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Effects of theophylline and rolipram on antigen-induced airway responses in neonatally immunized rabbits

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1 The effects of the xanthine, theophylline, a non-selective phosphodiesterase (PDE) inhibitor, and the phosphodiesterase type 4 (PDE 4) inhibitor, rolipram, were evaluated in a model of antigen-induced airway responses in the allergic rabbit.

2 Adult litter-matched NZW rabbits (2.5–3.9 kg), immunized within 24 h of birth with *Alternaria tenuis* antigen, were pretreated twice daily for 3 days with theophylline (3 mg kg^{-1}, i.p.) or rolipram (1 mg kg^{-1}, i.p.) prior to antigen challenge (*Alternaria tenuis*). For each drug-treated group, a parallel group of rabbits were pretreated with the appropriate vehicle. In all groups airway responsiveness to inhaled histamine and bronchoalveolar lavage (BAL) was performed 24 h before and after antigen-challenge.

3 Basal lung function in terms of resistance (R_L, cmH_2O l^{-1} s^{-1}) and dynamic compliance (C_{dyn}, ml cmH_2O^{-1}) were unaltered by pretreatment with theophylline or rolipram compared to their respective vehicles 24 h prior to or post antigen challenge.

4 The acute bronchoconstriction induced by inhaled *Alternaria tenuis* aerosol was unaffected by pretreatment with theophylline or rolipram.

5 Airway hyperresponsiveness to inhaled histamine was indicated by reduced R_L PC_{50} (2.4–3.5 fold) and C_{dyn} PC_{35} (2.5–2.6 fold) values 24 h after antigen challenge. Treatment with rolipram, but not theophylline, prevented the increase in responsiveness to inhaled histamine 24 h after antigen challenge.

6 Total cells per ml of BAL fluid increased 24 h after antigen challenge due to the recruitment of neutrophils and eosinophils. Antigen-induced increases in pulmonary neutrophils were unaffected; however, eosinophils were reduced 57.5% in theophylline and 82% in rolipram-treated rabbits.

7 Inhalation of *Alternaria tenuis* aerosol elicits an acute bronchoconstriction, followed 24 h later by an increased responsiveness to inhaled histamine and pulmonary neutrophil and eosinophil recruitment in the immunized rabbit. With the dosing regimes used, both rolipram and theophylline inhibited eosinophil recruitment, whilst only rolipram prevented the development of airway hyperresponsiveness. Neither agent inhibited the acute bronchoconstriction due to inhaled antigen.

Keywords: Xanthine; phosphodiesterase type-4 inhibitor; inflammation; airways; hyperresponsiveness; neonatally immunized rabbit

Introduction

Asthma is characterized by reversible airway obstruction, inflammation and airway hyperresponsiveness. Pathological features of asthma include microvascular leakage, mucus hypersecretion and epithelial shedding (Barnes, 1989). Such findings are thought to be the result of repeated mediator release from resident and recruited inflammatory cells, principally eosinophils and T-lymphocytes (Holgate *et al.*, 1987).

Theophylline has been used in the treatment of asthma for over 40 years and has been considered primarily as a bronchodilator (Weinburger *et al.*, 1980). However, recent clinical studies have revealed that theophylline may possess anti-inflammatory (Pauwells, 1987; Sullivan *et al.*, 1994) and immunomodulatory activity (Ward *et al.*, 1993; Jaffer *et al.*, 1994; Kidney *et al.*, 1993).

The cellular activities of theophylline remain unclear but include non-selective inhibition of cyclic nucleotide phosphodiesterase (PDE) (Beavo & Reifsnyder, 1990) and adenosine receptor antagonism (Fredholm *et al.*, 1980).

Recent work has suggested that the adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-specific PDE 4 isoenzyme is prevalent in many inflammatory cells pertinent to asthma including eosinophils, lymphocytes, mast cells, neutrophils, basophils and monocytes. Inhibition of PDE 4 with the subsequent elevation of cyclic AMP has been shown to inhibit the action of a range of inflammatory cells (Torphy & Undem, 1991; Giembycz & Dent, 1992) and it is plausible that theophylline may exert its effects through inhibition of PDE 4. Therefore a range of selective PDE 4 inhibitors have been developed which may share the efficacy of theophylline but have an improved side effect profile.

Neonatal immunization of rabbits with subsequent repeated exposure to allergen over the first three months of life leads to allergic adult rabbits (Larsen *et al.*, 1987; Minshall *et al.*, 1993). Inhaled antigen in adult rabbits can elicit a similar sequence of events as seen in atopic individuals, including, an acute bronchospasm and late phase obstruction (Larsen *et al.*, 1987), increased airway responsiveness (Bloom *et al.*, 1988; Herd *et al.*, 1994) and pulmonary eosinophil recruitment (Marsh *et al.*, 1985; Herd *et al.*, 1994).

In the present study, we have investigated the effects of the non selective PDE inhibitor, theophylline and the PDE 4 inhibitor, rolipram, on antigen-induced acute bronchspasm, hyperresponsiveness to inhaled histamine and pulmonary inflammatory cell recruitment in adult allergic rabbits. Part of this work has been presented to the British Pharmacological Society (Gozzard *et al.*, 1995).

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Methods

Immunization

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hampshire) of either sex were used throughout the study. The neonatal immunization of rabbits has been described previously (Minshall *et al.*, 1993). Rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract (40,000 PNU ml^{-1}) in aluminium hydroxide ($Al(OH)_3$) moist gel adjuvant and saline in the ratio of 2:1:1 v/v. Antigen and adjuvant administration was repeated weekly for the first month of life and then biweekly for the following two months. The methods described in this study were subject to Home Office approval and performed under the Animals (Scientific Procedures) Act, 1986.

Experimental protocol

At three months of age, adult immunized rabbits (2.5–3.9 kg) were treated with either theophylline, (3 mg kg^{-1}) or vehicle (5% dimethyl sulphoxide [DMSO] in saline). In a separate study, 2 groups of 9 rabbits were treated with rolipram (1 mg kg^{-1}) or vehicle (20% ethanol in saline). Both drugs and their respective vehicles were given twice daily (09 h 00 min and 17 h 00 min) intraperitoneally (2 ml kg^{-1}) on days 1–3 and 1 h prior to aerosolized antigen challenge on day 4.

On Day 3, dose-response curves to inhaled histamine and bronchoalveolar lavage were performed. On Day 4, rabbits were given a final dose of drug or vehicle 1 h prior to antigen challenge with inhaled *Alternaria tenuis* extract. On Day 5, dose-response curves to inhaled histamine and bronchoalveolar lavage were performed as on day 3. Additionally, two groups of 6 rabbits were treated with either the theophylline vehicle or rolipram vehicle and were subjected to the above protocol but challenged with saline in place of *Alternaria tenuis* extract on day 4.

Measurement of lung function

Immunized rabbits were pre-medicated with diazepam (2.5 mg kg^{-1}, i.p.) followed by Hypnorm (0.4 ml kg^{-1}, i.m.). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of Hypnorm (0.2 ml kg^{-1}, i.m.) every 15–30 min (Flecknall, 1987). Spontaneously breathing rabbits were intubated with a cuffed endotracheal tube (3.0 mm internal diameter; Mallinckrodt Laboratories, Athlone, Ireland), connected to a thermoregulated (37°C) Fleisch pneumotachograph (size 00) to allow measurement of tidal air flow. An oesopharyngeal balloon catheter was inserted to provide a measure of intra-pleural pressure and transpulmonary pressure (the difference between thoracic and pleural pressure). Measurements were made as previously described (Minshall *et al.*, 1993). Total lung resistance (R_L, $cmH_2O\ l^{-1}\ s^{-1}$) and dynamic compliance (C_{dyn}, ml cmH_2O^{-1}) values were calculated by an on-line respiratory analyser (Pulmonary Monitoring System, version 5.1 Mumed Ltd., London) according to the method of Von Neergaard & Wirtz (1927).

Airway responsiveness

Airway responsiveness was determined by performing a cumulative dose-response curve (1.25–80 mg ml^{-1}) to inhaled aerosolized histamine (aerosols generated with a Devilbiss ultrasonic nebuliser, particle size 0.5–5 μM). Following each 2 min aerosol of histamine, 10 breaths were recorded and the mean values of total lung resistance (R_L) and dynamic complicance (C_{dyn}) were calculated.

Antigen challenge

Antigen challenge was performed with rabbits maintained under neuroleptanalgesia and instrumented for lung function measurement. Antigen challenge with inhaled aerosolised *Alternaria tenuis* extract in 0.9% saline (20,000 PNU ml^{-1}, De Vilbiss ultrasonic nebuliser), consisted of a 4 min aerosol of saline followed by two 2 min antigen aerosols, then 4 consecutive antigen aerosols of 4 min duration (total 20 min with 2 min dose cycle). After each challenge, respiratory function was recorded as described above. A further 2 recordings at 15 and 30 min post antigen were also recorded.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately following histamine dose-response curves. Saline (5 ml) was injected into the lungs through a polyethylene catheter (positioned at the carina through the endotracheal tube) and then immediately aspirated and collected on ice. Total cell counts were enumerated under light microscopy with an improved Neubauer haemocytometer. For differential cell counts cytospin preparations were made. Aliquots of BAL fluid (75 μl) were centrifuged at 1300 r.p.m. for 1 min in a Shandon Cytospin 2 (Shandon Southern Instruments, Sewickley, PA, U.S.A.) at room temperature. Cells were then stained with a combination of haemotoxylin and chromotrope 2R according to the method of Lendrum (1944).

Drugs and chemicals

All reagents were of analytical grade. Drugs and chemicals used were: rolipram, (Celltech Therapeutics Ltd); *Alternaria tenuis* extract (40,000 PNU ml^{-1}, $\cong$ 1 mg ml^{-1}; Greer Laboratories Inc. Lenoir, NC, U.S.A.); aluminium hydroxide ($Al(OH)_3$) moist gel (FSA Laboratory Supplies, Loughborough, Leicestershire); theophylline, Chromotrope 2R, histamine diphosphate, ethanol, (Sigma Chemical Co., Poole, Dorset); haematoxylin (BDH Chemicals, Poole, Dorset); Diazapam (Valium 5 mg ml^{-1}; Roche Products Ltd., Welwyn Garden City, Herts); Hypnorm (fentanyl citrate 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1}; Janssen Pharmaceutical Ltd, Grove, Oxon.); sterile pyrogen-free 0.9% sodium chloride solution (saline; Baxter Healthcare Ltd., Thetford, Norfolk).

Expression and analysis of results

All values are means $\pm$ s.e.mean, statistical comparisons were considered significant if $P<0.05$.

Acute bronchoconstriction Acute bronchoconstriction is expressed as the maximum percentage change in R_L and C_{dyn} from baseline during the antigen challenge and 30 min post challenge. Student's unpaired t test was used to compare differences between treatment groups.

Airway responsiveness Airway responsiveness to inhaled histamine is expressed as the percentage change in R_L and C_{dyn} from baseline values in response to increasing doses of inhaled histamine. The dose of histamine required to provoke a 50% increase (PC_{50}) in R_L and a 35% decrease (PC_{35}) in C_{dyn} was determined. The maximum percentage increase in R_L and decrease in C_{dyn} within the dose-range of 1.25–80 mg ml^{-1} were also recorded, with both parameters used as indices of airway responsiveness. Student's paired t tests were used to compare histamine responsiveness data before and after antigen challenge using the geometric mean of $\log_{10}$ transformed PC_{50} and PC_{35} values or percentage maximum R_L and minimum C_{dyn} values. Student's unpaired t tests were used to compare between drug and vehicle-treated rabbits.

Bronchoalveolar lavage At least 300 cells were differentiated as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria and expressed as absolute cell counts per ml of lavage fluid. Cell counts were performed 'blind' with respect to the observer and compared by Wilcoxan rank analysis.

Results

Basal lung function

Basal airway resistance (R_L) and dynamic compliance (C_{dyn}) were not significantly different between theophylline (Table 1A) and rolipram (Table 1B)-treated rabbits and their respective vehicle-treated groups, either 24 h before challenge, immediately before challenge, or 24 h post challenge with either antigen or saline.

Acute bronchoconstriction

Effect of theophylline (Figure 1a) In vehicle-treated rabbits, antigen challenge caused approximately a 2 fold greater increase in R_L and a 3 fold decrease in C_{dyn} than observed in saline challenged rabbits. This antigen-induced acute bronchoconstriction was similar in magnitude in theophylline treated rabbits.

Effect of rolipram (Figure 1b) In vehicle-treated rabbits, inhaled antigen caused an acute bronchoconstriction with approximately a 2 fold greater increase in R_L and decrease in C_{dyn} than observed in saline challenged rabbits. Pretreatment with rolipram failed to inhibit the antigen-induced acute bronchoconstriction.

Airway responsiveness to histamine

Effect of theophylline In vehicle-treated rabbits (Table 2A), no difference in responsiveness to inhaled histamine was observed 24 h before or after challenge with saline.

Prior to antigen challenge (Table 2A, Figure 2a, b), histamine responsiveness was similar in terms of R_L PC_{50}, and C_{dyn} PC_{35} in both vehicle and theophylline-treated rabbits. In vehicle-treated rabbits, antigen-induced airway hyperresponsiveness was shown by a 2.4 fold reduction in R_L PC_{50} and a 2.6 fold reduction in C_{dyn} PC_{35} values. In theophylline-treated rabbits a similar 1.8 fold reduction in R_L PC_{50} and 1.9 fold reduction C_{dyn} PC_{35} was observed 24 h after antigen challenge.

In vehicle-treated rabbits the maximum percentage increase in R_L to inhaled histamine (R_{Lmax}) tended to be increased (79±20.1%) 24 h after antigen challenge (Figure 2a). A similar 50.0±23.7% increase in R_{Lmax} was observed in theophylline-treated rabbits. There was no change in the minimum compliance ($C_{dyn\ min}$) values before or after antigen challenge in either theophylline or vehicle groups (Figure 2b).

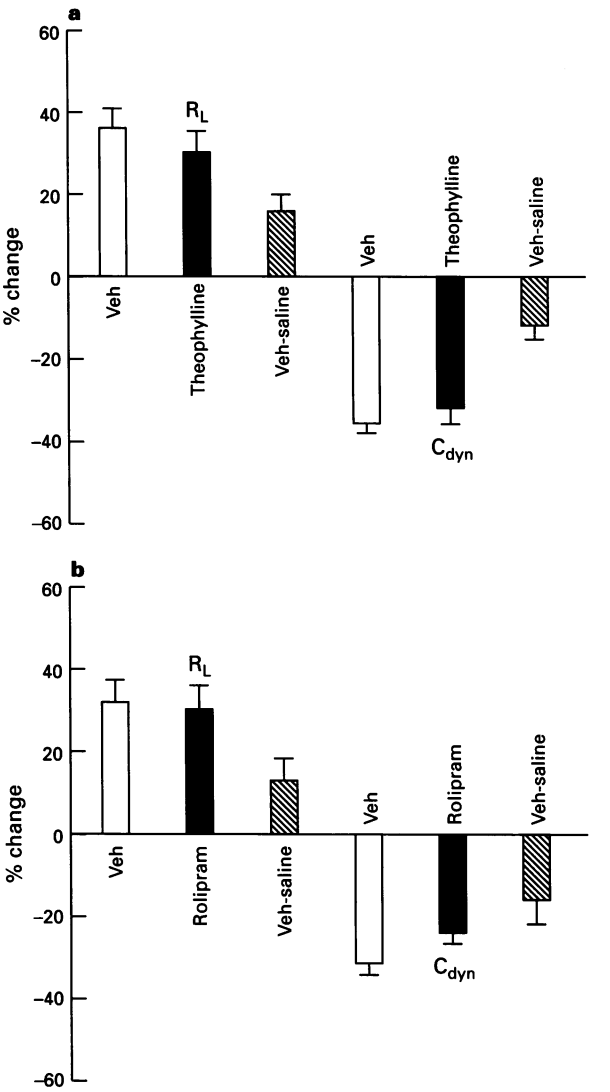

Figure 1 Antigen-induced acute bronchoconstriction is shown as percentage increase in total lung resistance (R_L) and decrease in dynamic compliance (C_{dyn}) after (a) theophylline vehicle ($n=9$) and theophylline ($n=9$) and (b) rolipram vehicle ($n=9$) and rolipram ($n=9$). The response to inhaled saline in (a) theophylline vehicle ($n=6$) and (b) rolipram vehicle ($n=6$)-treated rabbits.

Table 1 **A** Basal lung function (R_L and C_{dyn}) 24 h prior to challenge, immediately before challenge and 24 h post challenge with (a) saline and (b) antigen in vehicle and theophylline (Theoph) pretreated immunized rabbits

	R_L ($cmH_2O\ l^{-1}\ s^{-1}$)			C_{dyn} (ml cmH_2O^{-1})		
Treatment	*Pre*	*Challenge*	*Post*	*Pre*	*Challenge*	*Post*
(a) Theoph vehicle ($n=6$)	37.2±2.8	33.8±2.0	37.8±3.7	4.2±0.3	4.1±0.2	4.2±0.3
(b) Theoph vehicle ($n=9$)	41.4±1.8	38.6±1.2	38.9±1.9	4.1±0.1	4.1±0.1	4.2±0.1
(b) Theoph ($n=9$)	39.8±2.1	39.0±1.7	41.0±2.8	4.2±0.1	4.3±0.1	4.4±0.2

B Basal lung function (R_L and C_{dyn}) 24 h prior to challenge, immediately before challenge and 24 h post challenge with (a) saline and (b) antigen in vehicle and rolipram pretreated immunized rabbits

	R_L ($cmH_2O\ l^{-1}\ s^{-1}$)			C_{dyn} (ml cmH_2O^{-1})		
Treatment	*Pre*	*Challenge*	*Post*	*Pre*	*Challenge*	*Post*
(a) Rolipram vehicle ($n=6$)	36.5±2.8	35.4±1.6	36.8±3.5	4.2±0.3	4.6±0.2	4.4±0.2
(b) Rolipram vehicle ($n=9$)	32.0±1.7	35.6±2.0	31.5±1.5	4.2±0.1	4.5±0.2	4.4±0.2
(b) Rolipram ($n=9$)	32.2±1.6	32.7±1.1	33.6±1.0	4.6±0.2	4.3±0.1	4.7±0.1

Table 2 **A** The dose of inhaled histamine (mg ml^{-1}) required to provoke a 50% (PC_{50}) increase in R_L and 35% (PC_{35}) decrease in C_{dyn} in vehicle and theophylline pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

Treatment	R_L PC_{50} (mg ml^{-1})		C_{dyn} PC_{35} (mg ml^{-1})	
	Pre	*Post*	*Pre*	*Post*
(a) Theoph vehicle ($n=6$)	22.3±1.2	18.2±1.6	17.8±1.9	19.5±1.4
(b) Theoph vehicle ($n=9$)	15.5±1.3	6.5±1.2†	16.5±1.3	6.4±1.1†
(b) Theophylline ($n=9$)	18.7±1.1	10.2±1.3†	17.2±1.1	8.9±1.2†

B The dose of inhaled histamine (mg ml^{-1}) required to provoke a 50% (PC_{50}) increase in R_L and 35% (PC_{35}) decrease in C_{dyn} in vehicle and rolipram pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

Treatment	R_L PC_{50} (mg ml^{-1})		C_{dyn} PC_{35} (mg ml^{-1})	
	Pre-antigen	*Post-antigen*	*Pre-antigen*	*Post-antigen*
(a) Rolipram vehicle ($n=6$)	21.7±1.9	20.7±1.9	19.0±1.7	19.5±1.7
(b) Rolipram vehicle ($n=9$)	16.6±1.4	4.8±1.4†	12.7±1.4	5.0±1.3†
(b) Rolipram ($n=9$)	17.4±1.3	11.0±1.2$^{\dagger\#}$	11.1±1.2	10.6±1.3$^{\#}$

†Indicates $P<0.05$ compared to pre-antigen value (paired t test); $^{\#}$Indicates $P<0.05$ compared to vehicle-treated group (unpaired t test).

In vehicle-treated rabbits, R_L PC_{50} values before challenge with saline tended to be higher than in the vehicle group challenged with antigen.

Rolipram In vehicle-treated rabbits challenged with saline (Table 2B) there was no difference in histamine responsiveness 24 h prior to or post saline.

Before antigen challenge (Table 2B, Figures 3a, b), histamine responsiveness was similar in terms of R_L PC_{50}, in both vehicle and rolipram-treated rabbits. Antigen-induced airway hyperresponsiveness was shown in vehicle-treated rabbits by a 3.5 fold reduction in RL PC_{50} and a 2.5 fold reduction in C_{dyn} PC_{35} values. In rolipram-treated rabbits there was a significantly lower 1.6 fold reduction in R_L PC_{50} and no change in C_{dyn} PC_{35} 24 h after antigen challenge ($P<0.05$). In vehicle-treated rabbits an increase in R_{Lmax} (79.5±16.0%) to inhaled histamine was observed 24 h after antigen challenge (Figure 3a). A significantly reduced 21.3±7.7% increase in R_{Lmax} was observed in rolipram-treated rabbits ($P<0.05$). There was no change in the minimum compliance ($C_{dyn\ min}$) values before or after antigen challenge in either rolipram or vehicle groups.

In vehicle-treated rabbits, R_L PC_{50} and C_{dyn} PC_{35} values prior to challenge with saline tended to be higher than in the vehicle group challenged with antigen.

Bronchoalveolar lavage (BAL)

The volume of fluid recovered from BAL was not significantly different between drug and vehicle-treated rabbits before or after antigen challenge (2.0–3.0 ml, 40–60% recovery, data not shown).

Effect of theophylline In vehicle-treated rabbits challenged with saline, there was no change total cells, mononuclear cells or eosinophils, although neutrophils were increased (8.4 fold) 24 h after saline challenge (Table 3A).

Total leucocytes recovered in BAL fluid 24 h after antigen challenge were elevated 27.0% in vehicle-treated rabbits whilst only a 9.0% increase was observed in theophylline-treated rabbits ($P<0.05$). This increase was due to the recruitment of eosinophil, neutrophil and mononuclear cell types.

Neutrophil numbers were significantly elevated 5.8 fold after antigen challenge in vehicle-treated rabbits with a similar 5.3 fold increase observed in theophylline-treated rabbits. Eosinophils were virtually undetectable before antigen challenge and increased after antigen challenge by 47 fold in vehicle-treated rabbits with a significantly lower 20 fold increase observed in theophylline-treated rabbits ($P<0.05$). Mononuclear cells remained unchanged in both vehicle and theophylline-treated rabbits.

Effect of rolipram In vehicle-treated rabbits challenged with saline, there was no change in total cells, mononuclear cells or eosinophils; however, a 10.0 fold increase in neutrophils was observed 24 h after saline challenge (Table 3B).

Total leucocytes increased significantly (28.0%) 24 h after antigen challenge in vehicle-treated rabbits only. This increase was largely due to the recruitment of neutrophils and eosinophils. Total leucocytes tended to be reduced (25.5%) in rolipram-treated rabbits before antigen challenge and remained unchanged after antigen challenge ($P<0.05$).

Neutrophils increased 6.6 fold after antigen in the vehicle group with a similar 6.5 fold increase in the rolipram group. Eosinophil recruitment due to antigen was shown in vehicle-treated rabbits by over an 170 fold increase in eosinophil counts. A significantly reduced 30 fold increase in eosinophils was observed in rolipram-treated rabbits ($P<0.05$).

Discussion

The mechanisms underlying the efficacy of theophylline in respiratory disease is poorly understood (Barnes, 1988) but increasingly it appears to be due to a combination of bronchodilator (Weinburger *et al.*, 1980) and anti-inflammatory activity (reviewed in Barnes & Pauwels, 1994).

The present study supports the suggestion that theophylline is an anti-inflammatory agent and is consistent with clinical studies showing that theophylline inhibits the late asthmatic response following antigen challenge in man (Pauwels *et al.*, 1985; Mapp *et al.*, 1987; Hendeles *et al.*, 1995).

Low dose theophylline ($\cong$3 mg kg^{-1}) given twice daily over 5 weeks inhibited antigen-induced recruitment of activated eosinophils into the lungs of asthmatic individuals (Sullivan *et al.*, 1994). Consistent with the effects of low dose theophylline, the present study demonstrates that theophylline (3 mg kg^{-1}) inhibited eosinophil recruitment with no effect on the acute bronchoconstriction or the heightened airway responsiveness following antigen challenge in the allergic rabbit. Interestingly, the anti-inflammatory activity of theophylline has been observed in man with plasma levels lower than that required to cause significant bronchodilatation (Pauwels, 1987; Ward *et al.*, 1993) an observation consistent with the present study.

We have also demonstrated that the PDE 4 inhibitor, roli-

pram (1 mg kg^{-1}), has a similar profile of effects to theophylline which may suggest inhibition of PDE 4 as a common mechanism.

In the present study, a three day pretreatment with theophylline and rolipram was used since Banner & Page (1995) demonstrated, with a variety of PDE inhibitors, that chronic pretreatment was more effective than acute single dose pretreatment in preventing antigen-induced eosinophil recruitment into the airways of ovalbumin immunized, airway challenged guinea-pigs.

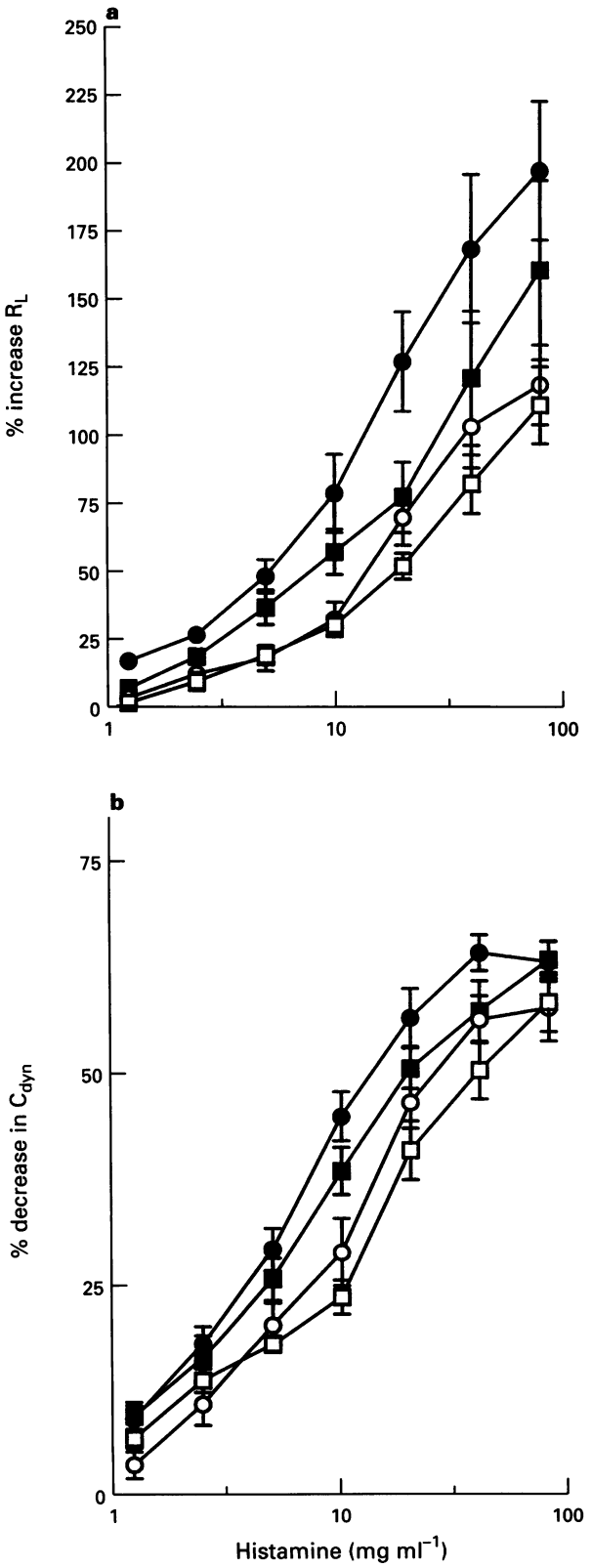

Figure 2 Percentage change in (a) total lung resistance (R_L) and (b) dynamic compliance (C_{dyn}) in response to inhaled histamine 24 h prior to or 24 h post antigen (Ag) challenge in vehicle (pre Ag ○; post Ag ●; $n=10$) and theophylline (pre Ag □; post Ag ■; $n=10$)-treated rabbits.

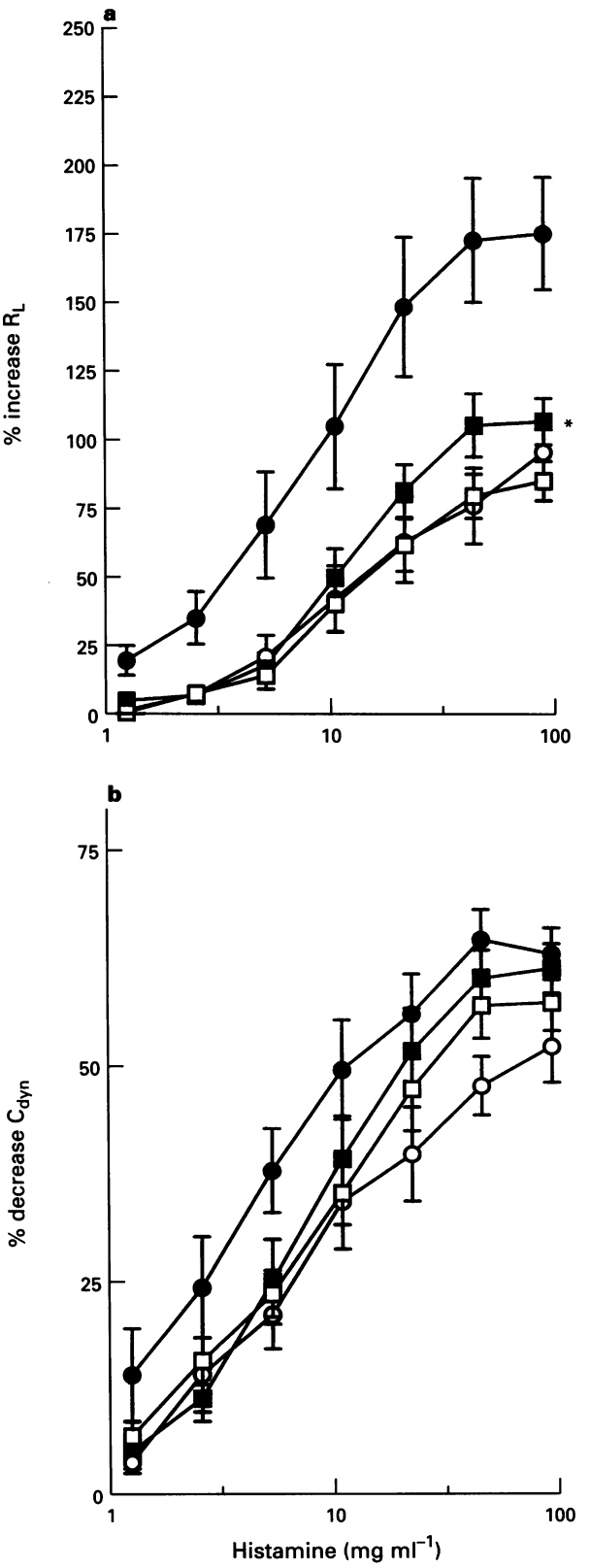

Figure 3 Percentage change in (a) total lung resistance (R_L) and (b) dynamic compliance (C_{dyn}) in response to inhaled histamine 24 h prior to or 24 h post antigen (Ag) challenge in vehicle (pre Ag ○; post Ag ●; $n=9$) and rolipram (pre Ag □; post Ag ■; $n=9$)-treated rabbits. *$P<0.05$ vs vehicle group 24 h post antigen.

Pretreatment with either theophylline or rolipram had no effect on basal airway tone or basal histamine responsiveness before antigen challenge suggesting that neither agent, at the doses used, exhibited bronchodilator activity or functionally antagonized histamine-induced bronchoconstriction. Most studies report that PDE inhibitors bronchodilate pre-constricted airways rather than prevent bronchoconstriction or reverse basal airway tone (Turner *et al.*, 1994).

The antigen-induced acute bronchoconstriction was similar in magnitude in both theophylline and rolipram vehicle groups. Antigen-induced acute bronchoconstriction was unaffected by treatment with theophylline or rolipram. The failure of rolipram to inhibit the acute bronchoconstriction is consistent with the findings of Turner *et al.* (1994) in *Ascaris*-sensitive cynomolgous monkeys. In contrast, rolipram is reported to inhibit antigen-induced acute bronchoconstriction in guinea-pigs (Howell *et al.*, 1992; Underwood *et al.*, 1994) and furthermore, Heaslip *et al.* (1992) reported that rolipram inhibited leukotriene production from homogenized sensitized guinea-pig lung after challenge with antigen. It is plausible that had we increased the dose of either drug in the present study we might have observed inhibition of this response.

Airway hyperresponsiveness (AHR) to inhaled aerosolized histamine was evident in both vehicle-treated groups 24 h after challenge with antigen, but not saline. AHR was shown by an increased sensitivity (reduction in PC_{50} and PC_{35} values) and degree of bronchoconstriction (increased R_{Lmax} values). This hyperresponsiveness was unaltered with theophylline, consistant with studies in man (Cockcroft *et al.*, 1989), but significantly reduced with rolipram-treatment, suggesting PDE 4 to be functional at sites relevant for the generation of airway hyperresponsiveness in the immunized rabbit. Inhibition of antigen-induced AHR with rolipram has been previously reported in primates (Turner *et al.*, 1994).

The development of airway hyperresponsiveness in this model can be inhibited by pretreatment with capsaicin (Herd *et al.*, 1995) and the mixed 5-LO/LTD_4 antagonist, PF5901 (Herd *et al.*, 1994) which may suggest that a leukotriene-mediated activation of sensory C-fibres could contribute to antigen-induced hyperresponsiveness in the immunized rabbit. Interestingly, Qian *et al.* (1994) demonstrated that rolipram inhibited electrical field stimulated excitatory non-cholinergic (eNANC) contraction of guinea-pig isolated trachea *in vitro* whilst having no effect on contraction due to exogenous substance P, an observation that has now been extended to a range of PDE 4 inhibitors (Spina *et al.*, 1995). Inhibition of this response with theophylline is also reported (Barlinski *et al.*, 1992). It is therefore possible that inhibition of antigen-induced AHR by rolipram may be due to modulation of C-fibre activity to reduce tachykinin release. Furthermore, this may explain the inhibitory effect of rolipram on ozone-induced AHR to inhaled histamine (Holbrook & Hughes, 1992) where the AHR is capsaicin sensitive and thought to be due to increased tachykinin effects resulting from oxidant damage of airway lumen neutral endopeptidase (Yeadon *et al.*, 1992).

Total cells, principally neutrophils and mononuclear cells, recovered in BAL fluid 24 h before antigen challenge were reduced though not significantly in rolipram-treated rabbits. This effect on basal lung cellularity has also been observed in primates (Turner *et al.*, 1994) and therefore the PDE 4 inhibitors may affect basal trafficking of leucocytes into the airways.

Both theophylline and rolipram signifiantly inhibited antigen-induced eosinophil recruitment in the rabbit, consistent with findings of PDE inhibitors in the guinea-pig (Griswood *et al.*, 1991; Underwood *et al.*, 1994; Lagente *et al.*, 1994) and primate (Turner *et al.*, 1994). Surprisingly, rolipram had no effect on neutrophil recruitment. In contrast, inhibition of antigen-induced neutrophil recruitment with rolipram has been observed in primate (Turner *et al.*, 1994) and guinea-pig (Underwood *et al.*, 1994).

The precise mechanisms by which theophylline and rolipram inhibit eosinophil recruitment in this model is unclear, though both may act by elevation of cyclic AMP (Torphy & Undem, 1991). The suppressant effect of elevated cyclic AMP levels within immune and inflammatory cells may lead to reduced release of eosinophil specific chemoattractants, the diapedesis process or the ability of eosinophils to respond to chemoattractive mediators. The release of the inflammatory cytokine, tumour necrosis factor (TNFα) from human T-lymphocytes (Schudt *et al.*, 1992) and monocytes (Endres *et al.*, 1991; Verghese *et al.*, 1995; Sinha *et al.*, 1995) is inhibited by rolipram. Additionally, Turner *et al.* (1994) reported that rolipram inhibited TNFα in BAL fluid 4 h post antigen challenge in primates. TNFα can increase the expression of a variety of adhesion molecules necessary for the passage of leucocytes into the airway mucosa, (Gundel *et al.*, 1991; Leung *et al.*, 1991). Therefore, the PDE inhibitors may inhibit the migration processes by reducing TNFα-mediated adhesion molecule expression or have a direct inhibitory effect on ad-

Table 3 **A** Total and differential cell counts ($\times 10^4$ cells ml^{-1}) recovered in BAL fluid from vehicle and theophylline pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

Treatment	*Total*		*Mononuclear cells*		*Neutrophils*		*Eosinophils*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
(a) Theophylline vehicle ($n=6$)	29.8±6.9	31.0±5.3	29.4±6.9	24.2±6.6	0.8±0.3	6.7±3.6†	0.1±0.1	0.1±0.1
(b) Theophylline vehicle ($n=9$)	32.6±1.7	41.4±2.8†	31.3±1.5	31.0±2.5	1.0±0.3	5.8±1.0†	0.1±0.1	4.7±0.9†
(b) Theophylline ($n=9$)	30.5±2.0	33.2±1.3$^{\#}$	29.7±2.1	27.5±1.6	0.7±0.2	3.7±0.7†	0.1±0.1	2.0±0.3$^{\dagger\#}$

B Total and differential cell counts ($\times 10^4$ cells ml^{-1}) recovered in BAL fluid from vehicle and rolipram pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

Treatment	*Total*		*Mononuclear cells*		*Neutrophils*		*Eosinophils*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
(a) Rolipram vehicle ($n=6$)	32.0±6.6	36.4±7.3	31.6±6.6	32.4±8.6	0.4±0.4	4.0±2.4†	0.0±0.0	0.02±0.04
(b) Rolipram vehicle ($n=9$)	28.2±2.6	36.1±6.3	27.0±2.5	26.5±6.8	1.2±0.5	7.9±2.2†	0.0±0.0	1.7±0.4†
(b) Rolipram ($n=9$)	21.0±2.6	19.2±2.1$^{\#}$	20.7±1.4	15.1±2.3	0.6±0.1	3.9±1.3†	0.01±0.01	0.3±0.01$^{\dagger\#}$

†Indicates $P<0.05$ compared to pre-antigen value; $^{\#}$indicates $P<0.05$ compared to vehicle-treated group (Wilcoxon analysis).

hesion molecule expression (Wellicombe *et al.*, 1990; Pober *et al.*, 1993; De Luca *et al.*, 1994). Rolipram has been shown to inhibit exogenous IL-5-mediated eosinophil recruitment into the pleural cavity of rats (Lisle *et al.*, 1995) indicating a reduced responsiveness of the eosinophil to chemoattractive mediators or inhibition of the diapedesis process. However, due to the ubiquitous distribution of the PDE 4 isoenzymes in the airways, theophylline and rolipram probably act at a number of cellular sites.

In conclusion, with the dosing regimes used, theophylline exhibits anti-inflammatory activity in preventing antigen-induced eosinophil recruitment in the immunized rabbit. This effect is comparable to that observed in man at a similar dose. Furthermore, this activity is shared by the selective PDE 4 inhibitor, rolipram, suggesting that the anti-inflammatory efficacy of theophylline could be due to inhibition of PDE 4. Rolipram also inhibited antigen-induced AHR which might suggest that selective PDE 4 inhibitors may exhibit greater potency or efficacy than theophylline in the treatment of asthma.

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