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Acute versus chronic administration of phosphodiesterase inhibitors on allergen-induced pulmonary cell influx in sensitized guinea-pigs

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1 The aims of this study were to determine which phosphodiesterase (PDE) isoenzymes are involved in the control of eosinophil accumulation in the airways of ovalbumin (OVA)-immunized guinea-pigs by the use of isoenzyme selective inhibitors and to compare the effects of acute versus chronic administration of PDE isozyme inhibitors on pulmonary cell influx in ovalbumin-immunized guinea-pigs.

2 Guinea-pigs were sensitized and subsequently challenged with aerosolized OVA. Twenty four hours later bronchoalveolar lavage (BAL) was performed to permit assessment of inflammatory cell accumulation. A significant increase in the number of eosinophils was observed in the lavage fluid from OVA-immunized ($13.6 \pm 1.4 \times 10^4$ ml^{-1} in acute experiments and $10.1 \pm 1.4 \times 10^4$ ml^{-1} in chronic experiments) animals compared with sham-treated controls ($5.6 \pm 0.6 \times 10^4$ ml^{-1} in acute experiments and $5.1 \pm 0.6 \times 10^4$ ml^{-1} in chronic experiments). There was no difference in neutrophil, mononuclear cell or total cell numbers between the two groups.

3 Acute administration of a high dose of selective and non-selective PDE inhibitors by the i.p. route had no significant effect on eosinophil accumulation in the airways.

4 Chronic administration of a low dose (3 mg kg^{-1}, i.p., twice daily for 7 days) of the type IV PDE inhibitor, RO 20-1724, and the PDE III/IV inhibitor, zardaverine, produced a significant inhibition of eosinophil accumulation (46% and 59% respectively).

5 These results suggest that the type IV PDE isoenzyme plays a role in the control of allergen-induced eosinophil infiltration into the airways, but indicate that a period of low dose chronic treatment with a type IV or mixed type III/IV PDE inhibitor is necessary for eosinophil accumulation in the airways to be reduced.

Keywords: Sensitized guinea-pig; cyclic nucleotide phosphodiesterase isoenzymes, isoenzyme selective inhibitors

Introduction

Xanthines have been used in the treatment of bronchial asthma for many years with their primary therapeutic activity initially considered to be through bronchodilatation (Weinburger, 1980; Persson, 1986). Recent evidence suggests that theophylline, the most widely prescribed xanthine, may derive its therapeutic activity in asthma from anti-inflammatory effects (Pauwels, 1987; Ward *et al.*, 1993; Sullivan *et al.*, 1994). The second messengers adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP) regulate the function of several cell types involved in the pathophysiology of asthma, including inflammatory cells, and there is much evidence to suggest that elevation of cyclic AMP levels suppresses the activities of these cells (Torphy & Undem, 1991; Giembycz & Dent, 1992). Therefore, the cellular mechanism underlying the putative anti-inflammatory activity of theophylline may be related to the ability of this drug to inhibit the cyclic AMP and cyclic GMP regulatory enzyme, phosphodiesterase.

Currently there are at least seven different PDE isoenzyme families with specific and selective inhibitors (Nicholson *et al.*, 1991; Giembycz, 1994). Specific PDE isoenzymes are differentially regulated and expressed (Dent *et al.*, 1991); the PDE IV isoenzyme is the major isoenzyme in many inflammatory cells although PDE III predominates in platelets and is also found together with PDE IV in T-lymphocytes (Robicsek *et al.*, 1991). Drugs capable of selectively inhibiting specific PDE isoenzymes can be expected to be more useful therapeutically than non-selective PDE inhibitors because of a lower expected systemic side effect profile.

Eosinophils are the predominant inflammatory cell type in the airways of asthmatics and there is strong evidence that they play a critical role in the pathogenesis of bronchial asthma (Wardlaw & Kay, 1987). The number of eosinophils found in the airways of asthmatics correlates with the disease severity (Horn *et al.*, 1975). Activated eosinophils are able to synthesize and release toxic and pro-inflammatory mediators in response to specific stimuli, (Frigas & Gleich, 1986) and thus one possible therapeutic strategy for the therapy of asthma would be to target the mechanisms involved in the control of eosinophil accumulation and activation in the airways. There is evidence that PDE and cyclic AMP play a pivotal role in the accumulation and activation of eosinophils (Yukawa *et al.*, 1989).

Acute administration of theophylline and selective PDE isoenzyme inhibitors has been reported to reduce eosinophil infiltration induced by allergen challenge in sensitized guinea-pigs (Gristwood *et al.*, 1991; Schudt *et al.*, 1991; Manzini *et al.*, 1993; Underwood *et al.*, 1993). However, the majority of studies which have examined the effect of theophylline and selective isoenzyme inhibitors on eosinophil accumulation are not relevant to the clinical situation for two reasons; firstly the doses which have been used in these studies have been given as one dose acutely, prior to antigen challenge, and secondly because the doses which have been employed are much higher than those used clinically.

The aims of the present study have been firstly to try to determine which PDE isoenzymes are important in regulating eosinophil accumulation in the airways by utilising selective isoenzyme inhibitors, and secondly to compare the effects of acute high dose administration of isoenzyme selective inhibitors with that of more clinically relevant chronic low dose treatment for 7 days.

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Part of this work has been presented to the British Pharmacological Society (Banner & Page, 1994a).

Methods

Sensitization and challenge procedure

Male Dunkin Hartley guinea-pigs (Charles River) (200–250 g) were injected i.p. with ovalbumin (OVA) (0.5 ml per animal; 20 μg OVA in $Al(OH)_3$ (moist gel)); this preparation produced an injectable stable suspension containing excess $Al(OH)_3$. Sham animals were injected with 0.5 ml $Al(OH)_3$ alone. After a period of 18–21 days, animals were exposed to an aerosol of OVA (100 μg ml^{-1}) for 1 h in an exposure chamber. The rate of flow of ovalbumin in the chamber was approximately 10 ml h^{-1}.

Bronchoalveolar lavage

Animals were anaesthetized, 24 h after aerosol exposure, with urethane (25%, w/v, 7 ml kg^{-1}, i.p.) and the trachea cannulated. BAL was performed by instilling 5 ml sterile saline into the lungs via the tracheal cannula and the fluid was immediately removed. The same fluid was then re-injected and the procedure repeated 5 times in total. This procedure resulted in a 40–60% recovery of BAL fluid from the lungs of the guinea-pig. Total cell counts were performed on the resultant BAL fluid using an improved Neubauer haemocytometer. Cytospin preparations were prepared in a Shandon Cytospin 2 centrifuge: 100 μl of BAL fluid was added to each cytospin cup and the samples were centrifuged for 1 min at 1300 r.p.m. Slides were fixed in acetone and stained with haematoxylin and carbol chromotrope according to the method described by Lendrum (1944). Differential cell counts were performed on each slide by counting 200 cells at random, the cell types were classified as neutrophils, eosinophils and mononuclear cells according to standard morphological criteria. Cells were counted 'blind'. The results are expressed as the number of neutrophils, eosinophils and mononuclear cells per ml of BAL fluid. The remaining BAL fluid was centrifuged (10 min, 1000 *g*) and the resultant cells and cell free supernatants were divided into aliquots and frozen for later assays.

Acute dosing protocol

Eighteen to twenty one days after immunization, guinea-pigs were given one i.p. injection of a PDE inhibitor [vinpocetine (PDE I inhibitor), SK&F 94836 (PDE III inhibitor), RO 20-1724 (PDE IV inhibitor), zardaverine (mixed type III/IV inhibitor), zaprinast (PDE V inhibitor), 3-isobutyl-1-methylxanthine (IBMX) and theophylline (non-selective PDE inhibitors)] or the appropriate vehicles 1 h prior to OVA exposure in the chamber. In a similar manner sham-treated animals were given a single i.p. injection of the appropriate vehicle 1 h prior to antigen, OVA, challenge.

All drugs were dissolved in dimethylsulphoxide (DMSO) except IBMX which was dissolved in saline. PDE inhibitors were administered at a dose of 30 mg kg^{-1} except for IBMX which was given at doses of 250 μg kg^{-1}, 500 μg kg^{-1}, 1 mg kg^{-1} and 2 mg kg^{-1} due to the toxicity of this drug at higher doses. Bronchoalveolar lavage was performed 18–24 h later after exposure to OVA according to the method described above. Total and differential cell counts were then performed on BAL fluid.

Multiple dosing protocol

Eleven to fourteen days after immunization animals were injected i.p. twice daily with 3 mg kg^{-1} of one of the PDE inhibitors used in the acute studies or the appropriate vehicle, which was DMSO, for 7 days. This dosing regime approximates to recent clinical studies demonstrating anti-inflammatory activity of theophylline in asthmatics. A group of sham-treated animals were injected with the vehicle, DMSO, in the same manner. On day 7, 1 h after a final injection, all animals were exposed to an aerosol of OVA as described above in an exposure chamber for 1 h. BAL was performed 18–24 h later and total and differential cell counts conducted on the BAL fluid recovered.

Statistical tests

Data are expressed as mean ± s.e.mean. Each group of animals treated with a specific drug was compared with a time-matched control group by unpaired *t* test. For simplicity, control groups have been combined for purposes of data presentation.

Drugs

The following drugs were used: vinpocetine (Gedeon Richter Ltd, Budapest, Hungary), SK&F 94836 (6-[4-(N2-methyl-N3-cyanoguanidino) phenyl]-4,5-dihydro-5-methylpyridazin-3 (2H)-one) (SmithKline Beecham Pharmaceuticals Inc., King of Prussia, PA, U.S.A), RO 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) (Calbiochem), zardaverine (B 842-90) (Forschungslaboratorien Byk Gulden, Germany), zaprinast (M&B 22,948) (Rhone-Poulenc Rorer, Dagenham, Essex), 3-isobutyl-1-methyl xanthine (IBMX), theophylline, chicken egg ovalbumin (Grade V), chromotrope 2R, Erhlichs Haematoxylin, Brilliant blue, urethane (Sigma, Poole, Dorset), aluminium hydroxide gel (BDH Chemicals, Poole, Dorset).

Results

Acute experiments

The results from the acute studies are shown in Table 1. Total and differential cell counts were performed on the BAL fluid recovered from animals post antigen, OVA, challenge. There was no significant difference between the total numbers of cells ml^{-1} found in the BAL fluid of sham-treated and OVA-immunized animals ($35.5 \pm 2.5 \times 10^4$ cells ml^{-1} and $42.3 \pm 2.6 \times 10^4$ cells ml^{-1} respectively). Of the total cells counted the number of neutrophils and mononuclear cells recovered from immunized animals ($1.4 \pm 0.2 \times 10^4$ cells ml^{-1} and $27.3 \pm 2.2 \times 10^4$ cells ml^{-1} respectively) was not significantly different from the numbers of neutrophils and mononuclear cells ($2.4 \pm 0.6 \times 10^4$ cells ml^{-1} and $27.5 \pm 2.2 \times 10^4$ cells ml^{-1} respectively) counted in the BAL fluid from sham-treated animals. However a significant increase in the number of eosinophils ($P < 0.05$) in the BAL fluid from OVA immunized animals ($13.6 \pm 1.4 \times 10^4$ cells ml^{-1}) was seen compared with the number of eosinophils recovered from the BAL fluid from sham-treated animals ($5.6 \pm 0.6 \times 10^4$ cells ml^{-1}).

None of the PDE inhibitors examined had any significant effect on the total numbers of cell ml^{-1} recovered from BAL fluid, post challenge, compared with the appropriate time-matched immunized vehicle-treated group of animals (Table 1). There was also no significant difference in the relative numbers of eosinophils (Figure 1), neutrophils or mononuclear cells obtained from the drug-treated animals compared with the immunized vehicle-treated group.

Chronic experiments

The results from the chronic studies are shown in Table 2. Total and differential cell counts were performed on the BAL fluid recovered from animals post antigen challenge. There was no significant difference in the total number of cells ml^{-1} found in the BAL fluid of sham-treated and OVA-immunized

Table 1 Effect of PDE inhibitors administered 1 h before challenge on inflammatory cell composition in bronchoalveolar lavage fluid

Treatment	n	*Total cells*	*Neutrophils*	*Eosinophils*	*Mononuclear cells*
Sham	28	35.5 ± 2.5	2.4 ± 0.6	5.6 ± 0.6	27.5 ± 2.2
Control	33	42.3 ± 2.6	1.4 ± 0.2	13.6 ± 1.4	27.3 ± 2.2
Vinpocetine	12	48.0 ± 4.3	1.5 ± 0.3	16.0 ± 3.2	30.5 ± 3.2
SK&F 94836	10	28.5 ± 1.8	0.8 ± 0.4	11.4 ± 1.8	16.3 ± 0.9
RO 20-1742	11	53.1 ± 7.9	3.3 ± 1.6	20.4 ± 2.5	29.4 ± 5.1
Zardaverine	9	41.2 ± 6.1	6.7 ± 5.1	12.5 ± 3.3	22.1 ± 2.3
Zaprinast	11	44.5 ± 4.9	2.7 ± 0.7	14.5 ± 3.6	27.3 ± 4.5
Theophylline	13	53.2 ± 5.7	1.8 ± 0.6	11.7 ± 2.6	39.7 ± 3.9
Sham saline	7	33.9 ± 6.7	2.6 ± 1.1	4.0 ± 1.2	27.3 ± 5.6
Control saline	10	39.2 ± 4.1	2.3 ± 0.7	14.3 ± 3.8	22.6 ± 4.5
IBMX (2 mg kg^{-1})	7	19.6 ± 2.5	0.9 ± 0.3	9.0 ± 2.1	9.7 ± 1.0
IBMX (1 mg kg^{-1})	8	42.8 ± 4.3	2.4 ± 1.1	9.9 ± 1.2	30.5 ± 4.5
IBMX (500 μg kg^{-1})	10	44.5 ± 7.9	2.0 ± 0.5	12.8 ± 3.2	29.7 ± 5.6
IBMX (250 μg kg^{-1})	6	57.3 ± 9.4	2.2 ± 0.8	17.3 ± 5.7	37.8 ± 5.6

Values are expressed as 10^4 cells ml^{-1} (mean ± s.e.mean).
Doses of PDE inhibitors are 30 mg kg^{-1} except IBMX which was administered at the doses indicated.

Table 2 Effect of chronic treatment with PDE inhibitors for 7 days on inflammatory cell composition in the airways

Treatment	n	*Total cells*	*Neutrophils*	*Eosinophils*	*Mononuclear cells*
Sham	31	38.4 ± 2.0	2.0 ± 0.5	5.1 ± 0.6	31.3 ± 1.8
Control	33	43.7 ± 3.1	3.4 ± 1.2	10.1 ± 1.4	30.2 ± 2.0
Vinpocetine	9	40.0 ± 4.2	2.1 ± 0.8	10.2 ± 1.5	27.7 ± 3.8
SK&F 94836	11	38.3 ± 5.0	4.3 ± 2.3	8.0 ± 1.6	26.0 ± 3.0
RO-20 1724	9	37.4 ± 4.7	2.3 ± 1.9	*5.5 ± 1.3	29.6 ± 4.0
Zardaverine	12	*23.7 ± 3.0	2.1 ± 0.7	*4.1 ± 1.4	*17.5 ± 3.2
Zaprinast	10	45.3 ± 7.6	2.8 ± 0.8	10.2 ± 3.0	32.3 ± 4.8
Theophylline	13	36.2 ± 5.4	2.5 ± 1.4	6.5 ± 1.5	27.2 ± 5.6
IBMX	12	43.9 ± 4.3	1.5 ± 0.5	9.2 ± 1.9	33.2 ± 4.1

*$P < 0.05$ versus control.
Values are expressed as 10^4 cells ml^{-1} (mean ± s.e.mean). PDE inhibitors were administered at a dose of 3 mg kg^{-1} i.p. twice daily for 7 days.

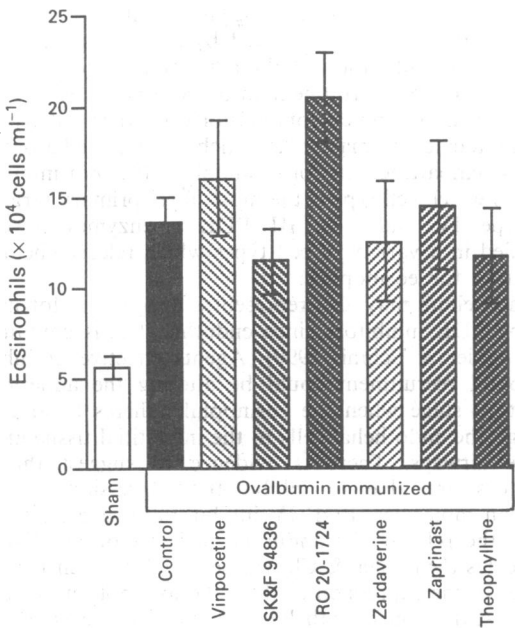

Figure 1 Effect of acute treatment with PDE inhibitors on antigen-induced eosinophil accumulation in the bronchoalveolar lavage (BAL) fluid of immunized guinea-pigs. Values are mean ± s.e.mean. Shading of histograms is designed to correspond with drug specificity of action on PDE isoenzymes. *$P < 0.05$ vs time-matched controls (note that all time matched control groups have been combined for simplicity of graphical presentation).

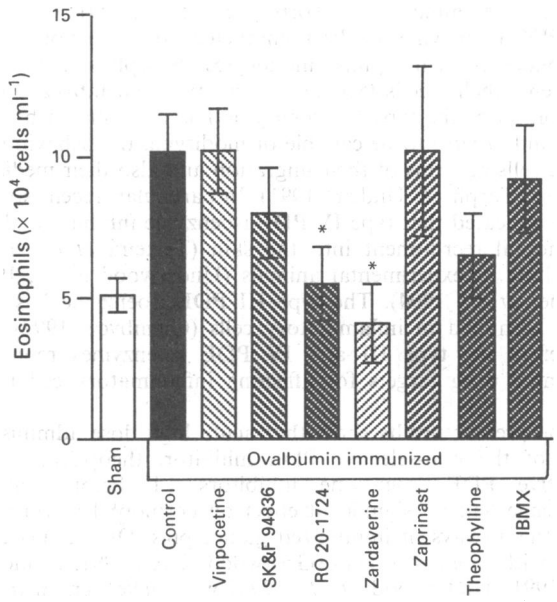

Figure 2 Effect of chronic treatment with PDE inhibitors on antigen-induced eosinophil accumulation in the bronchoalveolar lavage (BAL) fluid of immunized guinea-pig. Values are mean ± s.e.mean. Shading of histograms is designed to correspond with drug specificity of action on PDE isoenzymes. *$P < 0.05$ vs time-matched controls (note that all time matched control groups have been combined for simplicity of graphical presentation).

animals ($38.4 \pm 2.0 \times 10^4$ cells ml^{-1} and $43.7 \pm 3.1 \times 10^4$ cells ml^{-1} respectively). Of the total cells counted the numbers of neutrophils ($3.4 \pm 1.2 \times 10^4$ cells ml^{-1}) and mononuclear cells ($30.2 \pm 2.0 \times 10^4$ cells ml^{-1}) in the BAL fluid from immunized animals post challenge was not significantly different from the numbers of neutrophils ($2.0 \pm 0.5 \times 10^4$ cells ml^{-1}) and mononuclear cells ($31.3 \pm 1.8 \times 10^4$ cells ml^{-1}) recovered from the BAL fluid from sham-treated animals. However, a significant increase ($P<0.05$) in the number of eosinophils in BAL fluid of OVA-sensitized animals ($10.1 \pm 1.4 \times 10^4$ cells ml^{-1}) was seen compared with the number of eosinophils found in the BAL fluid from sham-treated animals ($5.1 \pm 0.6 \times 10^4$ cells ml^{-1}).

In contrast to the results obtained from the acute experiments, twice daily treatment with the type IV PDE inhibitor, RO 20-1724 (3 mg kg^{-1}) for 7 days resulted in a significant reduction in the numbers of eosinophils recovered ($5.5 \pm 1.3 \times 10^4$ cells ml^{-1}) compared with the immunized vehicle-treated group (Figure 2).

Also, in contrast to the effect of zardaverine administered acutely at a dose of 30 mg kg^{-1}, this mixed type III/IV PDE inhibitor when given twice daily for 7 days, at a dose of 3 mg kg^{-1}, produced a significant inhibition in the total number of cells recovered ($23.7 \pm 3.0 \times 10^4$ cells ml^{-1}). This inhibition in cell numbers was reflected in a significant reduction of eosinophils ($4.1 \pm 1.4 \times 10^4$ cells ml^{-1}) (Figure 2) and mononuclear cells ($17.5 \pm 3.2 \times 10^4$ cells ml^{-1}) whilst there was no change in the numbers of neutrophils in the BAL fluid compared with the immunized vehicle-treated animals.

None of the other PDE inhibitors examined had any significant effect on either the total numbers of cells ml^{-1} or on the numbers of neutrophils, eosinophils and mononuclear cells recovered from BAL fluid compared with their time-matched immunized vehicle-treated groups.

Discussion

Elevation of cellular cyclic nucleotide levels via the inhibition of PDE can inhibit both the activation and chemotaxis of a variety of immune and inflammatory cells including lymphocytes, monocytes and eosinophils (Thompson *et al.*, 1976; Averill & Kammer, 1985; Torphy & Undem, 1991). The type IV PDE isoenzyme has been characterized in eosinophils, T-lymphocytes, neutrophils, monocytes, basophils, mast cells and endothelial cells (Souness *et al.*, 1991; Giembycz, 1992). Inhibition of the type IV isoenzyme has been shown both *in vivo* and *in vitro* to be capable of modifying the behaviour of these cells in terms of their migration and also their mediator release (Torphy & Undem, 1991). In particular, recent studies have indicated that type IV PDE isoenzyme inhibitors inhibit eosinophil recruitment into the skin (Teixeira *et al.*, 1994) and lung of experimental animals (Underwood *et al.*, 1993; Turner *et al.*, 1994). The type III PDE isoenzyme has also been identified in inflammatory cells (Giembycz, 1992) and therefore the type III and IV PDE isoenzymes represent potential drug targets for affecting inflammatory cell function.

The present results show that acute high dose administration of the non-selective PDE inhibitor, theophylline and selective PDE isoenzyme inhibitors 1 h before antigen challenge had no significant effect on eosinophil recruitment into the airways of immunized guinea-pigs. Our results contrast with those of others (Gristwood *et al.*, 1991; Schudt *et al.*, 1991; Underwood *et al.*, 1993) who have demonstrated inhibitory effects on eosinophil accumulation by acute administration of PDE inhibitors. However, such differences may be attributable to differences in methodology between these studies and ours. Some of these studies have used higher doses than those used in our study and have also given PDE inhibitors prior to and following antigen challenge. For example the study conducted by Gristwood and co-workers (1991) gave two injections of theophylline (50 mg kg^{-1}) orally, the first 2 h before and the second 6 h after antigen challenge. Different routes of administration have also been employed by other groups and Underwood and co-workers (1993) found that rolipram administered at a dose of 10 mg kg^{-1} i.g. was effective in inhibiting eosinophil numbers in BAL fluid. However, we are not aware of any studies which have used the same immunization schedule as we have and given a single i.p. injection of a PDE IV inhibitor only 1 h before antigen challenge which have been able to demonstrate an inhibitory effect on eosinophil accumulation in the airways.

In contrast to the findings with acute administration of PDE inhibitors, zardaverine and RO 20-1724 when administered at a low dose chronically produced a significant inhibition of eosinophil accumulation in the airways. Zardaverine also reduced the numbers of mononuclear cells present in the airways. However, the type III PDE isoenzyme inhibitor, SK&F 94836, failed to inhibit eosinophil infiltration.

Zardaverine inhibits the type III and IV PDE isoenzymes and RO 20-1724 is a selective inhibitor of the type IV PDE isoenzyme (Nicholson & Shahid, 1994). These results therefore suggest that inhibition of the type IV PDE isoenzyme is most probably required for inhibition of eosinophil accumulation, and that if zardaverine and RO 20-1724 are acting through inhibition of the phosphodiesterase enzyme, cyclic nucleotide levels may need to be elevated chronically to achieve this effect.

If PDE IV inhibition is responsible for the effects of zardaverine and RO 20-1724, then there are several possible cellular targets for this action since a series of events lead to eosinophil accumulation in the airways. Theoretically the drugs could be acting by at least three ways to reduce eosinophil numbers in the airways: (i) by inhibiting eosinopoesis, (ii) by decreasing eosinophil survival or (iii) by redistributing or preventing eosinophils from moving from the blood to the airways. Evidence to support the first mechanism of action is provided by the finding that seven days of treatment with the PDE IV isoenzyme inhibitor, rolipram, decreased the number of circulating eosinophils in parasite-infected mice (DeBrito *et al.*, 1991) by inhibiting the development of eosinophil progenitor cells.

These effects may be achieved by actions on different cell targets: (i) by inhibition of the activation of eosinophils and their subsequent recruitment into the airways (ii) an action on the eosinophil precursors and/or (iii) other cell types such as lymphocytes or mast cells which release mediators which act as chemoattractants for eosinophils. It is not immediately obvious which cell type is the most likely primary target since the type IV and type III PDE isoenzymes have been identified in a variety of cell types which release chemotactic mediators for eosinophils.

Endothelium may also represent a drug target for modulation by PDE inhibitors since endothelial cells contain PDE IV (Lugnier & Schini, 1990). Agents effective at inhibiting eosinophil recruitment could be altering the adhesion and migratory stage when the eosinophil adheres to and moves through the endothelial cells to the interstitial tissue and then to the airways. There is evidence to suggest that PDE inhibitors are able to inhibit the expression of specific adhesion molecules. PDE IV inhibitors have been shown to inhibit the release of tumour necrosis factor (TNF-α) from monocytes (Maschler & Christensen, 1991) which is a potent inducer of the expression of the adhesion molecules, ICAM-1 and ELAM-1, on endothelial cells (Wellicome *et al.*, 1990). Since endothelial cells contain PDE IV (Lugnier & Schini, 1990) it is therefore conceivable that inhibition of the PDE IV enzyme could down regulate the expression of adhesion molecules, thus preventing eosinophil binding to endothelial cells. Finally, it is also possible that PDE inhibitors may be affecting the permeability of endothelial cells. Type III and IV inhibitors have been shown to reduce endothelial cell

permeability *in vitro* (Seiburt *et al.*, 1992; Suttorp *et al.*, 1993) and it has been demonstrated *in vivo* that rolipram can inhibit PAF-induced plasma extravasation in guinea-pig airways (Raeburn & Karlsson, 1992).

Monocytes and T-lymphocytes are a possible primary target. Theophylline and IBMX have been shown to inhibit the generation of potent eosinophil chemoattractants such as PAF and complement C2 from human monocytes (Lappin *et al.*, 1984). The activation and proliferation of T-lymphocytes which are believed to play an important role in eosinophil recruitment into the airways (Corrigan & Kay, 1989) can also be inhibited by mixed type III/IV PDE inhibitors (Robicsek *et al.*, 1991; Schudt *et al.*, 1992) and by type IV PDE inhibitors (Averill & Kammer, 1985; Banner & Page, 1994b).

Mast cells are another possible target. However, it is unlikely that the inhibitory effect of RO 20-1724 and zardaverine is on this cell type in asthma since the PDE IV isoenzyme inhibitor, rolipram, can prevent ovalbumin-induced eosinophil influx even when administered after antigen challenge (Sturm *et al.*, 1990) and hence after mast cell degranulation has already occurred. Furthermore, Turner and co-workers (1994) also found that rolipram had no effect on acute antigen-induced bronchospasm in atopic monkeys.

In conclusion these results suggest that the type IV PDE isoenzyme may be involved in the control of eosinophil accumulation in the airways of immunized guinea-pigs and indicate that a period of chronic treatment with type IV or type III/IV inhibitors is necessary before inhibition is observed.

K.H.B. is a Medical Research Council Scholar.

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