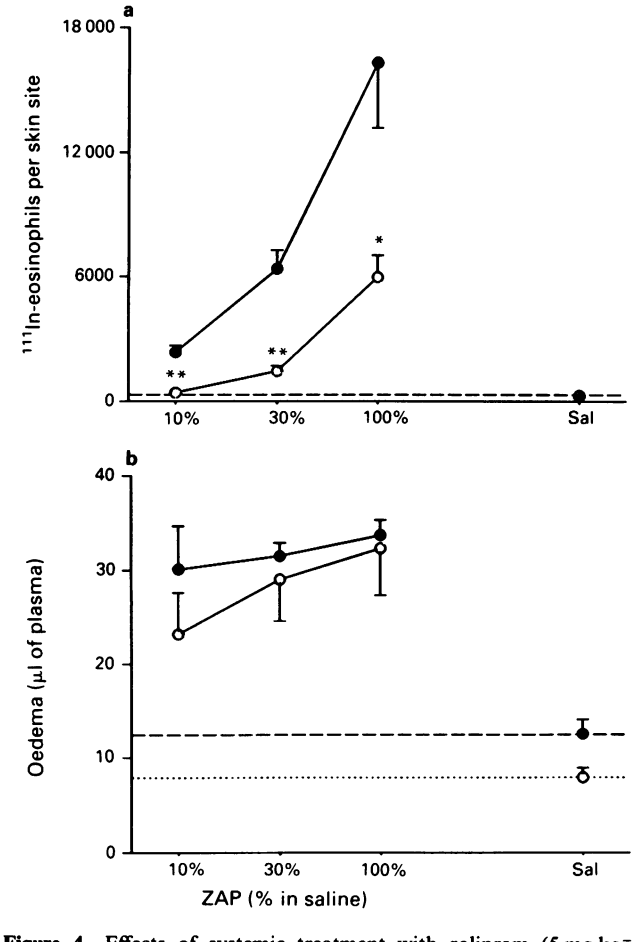

Figure 4 Effects of systemic treatment with rolipram (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) on zymosan-activated plasma (ZAP)-induced ^{111}In-eosinophil accumulation (a) and oedema formation (b) in guinea-pig skin. Control animals are shown by (●) and treated animals by (○). Inflammatory responses were assessed 2 h after i.d. injection of ZAP or saline (Sal). The lines across the graphs represent the background values in response to i.d. injection of saline in control (dashed line) and rolipram-treated (dotted line) animals. Results are mean ± s.e.mean of 5 pairs of animals where $*P<0.05$ and $**P<0.01$, respectively, when compared to control animals.

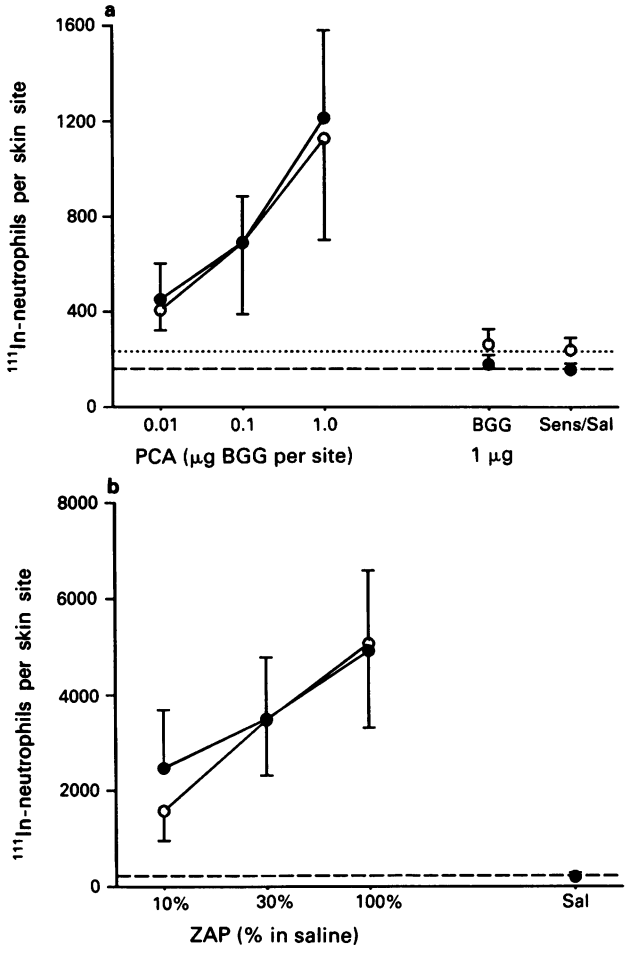

Figure 5 Effect of systemic treatment with rolipram (5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v.) on ^{111}In-neutrophil accumulation in (a) a passive cutaneous anaphylaxis (PCA) reaction and (b) in response to i.d. injection of zymosan-activated plasma (ZAP) in guinea-pig skin. Control animals are shown by (●) and treated animals by (○). Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine gamma-globulin, BGG) in saline-treated sites (shown as BGG), sites previously sensitized with an IgG_1-rich anti-sera (shown as PCA) or in sites injected with ZAP or saline (Sal). The lines across the graphs represent the background values in saline sites or sensitized skin sites injected with saline (Sens/Sal) in control (dashed line) and rolipram-treated (dotted line) animals. Results are mean ± s.e.mean of 5 pairs of animals.

Table 3 Effect of systemic treatment with rolipram on the ^{111}In-eosinophil accumulation and oedema formation in guinea-pig skin

Stimuli (mol per site)	*^{111}In-eosinophils per site* Control	Rolipram	*Oedema (μl of plasma)* Control	Rolipram
Saline	277 ± 46	173 ± 13	7.9 ± 1.0	12.5 ± 1.5
BK 10^{-10}	401 ± 77	196 ± 13	37.3 ± 2.9	36.6 ± 3.9
10^{-9}	448 ± 99	210 ± 19	44.5 ± 2.9	42.4 ± 3.1
Hist 2.5×10^{-9}	702 ± 192	207 ± 98	36.6 ± 3.7	25.5 ± 5.8
2.5×10^{-8}	766 ± 152	248 ± 26*	51.5 ± 6.7	51.8 ± 4.6
PAF 10^{-10}	1434 ± 275	358 ± 31*	34.1 ± 2.5	34.7 ± 3.3
10^{-9}	2997 ± 919	890 ± 353*	55.4 ± 4.0	53.8 ± 6.8

Rolipram was given at a dose of 5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v. 30 and 10 min, respectively, before the i.v. injection of ^{111}In-eosinophils and [^{125}I]-HSA. The inflammatory stimuli were injected i.d. and ^{111}In-eosinophil accumulation and oedema formation assessed 2 h later. The following stimuli were used: bradykinin (BK), histamine (Hist) and platelet-activating factor (PAF) which were administered at the doses shown. Results are mean ± s.e.mean of 5 pairs of animals. $*P<0.05$.

rolipram suppressed ^{111}In-eosinophil accumulation in our model, we decided to test if accumulation of ^{111}In-neutrophils was also inhibited. Figure 5 shows the effects of systemic rolipram on the ^{111}In-neutrophil accumulation in a PCA reaction (Figure 5a) and in response to i.d. injection of ZAP (Figure 5b). There was no inhibition of ^{111}In-neutrophil accumulation and oedema formation measured in the same sites was also unaltered (data not shown). PAF-induced ^{111}In-neutrophil accumulation was also not affected by systemic administration of rolipram (data not shown).

Effects of intradermal administration of rolipram on ^{111}In-eosinophil accumulation and oedema formation

In order to assess whether rolipram could also inhibit ^{111}In-eosinophil accumulation when given locally, increasing concentrations of rolipram were mixed with PAF (10^{-9} mol per site), histamine (2.5×10^{-8} mol per site) or antigen (1 μg of BGG) prior to their i.d. injection. At the doses used (0.1 to 10.0 μg per site), rolipram had little effect on ^{111}In-eosinophil accumulation and oedema formation induced by the inflammatory stimuli or in the PCA reaction (Figure 6). Only PAF-induced ^{111}In-eosinophil accumulation was partially suppressed by rolipram at 10 μg per site. Higher doses of rolipram could not be used because of limited solubility.

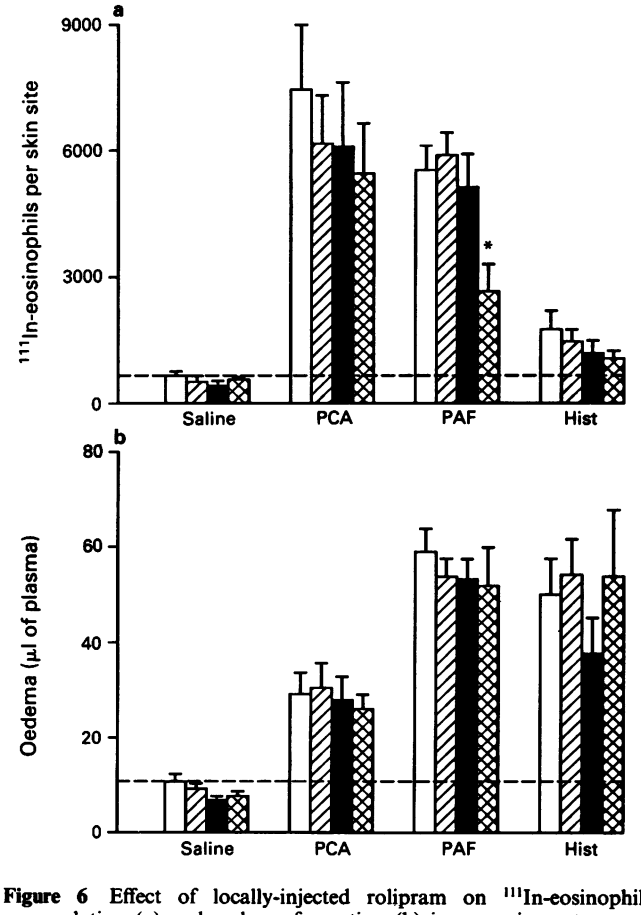

Figure 6 Effect of locally-injected rolipram on ^{111}In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylaxis (PCA, 1 μg of antigen per site) reaction and induced by PAF (10^{-9} mol per site) or histamine (Hist, 2.5×10^{-8} mol per site). Inflammatory responses were assessed 2 h after i.d. injection of mediators and antigen either alone (open columns) or with rolipram at 0.1 μg per site (hatched columns), 1.0 μg per site (solid columns) or 10.0 μg per site (cross-hatched columns). The line across the graphs represent the background values in response to i.d. injection of saline. Results are mean ± s.e.mean of 5 pairs of animals where *represents $P < 0.05$ when compared to i.d. injection of mediators or antigen alone.

Discussion

We have studied the effects of selective inhibitors of PDE isoenzymes III, IV and V on the local accumulation of radiolabelled eosinophils, neutrophils and plasma protein induced by antigen or different known mediators of inflammation in guinea-pig skin. Our findings can be summarized as follows: (1) Systemic treatment with a type III (SK&F 94120) or a type V (zaprinast) PDE inhibitor had no significant effect on the ^{111}In-eosinophil accumulation and oedema formation induced by the different inflammatory stimuli; (2) Systemic treatment with theophylline significantly inhibited ^{111}In-eosinophil accumulation but not oedema formation induced by ZAP, PAF and in the PCA reaction; (3) Systemic treatment with rolipram, a type IV PDE inhibitor, effectively suppressed the accumulation of ^{111}In-eosinophils, but had no effect on the accumulation of ^{111}In-neutrophils or oedema formation in response to the i.d. inflammatory stimuli; (4) Co-injection of rolipram with PAF, histamine or antigen had little effect on the accumulation of ^{111}In-eosinophils or oedema formation induced by these stimuli. These results show that systemic, but not local, PDE type IV inhibition was associated with strong inhibition of allergic and mediator-induced ^{111}In-eosinophil accumulation in guinea-pig skin.

Theophylline has been previously shown to inhibit the influx of eosinophils into the airways of different animal models of allergic inflammation (Spicer *et al.*, 1990; Sturm *et al.*, 1990; Gristwood *et al.*, 1991). In our studies in guinea-pig skin, theophylline also effectively inhibited the accumulation of ^{111}In-eosinophils induced by different mediators and in a PCA reaction when given at 50 mg kg^{-1}, but not at 5 mg kg^{-1}. The need for higher doses of theophylline to achieve effective inhibition of eosinophil accumulation is in agreement with previously published data (Spicer *et al.*, 1990; Sturm *et al.*, 1990; Gristwood *et al.*, 1991).

Griswold *et al.* (1993) recently reported that oral administration of rolipram inhibited neutrophil accumulation and oedema formation in the inflamed mouse ear and peritoneum with an ED_{50} of 1.7 mg kg^{-1} and 2.5 mg kg^{-1}, respectively. Zaprinast, up to 10 mg kg^{-1}, had no inhibitory effect. In the guinea-pig, oral administration of rolipram has been reported to dose-dependently inhibit eosinophil accumulation in the conjunctiva induced by topical application of leukotrienes (Newsholm & Schwartz, 1993). Maximum inhibition was observed at 10 mg kg^{-1} but greater than 80% inhibition was found with as little as 0.1 mg kg^{-1}. In our studies we found that administration of rolipram at 5 mg kg^{-1}, i.p. and 0.5 mg kg^{-1}, i.v. gave maximal inhibition of eosinophil accumulation although we did not conduct full dose-response analysis. Preliminary studies with rolipram at 0.5 mg kg^{-1} i.v. gave inconsistent inhibition of eosinophil accumulation and therefore the compound was also administered i.p. at the higher dose.

The effects of PDE inhibition on guinea-pig pulmonary function and cellular influx after antigen challenge has also been reported in recent publications (Howell *et al.*, 1993; Underwood *et al.*, 1993). Rolipram inhibited antigen-induced bronchoconstriction both *in vitro* and *in vivo*, but it had no effect on bronchoconstriction induced by LTC_4 or histamine (Howell *et al.*, 1993; Underwood *et al.*, 1993). In contrast, zaprinast at oral doses up to 200 mg kg^{-1} failed to inhibit antigen-induced bronchoconstriction (Howell *et al.*, 1993). However, airway hyperreponsiveness (AHR) and eosinophil influx measured 24 h after antigen challenge were also inhibited by rolipram. The observation that rolipram inhibited antigen-induced but not mediator-induced responses led the authors to suggest that inhibition of mast cells could account for some of the inhibitory effects of rolipram (Underwood *et al.*, 1993). However, rolipram may inhibit eosinophil influx even when given 12 h after antigen challenge mitigating against an early inhibitory effect only on the mast cell (Sturm *et al.*, 1990).

In guinea-pig skin, rolipram inhibited both antigen- and mediator-induced ^{111}In-eosinophil accumulation. We cannot rule out a role for mast cells as the cellular target for the inhibitory actions of rolipram, but several pieces of evidence suggest that these cells may not be the main site of action. Firstly, we have no evidence for a mast cell-dependent component to responses to PAF, but these were effectively inhibited by rolipram. Additionally, one would expect that, if rolipram were acting on the mast cell to inhibit mediator release, both oedema formation and ^{111}In-neutrophil accumulation would also be suppressed accordingly. Finally, i.d. injection of rolipram with PAF or antigen had little effect on the ^{111}In-eosinophil accumulation induced by these stimuli favouring an effect on circulating cells, rather than skin tissue cells.

If the mast cell is not the main cellular target for rolipram-induced inhibition of ^{111}In-eosinophil accumulation, what are other possible sites of action? Guinea-pig eosinophils have been previously shown to possess a membrane bound, cyclic AMP-specific, cyclic GMP-and calmodulin-insensitive, Ro-20-1724-inhibitable isoenzyme (Dent *et al.*, 1991; Souness *et al.*, 1991). These characteristics are of a type IV PDE isoenyzme which accounts for most of the cyclic AMP hydrolytic activity in these cells (Dent *et al.*, 1991). Systemic rolipram treatment may lead to an inhibition of the PDE type IV in circulating ^{111}In-eosinophils, thus inhibiting their accumulation. If that is the case, PDE IV inhibitors are capable of inhibiting not only eosinophil function *in vitro*, but also their accumulation *in vivo*. This may be of potential benefit in diseases where eosinophils play a major pathophysiological role.

Another putative site for the inhibitory action of rolipram is the endothelial cell. Analysis of the PDE isoenzyme profile of endothelial cells from different species has shown that these cells possess mainly types II, III and IV isoenzymes (Suttorp *et al.*, 1993). Whether inhibition of PDE type IV isoenzymes in endothelial cells can inhibit the transmigration of leukocytes *in vitro* or into inflamed tissues *in vivo* is presently unknown, but deserves further investigation. Nevertheless, it has been shown previously that increased levels of cyclic AMP in endothelial cells may differentially affect the expression of adhesion molecules (Pober *et al.*, 1993) which could be translated into decreased or preferential accumulation of leukocytes within a given tissue. We are at present investigating this hypothesis.

Human neutrophils possess a PDE type IV isoenzyme which accounts for most of the nucleotide hydrolytic activity in these cells (Nielson *et al.*, 1990; Schudt *et al.*, 1991). As found with eosinophils, human neutrophil activity is also suppressed *in vitro* by inhibitors of PDE type IV such as rolipram (Nielson *et al.*, 1990). However, in the present study systemic rolipram had no effect on the accumulation of ^{111}In-neutrophils induced by different inflammatory stimuli in guinea-pig skin. This is in accordance with a recent abstract by Boucheron *et al.* (1991) who showed that guinea-pig neutrophil function is less inhibited by PDE inhibitors than the human neutrophil. In the same studies however, rolipram effectively inhibited cyclic nucleotide hydrolytic activity in cell lysates (Boucheron *et al.*, 1991). Thus, it is possible that rolipram does not have access to neutrophil PDE enzymes or that cyclic AMP turnover in these cells is low and a stimulus which activates adenylate cyclase is also necessary (Boucheron *et al.*, 1991). The lack of effect of rolipram on ^{111}In-neutrophil accumulation does not exclude the endothelial cell as its main cellular target. In fact, agents which increase cyclic AMP levels in endothelial cells have been shown to inhibit preferentially the expression of VCAM-1 (the ligand for VLA-4, an integrin present on eosinophils but not neutrophils) but not ICAM-1 *in vitro* (Pober *et al.*, 1993). Interestingly, we have shown that other agents such as prostaglandins of the E series and isoprenaline which may enhance cyclic AMP *in vivo* inhibit ^{111}In-eosinophil accumulation but not ^{111}In-neutrophil accumulation (Teixeira *et al.*, 1993a).

Microvascular leakage of plasma protein in the hamster cheek pouch and in the guinea-pig lung was attenuated by inhibition of PDE types III or IV and PDE type IV (Raeburn *et al.*, 1991; Ortiz *et al.*, 1992; Svensjo *et al.*, 1992). In contrast, in the present study we found no inhibitory effect on local oedema formation in guinea-pig skin with any of the PDE inhibitors used. This discrepancy may relate, in part, to methodological differences since in the guinea-pig lung studies, leakage of plasma protein was measured either 5 or 10 min after PAF challenge whereas we measured local oedema formation after 2 h. Experiments *in vitro* using porcine endothelial cells have established a role for PDE types III and IV in protecting against H_2O_2-induced increased vascular permeability (Suttorp *et al.*, 1993). Endothelial cells also possess a PDE type II isoenzyme (Suttorp *et al.*, 1993). However, there is no inhibitor of this enzyme available at the moment. We are not aware of studies on the PDE isoenzyme profile of guinea-pig endothelial cells, but it is possible that a type II isoenzyme may account for a significant portion of cyclic nucleotide hydrolytic activity as it does in porcine endothelial cells (Suttorp *et al.*, 1993). Alternatively, inhibition of only PDE type III or IV separately may be insufficient to inhibit increases in vascular permeability and addition of both inhibitors may be necessary (as occurs in bronchial smooth muscle; Torphy *et al.*, 1993).

The importance of the eosinophil for the pathophysiology of allergic diseases such as asthma is well recognized (Djukanovic *et al.*, 1990). However, it is not known if drugs which inhibit eosinophil accumulation and/or function will be useful in the treatment of these diseases. Also unknown is the relevance of PDE inhibition for asthma treatment, even though at least some of the useful effects of theophylline may be mediated in this way (Kuehl *et al.*, 1987). The observation that rolipram can mimic the inhibitory effects of theophylline in this model is further evidence that at least some of the anti-inflammatory properties of theophylline are mediated via PDE inhibition. In addition, it suggests that PDE type IV inhibitors may also mimic the useful effects of theophylline in allergic diseases such as asthma. Interestingly, atopic subjects may have higher levels of PDE activity in their inflammatory cells (Hanifin & Chan, 1988; Townley, 1993) and this may contribute to altered inflammatory cell function in asthma (Morley, 1993). The suppressive activity of PDE type IV inhibitors on eosinophil accumulation shown in this and other recent studies (Griswold *et al.*, 1993; Newsholme & Schwartz, 1993; Underwood *et al.*, 1993) and on eosinophil function (Souness *et al.*, 1991; Dent *et al.*, 1991) warrant a search for better and safer drugs which could be tested in human diseases. Furthermore, ^{111}In-eosinophil accumulation in the guinea-pig skin is a useful model for testing the effects of putative PDE type IV inhibitors *in vivo*.

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References

BEAVO, J.A. & REIFSNYDER, D.H. (1990). Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol. Sci.*, **11**, 150–155.

BENTLEY, A.M., MENZ, G., STORZ, C.H.R., ROBINSON, D.S., BRADLEY, B., JEFFERY, P.K., DURHAM, S.R. & KAY, A.B. (1992). Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. *Am. Rev. Respir. Dis.*, **146**, 500–506.

BOUCHERON, J.A., VERGHESE, M.W., IRSULA, O. & STACY, L. (1991). Species differences in neutrophil superoxide modulation by cyclic nucleotide phosphodiesterase inhibitors. *FASEB. J.*, **5**, A510.

BRUIJNZEEL-KOOMEN, C., STORZ, E., MENZ, G. & BRUIJNZEEL, P. (1992). Skin eosinophilia in patients with allergic and nonallergic asthma and atopic dermatitis. *J. Allergy Clin Immunol.*, **89**, 52–59.

DE BOER, J., PHILPOTT, A.J., VAN AMSTERDAM, R.G.M., SHAHID, M., ZAAGSMA, J. & NICHOLSON, C.D. (1992). Human bronchial cyclic nucleotide phosphodiesterase isoenzymes: biochemical and pharmacological analysis using selective inhibitors. *Br. J. Pharmacol.*, **106**, 1028–1034.

DENT, G., GIEMBYCZ, M.A., RABE, K.F. & BARNES, P.J. (1991). Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-stimulated respiratory burst by 'type IV'-selective PDE inhibitors. *Br. J. Pharmacol.*, **103**, 1339–1346.

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.

FACCIOLI, L.H., NOURSHARGH, S., MOQBEL, R., WILLIAMS, F.M., SEHMI, R., KAY, A.B. & WILLIAMS, T.J. (1991). The accumulation of ^{111}In-eosinophils induced by inflammatory mediators in vivo. *Immunology*, **73**, 222–227.

FOSTER, C.S., RICE, B.A. & DUTT, J.E. (1991). Immunopathology of atopic keratoconjuctivitis. *Ophthalmology*, **98**, 1190–1996.

GIEMBYCZ, M.A. & DENT, G. (1992). Prospects for selective cyclic nucleotide phosphodiesterase inhibitors in the treatment of bronchial asthma. *Clin. Exp. Allergy*, **22**, 337–344.

GRISTWOOD, R.W., LLUPIA, J. & BERGA, P. (1991). Effects of theophylline compared with prednisolone on late phase airway leukocyte infiltration in guinea pigs. *Int. Arch. Allergy Appl. Immunol.*, **94**, 293–294.

GRISWOLD, D.E., WEBB, E.F., BRETON, J., WHITE, J.R., MARSHALL, P.J. & TORPHY, T.J. (1993). Effect of selective phosphodiesterase type IV inhibitor, rolipram, on fluid and cellular phases of inflammatory response. *Inflammation*, **17**, 333–344.

GUNDEL, R.H., LETTS, L.G. & GLEICH, G.J. (1991). Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J. Clin. Invest.*, **87**, 1470–1473.

HANIFIN, J.M. & CHAN, S.C. (1988). Characterization of cAMP-phosphodiesterase as a possible laboratory marker of atopic dermatitis. *Drug Dev. Res.*, **13**, 123–136.

HOWELL, R.E., SICKELS, B.D. & WOEPPEL, S.L. (1993). Pulmonary antiallergic and bronchodilator effects of isoenzyme-selective phosphodiesterase inhibitors in guinea pigs. *J. Pharmacol. Exp. Ther.*, **264**, 609–615.

JOHNSTON, I.D.A. (1990). Theophylline in the management of airflow obstruction. Difficult drug to use, few clinical indications. *Br. Med. J.*, **300**, 929–931.

KLEMENTSSON, H. (1992). Eosinophils and the pathophysiology of allergic rhinitis. *Clin. Exp. Allergy*, **22**, 1058–1064.

KUEHL, F.A., ZANETTI, M.E., SODERMAN, D.D., MILLER, D.K. & HAM, E.A. (1987). Cyclic AMP-dependent regulation of lipid mediators in white cells. A unifying concept for explaining the efficacy of theophylline in asthma. *Am. Rev. Respir. Dis.*, **136**, 210–213.

LEBOWITZ, M.D. & SPINACI, S. (1993). The epidemiology of asthma. *Eur. Respir. Rev.*, **3**, 415–423.

MONTEFORT, S., HERBERT, C.A., ROBINSON, C. & HOLGATE, S.T. (1992). The bronchial epithelium as a target for inflammatory attack in asthma. *Clin. Exp. Allergy*, **22**, 511–520.

MORLEY, J. (1993). Immunopharmacology of asthma. *Immunol. Today*, **14**, 317–322.

NASSIF, E.G., WEINBERGER, M., THOMPSON, R. & HUNTLEY, W. (1981). The value of maintenance theophylline in steroid-dependent asthma. *N. Engl. J. Med.*, **30**, 71–75.

NEWSHOLME, S.J. & SCHWARTZ, L. (1993). cAMP-specific phosphodiesterase inhibitor, rolipram, reduces eosinophil infiltration evoked by leukotrienes or by histamine in guinea pig conjuctiva. *Inflammation*, **17**, 25–31.

NICHOLSON, C.D., CHALLISS, R.A.J. & SHAND, M. (1991). Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends Pharmacol. Sci.*, **12**, 19–27.

NIELSON, C.P., CROWLEY, J.J., MORGAN, M.E. & VESTAL, R.E. (1988). Polymorphonuclear leukocyte inhibition by therapeutic concentrations of theophylline is mediated by cyclic-3',5'-adenosine monophosphate. *Am. Rev. Respir. Dis.*, **137**, 25–30.

NIELSON, C.P., VESTAL, R.E., STURM, R.J. & HEASLIP, R. (1990). Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. *J. Allergy Clin. Immunol.*, **86**, 801–808.

ORTIZ, J.L., CORTIJO, J., VALLES, J.M., BOU, J. & MORCILLO, E.J. (1992). Rolipram inhibits PAF-induced airway microvascular leakage in guinea pig: a comparison with milrinone and theophylline. *Fundam. Clin. Pharmacol.*, **6**, 247–249.

PERSSON, C.G.A. (1986). Overview of effects of theophylline. *J. Allergy Clin. Immunol.*, **78**, 780–787.

POBER, J.S., SLOWIK, M.R., DE LUCA, L.G. & RITCHIE, A.J. (1993). Elevated cyclic AMP inhibits endothelial cell synthesis and expression of TNF-induced endothelial leukocyte adhesion molecule-1, and vascular cell molecule-1, but not intercellular adhesion molecule-1. *J. Immunol.*, **150**, 5114–5123.

RAEBURN, D., WOODMAN, V., BUCKLEY, G. & KARLSSON, J.A. (1991). Inhibition of PAF-induced microvascular leakage in the guinea-pig lung in vivo: the effect of rolipram and theophylline. *Eur. Respir. J.*, **4**, 590s.

SCHUDT, C., WINDER, S., FORDERKUNZ, S., HATZELMANN, A. & ULLRICH, V. (1991). Influence of selective phosphodiesterase inhibitors on human neutrophil functions and levels of cAMP and Ca_i. *Naunyn-Schmied. Arch. Pharmacol.*, **344**, 682–690.

SPICER, B.A., BAKER, R.C., HATT, P.A., LAYCOCK, S. & SMITH, H. (1990). The effects of drugs on sephadex-induced eosinophilia and lung hyper-responsiveness in the rat. *Br. J. Pharmacol.*, **101**, 821–828.

SOUNESS, J.E., CARTER, C.M., DIOCEE, B.K., HASSALL, G.A., WOOD, L.J. & TURNER, N.C. (1991). Characterization of guinea-pig eosinophil phosphodiesterase activity. *Biochem. Pharmacol.*, **42**, 937–945.

STURM, R.J., OSBORNE, M.C. & HEASLIP, R.J. (1990). The effect of phosphodiesterase inhibitors on pulmonary inflammatory cell influx in ovalbumin sensitized guinea pigs. *J. Cell Biochem.*, **14**, 337.

SUTTORP, N., WEBER, U., WELSCH, T. & SCHUDT, C. (1993). Role of phosphodiesterases in the regulation of endothelial permeability in vitro. *J. Clin. Invest.*, **91**, 1421–1428.

SVENSJO, E., ANDERSSON, K.E., BOUSKELA, E., CYRINO, F.Z.G.A. & LINDGREN, S. (1992). Effects of two vasodilatory phosphodiesterase inhibitors on bradykinin induced permeability increase in the hamster. *Int. J. Microcirc. Clin. Exp.*, **11**, A179.

TEIXEIRA, M.M. & HELLEWELL, P.G. (1994). Effect of a 5-lipoxygenase inhibitor, ZM 230487, on cutaneous allergic inflammation in the guinea-pig. *Br. J. Pharmacol.* (in press).

TEIXEIRA, M.M., REYNIA, S., ROBINSON, M., SHOCK, A., WILLIAMS, T.J., WILLIAMS, F.M., ROSSI, A.G. & HELLEWELL, P.G. (1994). Role of CD18 in the accumulation of eosinophils and neutrophils and local oedema formation in inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.*, **111**, 811–818.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1993a). E-type prostaglandins enhance local oedema formation and neutrophil accumulation but suppress eosinophil accumulation in guinea-pig skin. *Br. J. Pharmacol.*, **110**, 416–422.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1993b). Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.*, **110**, 1515–1521.

TORPHY, T.J. & UNDEM, B.J. (1991). Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. *Thorax*, **46**, 512–523.

TORPHY, T.J., UNDEM, B.J., CIESLINSKI, L.B., LUTTMANN, M.A., REEVES, M.L. & HAY, D.W.P. (1993). Identification, characterisation and functional role of phosphodiesterase isozymes in human airway smooth muscle. *J. Pharmacol. Exp. Ther.*, **265**, 1213–1223.

TOWNLEY, R.G. (1993). Elevated cAMP-phosphodiesterase in atopic disease: cause or effect? *J. Lab. Clin. Med.*, **121**, 15–17.

UNDERWOOD, D.C., OSBORN, R.R., NOVAK, L.B., MATTHEWS, J.K., NEWSHOLME, S.J., UNDEM, B.J., HAND, J.M. & TORPHY, T.J. (1993). Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. *J. Pharmacol. Exp. Ther.*, **266**, 306–313.

VENGE, P. (1990). The human eosinophil in inflammation. *Agents Actions*, **29**, 122–126.

WARD, A.J.M., MCKENNIFF, M., EVANS, J.M., PAGE, C.P. & COSTELLO, J.F. (1993). Theophylline – an immunomodulatory role in asthma? *Am. Rev. Respir. Dis.*, **147**, 518–523.

WARDLAW, A.J., DUNNETTE, S., GLEICH, G.J., COLLINS, J.V. & KAY, A.B. (1988). Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.*, **137**, 62–69.

WEG, V.B., WATSON, M.L., CORDEIRO, R.S.B. & WILLIAMS, T.J. (1991). Histamine, leukotriene D_4 and platelet activating factor in guinea pig passive cutaneous anaphylaxis. *Eur. J. Pharmacol.*, **204**, 157–163.

WEG, V.B., WATSON, M.L., FACCIOLI, L.H. & WILLIAMS, T.J. (1992). [^{111}In]-eosinophil accumulation during passive cutaneous anaphylaxis in the guinea-pig. *Br. J. Pharmacol.*, **105**, 127P.

WEG, V.B., WILLIAMS, T.J., LOBB, R.R. & NOURSHARGH, S. (1993). A monoclonal antibody recognising very late activation antigen-4 (VLA-4) inhibits eosinophil accumulation in vivo. *J. Exp. Med.*, **177**, 561–566.

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