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Phosphodiesterase inhibitors reduce bronchial hyperreactivity and airway inflammation in unrestrained guinea pigs

Ruud E. Santing *, Clemens G. Olymulder, Kees Van der Molen, Herman Meurs, Johan Zaagsma

Groningen / Utrecht Institute for Drug Exploration, Department of Medicinal Chemistry and Molecular Pharmacology, University of Groningen, A. Deusinglaan 2, 9713 AW Groningen, Netherlands

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Abstract

A new guinea pig model of allergic asthma was used to investigate the effects of low doses of the phosphodiesterase inhibitors, rolipram (phosphodiesterase IV selective), ORG 20241 (*N*-hydroxy-4-(3,4-dimethoxyphenyl)-thiazole-2-carboximidamide; dual phosphodiesterase III/IV inhibitor with some selectivity for the phosphodiesterase IV isoenzyme), and of theophylline (non-selective) on allergen-induced early and late phase asthmatic reactions, bronchial hyperreactivity to histamine inhalation, and airway inflammation. Theophylline (25 mg/kg i.p.) and ORG 20241 (5 mg/kg i.p.) did not affect histamine-induced bronchoconstriction, whereas rolipram (75 μg/kg i.p.) only slightly reduced the response to histamine at 7 h after administration. However, bronchial hyperreactivity after the early and after the late reaction was significantly reduced by theophylline, rolipram and ORG 20241, while bronchoalveolar lavage studies revealed a selective inhibition of airway inflammation by the phosphodiesterase inhibitors. Theophylline significantly reduced the number of eosinophils, neutrophils and macrophages; rolipram reduced the number of neutrophils and lymphocytes, and ORG 20241, the number of eosinophils and macrophages. None of the compounds at the dosage indicated reduced the early and late reaction when administered i.p. 1 h before allergen exposure to defined dual responding animals. These results indicate that non-bronchodilator doses of these phosphodiesterase inhibitors markedly reduce the allergen-induced development of bronchial hyperreactivity as well as airway inflammation, presumably by selectively inhibiting cellular migration. The results suggest that an orchestrated series of cellular interactions is involved in the development of bronchial hyperreactivity. It is concluded that phosphodiesterase inhibitors may be very useful in the treatment of bronchial asthma.

Keywords: Phosphodiesterase inhibitor; Bronchial hyperreactivity; Airway inflammation; Early and late asthmatic reaction; (Guinea pig, allergic)

1. Introduction

Cyclic nucleotide phosphodiesterases are a family of enzymes which hydrolyse the 3′-ribose phosphate bond of the naturally occurring second messenger nucleotides, 3′,5′-cyclic adenosine and guanosine monophosphates (cAMP and cGMP), to form the biologically inactive 5′-nucleoside monophosphates. At least five isoenzyme classes have been defined biochemically, each containing two or more subtypes (for review see Torphy and Undem, 1991). Given the apparent multiplicity of phosphodiesterase isoenzymes, it is not surprising that the distribution of these isoenzymes may vary markedly between different cells and tissues, providing a unique therapeutic opportunity for the development of highly family-selective phosphodiesterase inhibitors, which may permit selective manipulation of various pathophysiological processes, such as bronchial asthma.

The cAMP-specific phosphodiesterase IV isoenzyme has been functionally identified as regulating cAMP levels in human T-lymphocytes (Robicsek et al., 1989), guinea pig peritoneal macrophages (Turner and Gueremy, 1991; Giembycz and Dent, 1992), and hu-

* Corresponding author. Tel. 050-633323, fax 050-633311.

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man as well as guinea pig eosinophils and neutrophils (Wright et al., 1990; Dent et al., 1991; Schudt et al., 1991; Souness et al., 1991). Moreover, in vitro studies have established that inhibitors of the cGMP-inhibited phosphodiesterase III as well as phosphodiesterase IV relax effectively pre-contracted airway smooth muscle preparations of guinea pig, bovine and human origin (Harris et al., 1989; Shahid et al., 1991; De Boer et al., 1992).

Therefore, phosphodiesterase IV is presently considered as a major molecular target for novel anti-asthmatic agents (Torphy and Undem, 1991). Due to their cardiovascular side-effects, selective phosphodiesterase III inhibitors are not expected to prove useful in the treatment of bronchial asthma. However, hybrid drugs which combine phosphodiesterase III and IV selectivity in the same molecule may provide optimal anti-inflammatory and bronchodilator activity. Examples of these drugs are benzafentrine (AH 21-132), zardaverine (B 842-90), ORG 30029 and the more recently developed *N*-hydroxy-4-(3,4-dimethoxyphenyl)-thiazole-2-carboximidamide, ORG 20241 (Nicholson et al., 1992).

To investigate the potential importance of the anti-inflammatory effects of phosphodiesterase inhibitors in the treatment of bronchial asthma, we now studied the effectiveness of low, sub-bronchodilator dosages of the selective phosphodiesterase IV inhibitor, rolipram, the dual phosphodiesterase III/IV inhibitor, ORG 20241, and the non-selective phosphodiesterase inhibitor, theophylline, on allergen-induced early and late phase asthmatic reactions, bronchial hyperreactivity, and airway inflammation in a recently described guinea pig model of bronchial asthma (Santing et al., 1992, 1994).

2. Materials and methods

2.1. Animals

Male specific pathogen-free guinea pigs (Charles River SAVO, Kiszlegg, Germany), were sensitized to ovalbumin at 3 weeks of age as described previously (Santing et al., 1992). Briefly, 0.5 ml of an allergen solution containing 100 μg ovalbumin and 100 mg/ml $Al(OH)_3$ was injected i.p., while another 0.5 ml was divided over seven s.c. injection sites in the proximity of lymph nodes in the paws, lumbar regions and neck (Van Amsterdam et al., 1991). The animals were operated in week 3 following sensitization and were used experimentally in weeks 4–8. The animals were housed in individual cages in climate controlled animal quarters and were given water and food ad libitum.

All protocols described in this study were approved by the University of Groningen Animal Health Committee.

2.2. Measurement of airway function

Airway function was assessed by pleural pressure (P_{pl}) measurement as described previously (Santing et al., 1992). In brief, a small latex balloon (HSE, Freiburg, Germany), connected to a saline-filled cannula, was surgically implanted inside the thoracic cavity. The cannula was driven subcutaneously to and permanently attached in the neck of the animal. After connection via an external fluid-filled cannula to a pressure transducer (Gould P23ID, Gould Medical, Bilthoven, Netherlands), P_{pl} was continuously measured using an on-line computer system.

Using a combination of flow measurement with a pneumotachograph implanted inside the trachea, and pressure measurement with the pleural balloon, we had previously shown that changes in P_{pl} are linearly related to changes in airway resistance and hence can be used as a sensitive index for both allergic and non-allergic bronchoconstriction (Santing et al., 1992). Baseline P_{pl} measurements remained stable and no signs of inflammation were observed at the sites of surgery for several weeks. Using this model, airway function can be repeatedly and continuously measured for periods of at least 24 h, while the animals are unaware that measurements are being taken.

2.3. Provocation procedures

Ovalbumin and histamine provocations were performed by inhalation of aerosolized solutions. These provocations were carried out in a specially designed 9-l animal cage, in which the guinea-pigs could move freely (Santing et al., 1992). A DeVilbiss nebulizer (type 646, DeVilbiss, Somerset, PA, USA) driven by an airflow of 8 l/min provided the aerosol required with an output of 0.43 ml/min.

The animals were habituated to the experimental conditions and the provocation procedures during several training sessions. Each allergen and histamine provocation was preceded by an additional adaptation period of at least 30 min, which was followed by two saline challenges lasting 3 min each, separated by 10-min intervals. A baseline P_{pl} value was calculated by averaging the P_{pl} values from the last 20 min of the adaptation period.

Histamine provocations were performed starting with a 25 μg/ml solution in saline, followed by increasing dose steps of 25 μg/ml. Exactly as with the saline challenges, each provocation lasted 3 min and was separated by 10-min intervals. The animals were challenged until the P_{pl} increased by more than 100% for at least 3 consecutive min. Depending on the reactivity of the animal, three or more doses of histamine were applied. The provocation concentration causing a 100%

increase in P_{pl} (PC_{100}) was calculated by linear intrapolation.

Allergen provocations were performed by inhalation of increasing concentrations of 1.0, 3.0 and 5.0 mg/ml ovalbumin in saline for 3 min, separated by 10-min intervals. Allergen inhalations were discontinued when an increase in P_{pl} of more than 100% was observed. Under these conditions none of the animals developed anaphylactic shock after allergen provocation.

2.4. Experimental protocol

The phosphodiesterase inhibitors used in this study were dissolved in 10% dimethylsulfoxide in saline, this vehicle being used as control. Theophylline was studied in a dose of 25 mg/kg, rolipram of 75 μg/kg, and ORG 20241 of 5 mg/kg body weight. All drugs were administered i.p.

The effect of the phosphodiesterase inhibitors on histamine-induced bronchoconstriction was evaluated by measuring the PC_{100} values at 24 h before, and at 1, 7 and 25 h after administration. These time points were chosen to correspond to the allergen provocation protocol.

Allergen provocations were performed 24 h after a PC_{100} measurement with histamine. At 6 h after allergen provocation (between the early and late asthmatic response) and at 24 h (after the late response), the PC_{100} values for histamine were re-assessed to calculate changes in airway reactivity at these time points as ratios of PC_{100} pre/post allergen provocation. Airway functions were measured continuously during the whole procedure for the quantitative assessment of early and late phase asthmatic responses. Between the measurements of PC_{100} values at 6 h and 24 h, the animals were placed in a larger cage, where they could move around freely and eat and drink ad libitum. During this transfer, the animals remained connected to the measurement system.

The effect of treatment with vehicle or phosphodiesterase inhibitors on the allergic obstructive responses and the development of bronchial hyperreactivity was investigated 7 days later in the same animals. The second allergen provocation was identical to the first exposure with respect to allergen dose and was performed 1 h after treatment with vehicle or phosphodiesterase inhibitor. Only dual responding animals entered this part of the study, because it was found that bronchial reactivity was significantly increased in dual responders only (Santing et al., 1994).

2.5. Bronchoalveolar lavage procedure

At 24 h after the second allergen provocation, the animals were anaesthetized with 20 mg/ml Brietal-sodium, 35 mg/kg ketamine hydrochloride and 6 mg/kg Rompun i.p. The lungs were gently lavaged via a tracheal cannula using 5 ml of sterile saline at 37°C, followed by three subsequent aliquots of 8 ml of saline. The recovered lavage samples were cooled on ice, and centrifuged at $200 \times g$ for 10 min at 4°C. The pellets were combined and resuspended to a final volume of 1.0 ml in RPMI-1640 medium. Total cell numbers were counted in a Bürker-Türk chamber. For cytological examination, cytospinpreparations were stained with May-Grünwald and Giemsa. Cell differentiation was performed by counting at least 400 cells in duplicate.

2.6. Chemicals

Histamine hydrochloride, ovalbumin (grade III), aluminum hydroxide, theophylline and May-Grünwald/Giemsa stain were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and rolipram from Schering (Berlin, FRG). ORG 20241 (*N*-hydroxy-4-(3,4-dimethoxyphenyl)-thiazole-2-carboximidamide) was supplied by Organon Laboratories (Newhouse, Scotland).

Brietal-sodium (methohexital) was purchased from Eli Lilly (Amsterdam, Netherlands), ketamine hydrochloride from Parke-Davis (Barcelona, Spain), Rompun (2-(2,6-xylidino)-5,6-dihydro-4*H*-1,3-thiazine-hydrochloride, methylparaben) from Bayer (Leverkusen, Germany) and RPMI-1640 medium from Gibco Life Technologies (Paisley, Scotland).

2.7. Data analysis

The magnitudes of the allergen-induced early and late asthmatic responses were expressed as the area under the P_{pl} time-response curve (AUC) between 0 and 6 h after allergen provocation for the early asthmatic reaction, and between 8 and 24 h after provocation for the late asthmatic reaction. P_{pl} was expressed as percentage change from baseline and AUC was determined by trapezoid integration over discrete (5 min) time periods. Based on AUC values from saline control inhalations, threshold values (mean $\pm 2 \times$ standard deviation; 99% confidence interval) of 1185%*5 min for a positive early asthmatic reaction and 2790%*5 min for a positive late asthmatic reaction were defined (Santing et al., 1994). Using these criteria, animals were characterized as single early responders or dual responders (i.e. animals expressing an early asthmatic reaction as well as a late asthmatic reaction).

Changes in allergen-induced obstructive reactions as well as in bronchial reactivity were analyzed using Student's *t*-test for paired observations. Protection against histamine-induced bronchoconstriction due to treatment as well as cellular contents of bronchoalveolar lavage were analyzed using analysis of variance

Table 1
Effect of phosphodiesterase inhibitors on bronchial reactivity to histamine inhalation

Treatment [a]	PC_{100} histamine (μg/ml)			
	24 h before administration	1 h after administration	7 h after administration	25 h after administration
Vehicle	127.8 ± 9.7	120.3 ± 16.0	142.2 ± 18.2	131.9 ± 13.3
Theophylline	103.6 ± 11.5	108.9 ± 12.4	110.7 ± 11.7	108.9 ± 11.8
Rolipram	128.6 ± 15.4	154.7 ± 26.1	165.6 ± 18.3 [b]	146.4 ± 13.5
ORG 20241	119.9 ± 9.7	113.2 ± 9.4	112.3 ± 10.0	113.8 ± 10.7

[a] Administration i.p. in 10% DMSO in saline; rolipram: 75 μg/kg, ORG 20241: 5 mg/kg, theophylline: 25 mg/kg. Data represent mean values ± S.E.M. for five to seven animals. Statistical analysis: ANOVA, coupled with Dunnett's t-test: [b] $P < 0.05$, compared with PC_{100} 24 h before administration.

(ANOVA), coupled with Dunnett's t-test. Differences were considered to be statistically significant when $P < 0.05$. All data are presented as means ± S.E.M.

3. Results

3.1. Effect on bronchial reactivity to histamine

To determine the protective effect of the phosphodiesterase inhibitors on histamine-induced bronchoconstriction, the reactivity (PC_{100}) to histamine inhalation was measured 24 h before administration of phosphodiesterase inhibitors, as well as at 1 h, 7 h and 25 h thereafter (Table 1).

Administration of the vehicle did not significantly affect subsequent PC_{100} measurements. Treatment with theophylline (25 mg/kg i.p.) and ORG 20241 (5 mg/kg i.p.) did not change bronchial reactivity to histamine either. With rolipram (75 μg/kg), only a small reduction in bronchial reactivity to histamine inhalation was observed, which reached significance at 7 h after application.

3.2. Effect on allergen-induced asthmatic responses

Allergen provocation in this model induces a pronounced bronchoconstriction that could be clearly divided into an early (0–6 h) and a late component (8–24 h). Early asthmatic reactions evoked by the first allergen challenge were comparable between the groups of animals, although the 'rolipram' group had a somewhat greater initial early response (Table 2). This difference was, however, not statistically significant.

After the second allergen provocation, which was preceded by vehicle or phosphodiesterase inhibitor treatment, the magnitude of the early reaction was comparable to that of the first early reaction in all groups.

No statistically significant differences were observed in the severity of the late asthmatic reaction after the first and second, vehicle- or drug-treated, allergen provocation. Also, the magnitude of the late asthmatic reaction was comparable between all groups (Table 2).

Table 2
Effect of phosphodiesterase inhibitors on allergen-induced early and late phase reactions

Treatment [a]	AUC (% * 5 min)			
	Early asthmatic reaction		Late asthmatic reaction	
	Before treatment	After treatment	Before treatment	After treatment
Vehicle	2841 ± 615	2017 ± 640	6900 ± 1384	5455 ± 658
Theophylline	2180 ± 452	3737 ± 1039	9703 ± 3187	8699 ± 2931
Rolipram	4326 ± 484	3105 ± 886	6193 ± 933	4524 ± 895
ORG 20241	2338 ± 561	3271 ± 895	5541 ± 1008	4841 ± 1170

[a] Administration i.p. in 10% DMSO in saline 1 h before the second allergen provocation; rolipram: 75 μg/kg, ORG 20241: 5 mg/kg, theophylline: 25 mg/kg. Data are presented as area under the % change in P_{pl} time-response curve between 0 hand 6 h after allergen provocation for the early asthmatic reaction and between 8 h and 24 h after allergen provocation for the late asthmatic reaction. Data represent mean values ± S.E.M. for five to seven animals. Statistical analysis: Student's t-test for paired data (before/after treatment) showed no significant differences.

3.3. Effect on allergen-induced bronchial hyperreactivity

Allergen exposure to dual responding guinea pigs induced a considerable increase in bronchial reactivity to histamine aerosol, as determined after resolution of the early (6 h) as well as the late asthmatic response (24 h) (Fig. 1).

Bronchial reactivity was significantly enhanced at 6 h after the first allergen provocation in all groups. No significant differences were observed between these groups. At 6 h after the second allergen provocation a significant bronchial hyperreactivity was observed only in the control group ($P < 0.01$), which was very similar to that observed after the first allergen provocation. In the remaining three groups, treated with phosphodiesterase inhibitors, bronchial hyperreactivity at 6 h after allergen provocation was significantly reduced by theophylline ($P < 0.05$) and ORG 20241 ($P < 0.05$),

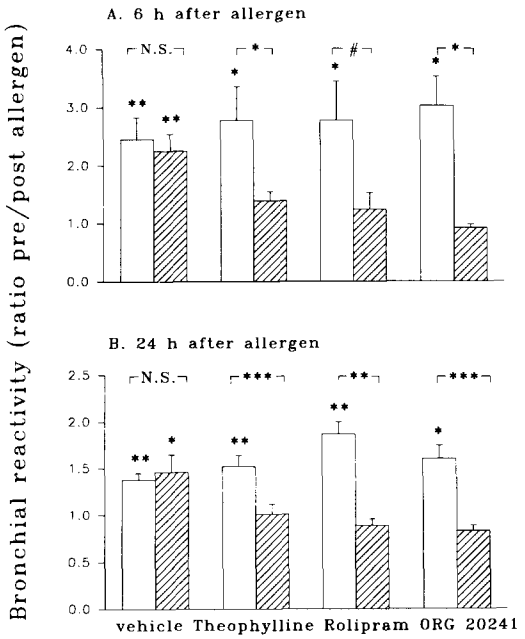

Fig. 1. Effects of theophylline (25 mg/kg i.p.), rolipram (75 μg/kg i.p.) and ORG 20241 (5 mg/kg i.p.) on allergen-induced bronchial hyperreactivity to histamine inhalation at 6 h (upper panel) and 24 h (lower panel) after allergen exposure. The open bars represent the ratio of PC_{100} before/after allergen provocation after the first allergen provocation (untreated), and the hatched bars that after the second allergen provocation (treated). PC_{100} values at 24 h before the first allergen provocation were 188.8 ± 36.9 (vehicle group), 104.5 ± 7.9 (theophylline group), 151.5 ± 23.2 (rolipram group), and 228.0 ± 69.3 (ORG 20241 group). At 24 h before the second allergen provocation, these values were 194.3 ± 40.7, 113.1 ± 7.8, 142.6 ± 22.5 and 216.8 ± 56.8, respectively (N.S.). Statistical analysis: paired Student's *t*-test compared to pre-challenge PC_{100}, and to first allergen provocation, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P = 0.06$, N.S.: not significant.

while the reduction by rolipram just did not reach significance ($P = 0.06$) (Fig. 1).

Bronchial hyperreactivity at 24 h after the first and second allergen provocation was very similar in the vehicle-treated group. However, in the remaining three groups, treated with phosphodiesterase inhibitors, allergen-induced development of bronchial hyperreactivity after the late reaction was completely prevented.

In all treatment groups, the baseline P_{pl} before assessment of histamine reactivity at 6 h and 24 h after allergen provocation was not statistically significantly different from the baseline values assessed at 24 h and 1 h before each allergen provocation, indicating that the bronchial hyperreactivity at these time points was not influenced by (effects on) bronchial tone.

3.4. Effect on allergen-induced inflammation

Bronchoalveolar lavage was performed at 24 h after the second allergen provocation (Table 3). In all groups the recovery of the lavage fluid was high, with an overall average of 85.6 ± 0.5% ($n = 25$). The viability of the recovered cells averaged 96.2 ± 0.4%.

Theophylline treatment significantly reduced the number of eosinophils ($P < 0.001$), neutrophils ($P < 0.05$) and macrophages ($P < 0.01$) in the bronchoalveolar lavage fluid, as well as the total number of cells ($P < 0.001$) (Table 3). Rolipram reduced the number of neutrophils ($P < 0.05$) and lymphocytes ($P < 0.05$), while the total number of cells was not significantly reduced ($P = 0.09$). Finally, ORG 20241 reduced the number of eosinophils ($P < 0.001$) and macrophages ($P < 0.05$) as well as the total number of cells ($P < 0.001$).

4. Discussion

In this study it was found that sub-bronchodilator dosages of phosphodiesterase inhibitors that did not reduce basal P_{pl}, histamine-induced bronchoconstriction and allergen-induced early and late phase reactions, markedly inhibited the subsequent development of bronchial hyperreactivity to histamine inhalation. This inhibition was accompanied by a selective reduction in the number of inflammatory cells infiltrated into the airway lumen.

Rolipram was previously shown to be effective against ozone-induced bronchial hyperreactivity to inhaled histamine in guinea pigs at a concentration of 100 μg/kg i.p. (Holbrook and Hughes, 1992). To investigate the effects of rolipram on allergen-induced bronchial hyperreactivity, we used a concentration of

Table 3
Total and differential leukocyte counts in bronchoalveolar lavage 24 h after the second allergen provocation

Treatment [a]	Total cells ($\times 10^6$)	Eosinophils ($\times 10^6$)	Neutrophils ($\times 10^6$)	Lymphocytes ($\times 10^6$)	Macrophages ($\times 10^6$)
Vehicle	32.30 ± 2.87	11.67 ± 0.61	1.64 ± 0.54	0.42 ± 0.05	18.40 ± 2.35
Theophylline	14.17 ± 1.18 [d]	4.20 ± 0.72 [d]	0.22 ± 0.04 [b]	0.28 ± 0.09	9.40 ± 0.98 [c]
Rolipram	24.92 ± 2.63	8.82 ± 1.62	0.10 ± 0.03 [b]	0.13 ± 0.05 [b]	15.63 ± 1.48
ORG 20241	16.28 ± 2.32 [d]	3.63 ± 0.60 [d]	0.79 ± 0.40	0.34 ± 0.12	11.50 ± 1.64 [b]

[a] Administration i.p. in 10% DMSO in saline 1 h before second allergen provocation; rolipram: 75 μg/kg, ORG 20241: 5 mg/kg, theophylline: 25 mg/kg. Data represent mean values ± S.E.M. for five to seven animals. Statistical analysis: ANOVA, coupled with Dunnett's *t*-test: [b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$, compared with vehicle.

75 μg/kg i.p., since this concentration had virtually no effect on histamine-induced bronchoconstriction 1 h after injection. Based on observations that the bronchodilating potency ratio for rolipram and theophylline ranges from 130 to 875 (Harris et al., 1989; Cortijo et al., 1993), we used theophylline at a dose of 25 mg/kg, which was also without effect on histamine-induced bronchoconstriction. The dose of ORG 20241 (5 mg/kg i.p., 60-fold higher than rolipram) was chosen because, for bovine tracheal smooth muscle, the potency of ORG 20241 to relax histamine- and methacholine-induced contraction was about 10–50 times lower than that of rolipram (Shahid et al., 1991; Zeilstra and Zaagsma, unpublished observations). This dose of ORG 20241 also did not affect histamine-induced bronchoconstriction.

Except for a small bronchodilator effect of rolipram on histamine-induced bronchoconstriction at 7 h after application, no substantial bronchodilator effects were observed that could account for the reduced histamine PC_{100} after allergen provocation. Consistent with the absence of direct bronchodilator activity against histamine-induced bronchoconstriction, treatment with the phosphodiesterase inhibitors did not affect the magnitude of allergen-induced early and late phase reactions. A recent study by Underwood and colleagues (1993), showed a dose-dependent inhibition of the allergen-induced early asthmatic reaction after intragastric application of rolipram to conscious guinea pigs. However, the lowest dose in their study (1 mg/kg i.g.), which only slightly inhibited the early reaction, was still 40 times higher than the intraperitoneal dose used in the present study.

Remarkably, the allergen-induced development of bronchial hyperreactivity was completely prevented by administration of all three phosphodiesterase inhibitors. Moreover, the allergen-induced influx of inflammatory cells into the airways was also inhibited by the non-bronchodilating concentrations of these compounds, indicating that the anti-inflammatory effects of the phosphodiesterase inhibitors may be important for the treatment of bronchial asthma.

Recently it has been shown that guinea pig peritoneal eosinophils express predominantly a membrane-bound phosphodiesterase IV (Dent et al., 1991; Souness et al., 1991). Functional studies in isolated intact eosinophils have shown that inhibitors of the phosphodiesterase IV isoenzyme, such as rolipram and denbufylline, but also ORG 20241, increase the cAMP content in the cell and markedly reduce respiratory burst activity (Dent et al., 1991; Souness et al., 1991; Nicholson et al., 1992). As in eosinophils, the predominant phosphodiesterase isoenzyme in human neutrophils is a membrane-associated phosphodiesterase IV (Wright et al., 1990). Interestingly, the kinetics of cAMP hydrolysis catalyzed by phosphodiesterase in guinea pig eosinophils and neutrophils are non-linear, suggesting that these cells may contain more than one phosphodiesterase IV (Giembycz and Dent, 1992).

Recent evidence emphasizes the importance of $CD4^+$ T-lymphocytes in allergic asthma. A functional subset of $CD4^+$ cells, Th_2-lymphocytes, produce a number of cytokines, including granulocyte-macrophage colony-stimulating factor, interleukin-3, tumor necrosis factor-α, which promote IgE production (Ricci et al., 1993) and bronchial mucosal eosinophilic inflammation (Gulbenkian et al., 1992). Recently, Kings et al. (1990) reported that eosinophil accumulation into guinea pig airways induced by subcutaneous and intraperitoneal injection of human recombinant granulocyte-macrophage colony-stimulating factor and interleukin-3, as well as mouse tumor necrosis factor-α was effectively prevented by prior administration of the phosphodiesterase III/IV inhibitor, benzafentrine. This is of particular interest since it implies that selective phosphodiesterase inhibitors are able to block the deleterious actions of both acute and chronic inflammatory mediators (Giembycz, 1992). Indeed, the phosphodiesterase isoenzyme population of human T-lymphocytes has been shown to consist of both phosphodiesterase III and IV isoenzymes (Robicsek et al., 1989).

Another important site of action for anti-inflammatory drugs is at the vascular endothelium where pro-inflammatory cells and plasma proteins leave the circulation. When fluorescene isothiocyanate-labelled dextran was used as a plasma protein marker, allergen-induced plasma extravasation in sensitized guinea pigs was inhibited by phosphodiesterase IV, but not phosphodiesterase I and III inhibitors (Raeburn et al., 1992). However, Suttorp et al. (1991) had shown earlier that the phosphodiesterase III inhibitor, motapizone, inhibited H_2O_2-induced increases in endothelial permeability, indicating that both phosphodiesterase III and IV may be active at this site.

The selective inhibition of inflammatory cell infiltration by the various phosphodiesterase inhibitors as found in this study may also point to an inhibitory effect on selective inducible adhesion molecules on the luminal surface of vascular endothelial cells. This is important since adherence is an obligatory step in the migration of leucocytes from the microvenules to the airways. To date, however, no conclusive evidence is available for an interaction between phosphodiesterase inhibitors and expression of endothelial cell adhesion molecules.

Eosinophilic infiltration of the airways is considered of primary importance for the development of allergen-induced bronchial hyperreactivity (Venge, 1990). The prevention of allergen-induced eosinophilic inflammation by theophylline and ORG 20241, as observed in this study, may thus underlie the reduction in

the development of bronchial hyperreactivity. Surprisingly, in the dosage applied, rolipram did not significantly inhibit eosinophilic infiltration into the airways. This observation is in contrast with reports that a reduction in the number of eosinophils was observed after treatment with rolipram, even when rolipram was administered after allergen provocation (Sturm et al., 1990). In atopic monkeys, rolipram (10 mg/kg, s.c.) inhibited allergen-induced increases in bronchoalveolar lavage fluid and neutrophils after both acute and chronic allergen exposure as well as the development of bronchial hyperreactivity after chronic exposure (Turner et al., 1994). Inhibition of bronchial hyperreactivity 24 h after acute allergen provocation by pretreatment with rolipram was recently reported for ovalbumin-sensitized guinea pigs (Howell et al., 1993). The dose of rolipram used (10 mg/kg, p.o.), caused inhibition of the allergen-induced early obstructive reaction as well as of histamine-induced bronchoconstriction, but showed no residual bronchodilator effect at the time of airway reactivity measurement, 24 h after the allergen provocation. Moreover, at 3 mg/kg i.g. (but not 1 mg/kg), rolipram significantly inhibited the eosinophil influx in the airway lumen of conscious, ovalbumin-challenged guinea pigs (Underwood et al., 1993). In the present study, however, lower doses of phosphodiesterase inhibitors were used, which did not substantially affect histamine-induced bronchoconstriction and allergen-induced early and late phase reactions. Nevertheless, rolipram potently reduced the increase in the number of neutrophils and lymphocytes in the bronchoalveolar fluid after allergen exposure. It is still unknown whether this reduction is directly responsible for the attenuation of bronchial hyperreactivity or acts indirectly, e.g. via modulation of (the activation state of) the eosinophils. This important question remains to be resolved.

In conclusion, this study has shown that low, subbronchodilating doses of the phosphodiesterase inhibitors theophylline (non-selective), rolipram (phosphodiesterase IV-selective) and ORG 20241 (dual phosphodiesterase IV/III inhibitor), which did not reduce histamine-induced bronchoconstriction and allergen-induced early and late phase reactions, were effective to preventing the allergen-induced development of bronchial hyperreactivity to histamine inhalation.

The inhibition of bronchial hyperreactivity was associated with a selective reduction in airway inflammation. The mechanism by which inflammatory cells induce bronchial hyperreactivity is still unclear, but probably involves an orchestrated complex series of cellular interactions. The anti-inflammatory effects of the phosphodiesterase inhibitors at non-bronchodilating concentrations suggest that these compounds may be of significant therapeutic value in the treatment of bronchial asthma.

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