Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor

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1 We have investigated the effects of RP 73401, a novel, potent and highly selective cyclic nucleotide phosphodiesterase (PDE) type IV inhibitor, in guinea-pig and rat models of bronchoconstriction and allergic inflammation. In some models, the effects of RP 73401 have been compared with those of the standard PDE type IV inhibitor, rolipram.

2 RP 73401 ($0.4\text{--}400\text{ }\mu\text{g kg}^{-1}$, intratracheally (i.t.) on lactose) inhibited antigen-induced bronchospasm in previously sensitized conscious guinea-pigs (ID_{50} : $7 \pm 1\text{ }\mu\text{g kg}^{-1}$) and in anaesthetized rats (ID_{50} : $100 \pm 25\text{ }\mu\text{g kg}^{-1}$). Rolipram inhibited the antigen-induced bronchospasm in guinea-pigs with an ID_{50} of $5 \pm 1\text{ }\mu\text{g kg}^{-1}$. In guinea-pig bronchoalveolar lavage (BAL) fluid, total inflammatory cell and eosinophil numbers were reduced by RP 73401 (ID_{50} s: $3.9 \pm 0.8\text{ }\mu\text{g kg}^{-1}$ and $3.2 \pm 0.7\text{ }\mu\text{g kg}^{-1}$, respectively). In the rat, inflammatory cell numbers are less affected. Only the highest dose of RP 73401 ($400\text{ }\mu\text{g kg}^{-1}$) significantly inhibited eosinophil influx ($41 \pm 16\%$ inhibition).

3 RP 73401 ($0.02\text{--}100\text{ }\mu\text{g kg}^{-1}$, i.v.) inhibited PAF-induced bronchial hyperreactivity to bombesin in the anaesthetized guinea-pig (ID_{50} : $0.09 \pm 0.03\text{ }\mu\text{g kg}^{-1}$) and inhibited ($0.4\text{--}40\text{ }\mu\text{g kg}^{-1}$, i.t.) histamine-induced airway microvascular leakage in the anaesthetized guinea-pig by approximately 60% at all doses.

4 RP 73401 relaxed guinea-pig isolated trachea under basal tone (EC_{50} : 9 nM) and when precontracted with histamine (IC_{50} : 2 nM), methacholine (IC_{50} : 29 nM) or leukotriene D_4 (LTD_4 , IC_{50} : 4 nM).

5 RP 73401 ($0.4\text{--}100\text{ }\mu\text{g kg}^{-1}$, i.t.) inhibited bronchospasm induced by histamine (ID_{50} : $34 \pm 6\text{ }\mu\text{g kg}^{-1}$), methacholine (ID_{50} : $66 \pm 12\text{ }\mu\text{g kg}^{-1}$) and LTD_4 (ID_{50} : $<4\text{ }\mu\text{g kg}^{-1}$) in the anaesthetized guinea-pig. Against these same bronchoconstrictors, rolipram (i.t.) had ID_{50} values of 44 ± 4 , 72 ± 18 and $<4\text{ }\mu\text{g kg}^{-1}$ respectively. RP 73401 (4 and $40\text{ }\mu\text{g kg}^{-1}$, i.t.) increased the magnitude and duration of bronchodilatation produced by salbutamol in the anaesthetized guinea-pig. At doses producing significant bronchodilatation, RP 73401 was without effect on heart rate or blood pressure in the anaesthetized guinea-pig. RP 73401 ($0.01\text{--}0.25\text{ mg kg}^{-1}$, i.v.) did not affect heart rate and produced only a small fall in blood pressure in the anaesthetized rat.

6 These data demonstrate that RP 73401 and rolipram inhibit antigen- and mediator-induced bronchospasm in guinea-pigs with the same potency. Furthermore, RP 73401 administered directly into the airways, protects against allergic airway inflammation. These results indicate the importance of PDE IV in regulating smooth muscle and inflammatory cell activity. At doses suppressing the inflammatory response in the lung, RP 73401 had little effect in the cardiovascular system. RP 73401 may have a role as a bronchodilator and, more importantly, as a prophylactic anti-inflammatory agent in the treatment of asthma.

Keywords: Phosphodiesterase inhibitor; RP 73401; bronchospasm; bronchodilator; inflammation; anti-inflammatory; anti-asthmatic; salbutamol; rolipram

Introduction

At least five different cyclic nucleotide phosphodiesterase (PDE) isoenzymes have now been identified on the basis of their functional characteristics such as substrate specificity and susceptibility to selective inhibitors (Beavo & Reifsnyder, 1990). The realization that there are differences in the relative functional importance of the PDE isoenzymes in specific cell types has fuelled the search for isoenzyme-selective PDE inhibitors which can be targeted to the cells implicated in the pathophysiology of asthma (Torphy & Undem, 1991; Giembycz & Dent, 1992; Raeburn *et al.*, 1993; Karlsson *et al.*, 1993).

Granulocytes, mononuclear cells and mast cells/basophils contain principally the PDE type IV isoenzyme. Inhibition of this enzyme is associated with increased intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) and suppression of cell activity (Torphy & Undem, 1991; Giembycz &

Dent, 1992; Raeburn *et al.*, 1993; Karlsson *et al.*, 1993). Although human airways smooth muscle contains at least five isoenzyme families (see Raeburn *et al.*, 1993 and references therein), the PDE III and PDE IV isoenzymes are functionally the most important as modulators of contractility (De Boer *et al.*, 1992; Qian *et al.*, 1993). In animal studies the functional profile of PDE isoenzymes in airways smooth muscle preparations both *in vitro* and *in vivo* appears similar to that in human tissue (Raeburn *et al.*, 1993).

RP 73401 (3-cyclopentylloxy-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide) is a novel, highly selective and very potent PDE type IV inhibitor (Karlsson *et al.*, 1993; Ashton *et al.*, 1994). PDE type IV isolated from various cell types is inhibited by RP 73401 with an IC_{50} of about 1 nM and it displays at least a 19,000 fold selectivity compared with the other PDE isoenzymes (Ashton *et al.*, 1994; Souness *et al.*, 1994b). We have now characterized the airways smooth muscle relaxant and anti-inflammatory effects of RP 73401 in the guinea-pig and rat. In some models, we have compared the

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effects of RP 73401 with those of the standard PDE type IV inhibitor rolipram. This study demonstrates potent inhibitory effects on a range of bronchoconstrictor and inflammatory responses in the tracheobronchial tree by RP 73401 and indicates that this compound has potential as an anti-asthma agent.

Methods

Guinea-pig isolated trachea

Male, Dunkin-Hartley guinea-pigs (350–500 g) were killed by cervical dislocation and the tracheas removed and placed in Krebs-Ringer bicarbonate (KRB) solution. The epithelium was removed by gently rubbing the luminal surface with a cotton swab. Transverse tracheal strips were prepared and suspended under an applied load of 2 g in 5 ml tissue baths containing KRB solution at 37°C gassed with 5% CO₂ in O₂. The load was determined from preliminary experiments (data not shown) to give maximum force of contraction in response to methacholine. The composition of the KRB was as follows (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 10.1. Changes in isometric force of contraction were measured with force-displacement transducers (Grass FT03) connected to a pen recorder (Lectromed Multitrace 8).

To examine the effects of RP 73401 on basal tone, tracheal tissues were equilibrated for 60 min, washing at 10 min intervals with KRB solution. Isoprenaline (2 µM) was then added to the baths to determine zero tone. The isoprenaline was subsequently washed from the tissues and when tone was fully recovered, the relaxant effects of RP 73401 were assessed following its cumulative addition.

To examine effects on spasmogen-induced increases in the force of contraction tissues were equilibrated as above but KRB solution containing indomethacin (5.6 µM) was used. Tissues were contracted to an EC₃₀ (concentration producing 30% maximal contraction) of histamine (0.49 µM, 0.94 ± 0.11 g), methacholine (0.10 µM, 0.82 ± 0.09 g) or leukotriene D₄ (LTD₄) (0.41 nM, 0.63 ± 0.04 g). Contractile responses to all agonists remained stable over the time-course of the experiment. When the contractile response to each agonist had plateaued, RP 73401 was added cumulatively. The vehicle for RP 73401 (dimethylsulphoxide, DMSO, 0.1%) produced less than 10% reduction in maintained force of contraction.

Responses to RP 73401 (mean ± s.e.mean from *n* animals) are expressed as % inhibition of spasmogen-induced contraction or % reduction in basal tone where appropriate. EC₅₀ values (basal tone) or IC₅₀ (precontracted tissues) were calculated by linear regression and are presented as geometric means with 95% confidence limits.

Spasmogen-induced bronchospasm in the anaesthetized guinea-pig

Male, Dunkin-Hartley guinea-pigs (350–450 g) were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.). The trachea was cannulated to allow mechanical ventilation (Harvard 683 rodent ventilator) at 60 strokes min⁻¹ with a stroke volume of 1 ml 100 g⁻¹. Pulmonary inflation pressure (PIP) was measured with a Druck (model PDCR75) pressure transducer connected to a side arm from the tracheal cannula. Blood pressure was measured by a second pressure transducer connected to a cannula tied into the left carotid artery. Heart rate was derived from the blood pressure signal. PIP, blood pressure and heart rate were recorded on a Lectromed (Multitrace 8) pen recorder. A cannula was tied into the right jugular vein to allow administration of spasmogen solutions in saline. RP 73401 or rolipram (4–100 µg kg⁻¹) were administered as a dry powder formulation (particle size, 4–8 µm) on lactose. A bolus (10 mg kg⁻¹) dose was blown from a tube into the airways by a plastic bulb (Underwood *et*

al., 1991; Raeburn & Karlsson, 1993). Vehicle control animals received lactose carrier (10 mg kg⁻¹) alone. We have shown (data on file) that a uniform distribution of drug throughout the lung occurs following administration as a dry powder.

Spasmogens (histamine, methacholine or LTD₄) were administered (i.v. bolus) at 6 min intervals and the dose titrated so that each produced an increase in PIP equivalent to about 60% of the maximum obtained by manually occluding the tracheal cannula. After two stable responses had been obtained, RP 73401 or rolipram were administered into the airways 1 min before the next dose of spasmogen. Administration of spasmogen was continued at 6 min intervals until responses had returned to pretreatment amplitude or for a maximum of 48 min. Each animal received only a single dose of the PDE inhibitor. Results are expressed as percentage inhibition (mean ± s.e.mean) of bronchospasm and duration of action (min). The ID₅₀ (the dose causing 50% inhibition of bronchospasm) in each case was calculated by linear regression.

Antigen-induced bronchospasm in vivo

Guinea-pigs were sensitized (i.p.) with ovalbumin (10 µg) and aluminium hydroxide (100 mg) in saline (1 ml). After 21–28 days, sensitization was confirmed by the intradermal injection of albumin (25 µl of 200 µg ml⁻¹) into the dorsal surface of the left ear. Animals not responding with marked erythema and oedema, maximal after 20–40 min, were excluded from the study. Sensitized animals were used 7–14 days later.

RP 73401 or rolipram (0.4–400 µg kg⁻¹) were administered intratracheally (i.t.). To facilitate dosing, animals were briefly anaesthetized with halothane (4% in oxygen) and placed in a dorsal recumbent position with the head elevated at an angle of 45°. A blunt-ended metal tube (external diameter 1.5 mm) was gently inserted through the larynx into the trachea. A bolus (10 mg kg⁻¹) of the dry powder formulation was blown from a tube into the airways as above. The dosing procedure took approximately 10 s, after which the animal rapidly recovered from anaesthesia. Vehicle control animals received lactose carrier (10 mg kg⁻¹) alone. Untreated control animals were not anaesthetized or dosed. Thirty min after administration of a PDE inhibitor or lactose, animals received mepyramine maleate (30 mg kg⁻¹, i.p.) to protect against fatal anaphylaxis during antigen challenge; 50 min after administration of a PDE inhibitor, animals were placed in a whole body, double chamber plethysmograph to allow measurement of specific airway resistance (sR_{aw}) by a method modified from that described by Pennock *et al.* (1979). A Buxco (model LS-14) respiratory mechanics analyser was used to measure the phase shift between nasal and thoracic air flows and compute sR_{aw} on a breath by breath basis. A bias flow of air (600 ml min⁻¹) was maintained through the nasal chamber to ensure a constant supply of fresh air to the animal. This flow was subtracted by the respiratory mechanics analyser from its calculations.

Sixty min after administration of RP 73401 or rolipram, animals were challenged by exposure for 5 min to an aerosol of ovalbumin, generated from a 20 mg ml⁻¹ solution in saline by a nebulizer (deVilbiss Pulmosonic) and passed through the nasal chamber by an air pump operating at a flow rate of 12 l min⁻¹. The mean sR_{aw} was calculated for the 5 min period immediately before antigen challenge (baseline measurement) and for 3 consecutive 5 min periods after challenge. The post-challenge mean measurement obtained during the 5 min period of peak change was used for subsequent analysis. For each treatment group, results were expressed as mean ± s.e.mean percentage inhibition of bronchospasm compared with that in the vehicle control group.

Male, Brown Norway rats (250–300 g) were sensitized with ovalbumin (1 mg) and aluminium hydroxide (100 mg) in 1 ml saline s.c. plus *B. pertussis* vaccine (0.25 ml, nominally 1 × 10¹⁰ cells) i.p. After 21–28 days, rats were anaesthetized

with sodium pentobarbitone (60 mg kg^{-1} , i.p.). The trachea was cannulated to allow mechanical ventilation (Harvard 683 rodent ventilator) at $80 \text{ strokes min}^{-1}$ with a stroke volume of $1 \text{ ml } 100 \text{ g}^{-1}$. PIP was recorded as before. A cannula was placed in a tail vein to allow ovalbumin administration.

RP 73401 (4 to $400 \mu\text{g kg}^{-1}$) was administered as described for the guinea-pig studies; 1 min after receiving RP 73401 or vehicle, rats were challenged with ovalbumin (1 mg in 0.1 ml saline, bolus i.v.). Bronchospasm was measured as an increase in PIP, which reached maximum within 3 min of challenge. This challenge dose was determined from preliminary experiments (data not shown) to give a bronchospasm of approximately 60% of maximum in vehicle control animals.

Antigen-induced inflammatory cell accumulation in vivo

Guinea-pigs used for studies of antigen-induced bronchospasm were concurrently assessed for pulmonary inflammatory cell accumulation. Rats were sensitized as before and used for experiments 28 days later. RP 73401 (4 – $400 \mu\text{g kg}^{-1}$) was administered during halothane anaesthesia; 30 min after administration of RP 73401, rats were placed in a 3.5 l perspex chamber and challenged by exposure for 20 min to an aerosol of ovalbumin generated from a 10 mg ml^{-1} solution in saline by a nebulizer (deVilbiss Pulmosonic) and pumped into the chamber by a respiration pump operating at $100 \times 5 \text{ ml min}^{-1}$. A further group of animals acted as untreated, unchallenged controls; 24 h after antigen exposure, animals were killed with sodium pentobarbitone (200 mg kg^{-1} , i.p.). This time-point was used because we have previously demonstrated that eosinophil influx into the airways is maximal at this time (Raeburn *et al.*, 1994). The trachea was immediately cannulated and the lungs lavaged with saline ($2 \times 5 \text{ ml}$, guinea-pigs; $2 \times 4 \text{ ml}$, rats) at 37°C . Each aliquot was left in the lungs for 3 min . The aliquots were pooled and the total volume measured. The sample was centrifuged (800 r.p.m. for 10 min) and the cell pellet resuspended in Hank's balanced salt solution (10 ml). Total cell counts were made with a Coulter (model S-plus IV) cell counter. Differential counts were made from Cytospin preparations stained with Hema-Tek modified Wright's stain. At least 200 cells were differentiated according to standard morphological criteria by an operator unaware of the identity of the treatment groups. In addition, basal cell populations were determined in unchallenged animals. For each treatment group, results were expressed as percentage inhibition (mean $\pm$ s.e.mean) of cell influx compared with that in the vehicle control group.

PAF-induced bronchial hyperreactivity (BHR) in the anaesthetized guinea-pig

Guinea-pigs were anaesthetized and ventilated as before. Two responses to bombesin (100 – 300 ng kg^{-1} , i.v. bolus), equivalent to 15% of the maximum PIP were obtained 15 and 5 min before the start of an infusion of PAF (600 ng kg^{-1} , i.v.) or its vehicle (0.1% bovine serum albumin, BSA, i.v.) delivered (Harvard 2681 infusion pump) over a period of 60 min . PAF was administered as three consecutive infusions: 180 ng kg^{-1} for 10 min , 360 ng kg^{-1} for 20 min and 900 ng kg^{-1} for 30 min . Two further doses of bombesin were administered 5 and 15 min after the end of the PAF infusion. BHR was assessed by comparing the mean bombesin responses before and after PAF and expressing the difference as $\%$ change. To determine its inhibitory action on PAF-induced bronchial hyperreactivity, RP 73401 (0.02 – $100 \mu\text{g kg}^{-1}$, i.v.) or vehicle (DMSO, 0.1%) was administered 1 min before infusion of PAF or its vehicle. RP 73401 was administered by the i.v. route because in preliminary experiments (data not shown) the duration of action following i.t. administration, but not i.v. administration, was such that there may have been residual bronchodilator activity at

the time after dosing when responses to bombesin were reassessed. Further, we have found that, although i.t. lactose has no effect on bronchospasm, this vehicle may augment hyperreactivity. For each treatment group, results are expressed as percentage inhibition (mean $\pm$ s.e.mean) of PAF-induced BHR.

Histamine-induced microvascular leakage in the anaesthetized guinea-pig

Measurements of microvascular leakage (MVL) in guinea-pigs (400 – 500 g), anaesthetized with xylazine (0.8 mg kg^{-1} , s.c.) and ketamine (3 mg kg^{-1} , s.c.), were made as previously described (Raeburn & Karlsson, 1993). RP 73401 (0.4 – $40 \mu\text{g kg}^{-1}$) was administered as a dry powder as for the cell influx studies. A fine bore polythene cannula (external diameter 0.63 mm) was introduced into the trachea via the metal tube which was then withdrawn. The end of the cannula was positioned just below the larynx; 10 min after drug or vehicle administration, fluorescein isothiocyanate (FITC)-dextran (mol.wt. $150,000$, 50 mg kg^{-1} , i.v., marginal ear vein) was injected as a marker of plasma extravasation. After a further 10 min , histamine (60 nmol) was administered over a period of 2 min by superfusion of the airways via the polythene cannula. This dose was determined in preliminary studies to induce maximum MVL over basal levels without causing bronchospasm. Negative control animals received vehicle (saline, 0.02 ml min^{-1}) to establish basal leakage.

Ten min after histamine or vehicle challenge, blood samples were removed by cardiac puncture and the animals were killed with sodium pentobarbitone (200 mg kg^{-1} , i.v.). The trachea and bronchi were removed and lavaged with phosphate-buffered saline (PBS, $3 \times 1 \text{ ml}$). FITC content of tracheal tissue and lavage fluid was measured by agitating the samples for 15 h in PBS (2 ml) and assaying FITC content by fluorimetry (Perkin Elmer LS5B fluorescence spectrophotometer set with excitation and emission wavelengths of 480 and 520 nm respectively). FITC content was expressed as ng FITC mg^{-1} tissue or $\mu\text{g FITC ml}^{-1}$ lavage fluid. These values were calculated from a standard calibration curve of FITC-dextran concentration versus fluorescence intensity. Percentage inhibition of histamine-induced FITC extravasation was then calculated.

Effects on salbutamol-induced bronchodilatation in the anaesthetized guinea-pig

Guinea-pigs were anaesthetized and prepared to allow stable responses to histamine to be obtained as described above. After 2 consecutive, reproducible responses RP 73401 (4 or $40 \mu\text{g kg}^{-1}$, i.t. on lactose) was administered into the airways as previously described; 2 min later, animals similarly received salbutamol (2 or $10 \mu\text{g kg}^{-1}$, i.t. on lactose) and 1 min later the next dose of histamine was given. Administration of histamine was continued at 6 min intervals until responses had returned to pretreatment amplitude. For each dose group, results (mean $\pm$ s.e.mean) were expressed as percentage inhibition of bronchospasm duration of action (min).

Cardiovascular effects of RP 73401

Rats ($300 \pm 50 \text{ g}$, Sprague-Dawley CD) were anaesthetized, ventilated and cannulated for blood pressure measurement as before. Drugs were administered (i.v.) into the jugular vein. Mean arterial blood pressure and heart rate were determined with a Modular Instruments Signal Processing Centre (MI² system). Cardiovascular parameters were allowed to stabilize before drug administration. RP 73401 (0.01 – 0.25 mg kg^{-1}) was administered over 30 s in a dose volume of 1 ml kg^{-1} followed by a wash-in of saline (0.2 ml). The RP 73401 vehicle was polyethylene glycol $200/0.1 \text{ M HCl}$. Animals received only one dose of RP 73401. Cardiovascular

parameters were measured before (baseline) and from 5 to 60 min after drug treatment. Results are expressed as mean $\pm$ s.e.mean from *n* animals.

To assess cardiovascular effects following i.t. dosing in the anaesthetized guinea-pig, RP 73401 (ID_{50} against histamine-induced bronchospasm) was administered into the airways as before. Each animal received only a single dose of the PDE inhibitor. Baseline mean arterial blood pressure and heart rate were measured and expressed as group mean $\pm$ s.e.mean. The peak change in arterial blood pressure and heart rate in the 20 min period after dosing were each expressed as percent change from baseline values.

Drugs and solutions

Drugs used were: ovalbumin (chicken egg grade V), histamine diphosphate, methacholine chloride, salbutamol hemisulphate, bovine serum albumin, FITC-dextran and DMSO (Sigma, U.K.); aluminium hydroxide (M&B Labchem, U.K.); *Bordetella pertussis* vaccine (Wellcome, U.K.); mepyramine maleate and sodium pentobarbitone (Rhône-Poulenc Rorer, U.K.); xylazine (Bayer, U.K.); ketamine (Parke Davis, U.K.); PAF and bombesin (NovaBiochem, U.K.); Hank's balanced salt solution (Gibco, U.K.); lactose (median particle size 60 μ m, Lactochem, U.K.). RP 73401 and rolipram were synthesized by Rhône-Poulenc Rorer, U.K. Saline was prepared as 0.9% sodium chloride solution. Dry powder formulations of RP 73401 and rolipram were prepared as described previously (Underwood *et al.*, 1991). Median particle size of RP 73401 and rolipram in all formulations was 4–8 μ m.

Statistical analysis

The statistical significance of differences between treatment group means was determined by applying parametric (ANOVA and Dunnett's multiple comparison) or non-parametric (Kruskal-Wallis multiple comparison) tests as appropriate. $P < 0.05$ was accepted as significant.

Results

Spasmogen-induced smooth muscle contraction

RP 73401 produced concentration-dependent relaxation of guinea-pig tracheal strips under basal tone and reversed contractions induced by histamine, methacholine and LTD₄ (Figure 1). RP 73401 vehicle was without effect. RP 73401

Figure 1 The relaxant effects of RP 73401 on guinea-pig trachealis under basal tone ($\diamond$) or contracted with histamine ($\bullet$), methacholine ($\blacksquare$) or leukotriene D₄ ($\blacktriangle$). Results are expressed as mean $\pm$ s.e.mean, $n = 6-8$.

EC₅₀ (basal tone) and IC₅₀ (pre-contracted) values are given in Table 1. *In vivo*, RP 73401 and rolipram each caused a dose-related inhibition of bronchospasm induced by histamine (Figure 2a), methacholine (Figure 3a) or LTD₄ (Figure 4a) in the anaesthetized guinea-pig. Lactose alone had no significant effect. ID₅₀ values for RP 73401 and rolipram against histamine, methacholine and LTD₄ are given in Table 2. Both RP 73401 and rolipram were more potent as inhibitors of LTD₄-induced bronchospasm than as inhibitors of either histamine or methacholine. In addition the degree of inhibition of LTD₄-induced bronchospasm was greater than that seen with the other spasmogens. Since the PIP induced by each agonist was similar, the greater effect of RP 73401 and rolipram on the LTD₄ response cannot be due to the smaller maximum contractile effects seen with this agent *in vitro*. RP 73401 and rolipram each had a similar duration of action against histamine (Figure 2b) and methacholine (Figure 3b) but a markedly longer duration of action against LTD₄ (Figure 4b).

Table 1 Effects of RP 73401 on basal tone (EC₅₀) and against spasmogen-induced contractions in guinea-pig tracheal preparation *in vitro* (IC₅₀)

	n	IC ₅₀ (nM)
Histamine	7	2 (1–5)
Methacholine	6	29 (14–59)
LTD ₄	8	4 (2–9)
Basal tone (EC ₅₀)	7	9 (3–24)

Results are presented as geometric mean (confidence limits).

Figure 2 (a) The effects of RP 73401 ($\bullet$) and rolipram ($\circ$) against bronchospasm induced by histamine in the anaesthetized guinea-pig. (b) Duration of action of RP 73401 ($\bullet$) and rolipram ($\circ$) against bronchospasm induced by histamine in the anaesthetized guinea pig. Results are expressed as mean $\pm$ s.e.mean, $n = 3-5$.

Figure 3 (a) The effects of RP 73401 (●) and rolipram (○) against bronchospasm induced by methacholine in the anaesthetized guinea-pig. (b) Duration of action of RP 73401 (●) and rolipram (○) against bronchospasm induced by methacholine in the anaesthetized guinea pig. Results are expressed as mean $\pm$ s.e.mean, $n = 3-5$.

Figure 4 (a) The effects of RP 73401 (●) and rolipram (○) against bronchospasm induced by leukotriene D_4 (LTD_4) in the anaesthetized guinea-pig. (b) Duration of action of RP 73401 (●) and rolipram (○) against bronchospasm induced by LTD_4 in the anaesthetized guinea pig. Results are expressed as mean $\pm$ s.e.mean, $n = 3-5$.

Table 2 Effects of RP 73401 and rolipram against spasmogen-induced bronchospasm in the anaesthetized guinea-pig

	n	RP 73401 ID_{50} ($\mu\text{g kg}^{-1}$)	n	Rolipram ID_{50} ($\mu\text{g kg}^{-1}$)
Histamine	5	34 ± 6	5	44 ± 4
Methacholine	5	66 ± 12	5	72 ± 18
LTD_4	5	<4	5	<4

Antigen-induced bronchospasm

Aerosolized antigen caused an acute bronchospasm which peaked within 15 min in conscious guinea-pigs. sR_{aw} increased from 4.7 ± 0.8 to 24.5 ± 2.9 ml $\text{cmH}_2\text{O}^{-1}$ in vehicle control animals. RP 73401 and rolipram, but not lactose vehicle, caused a dose-related inhibition of antigen-induced bronchospasm (Figure 5). The ID_{50} values were RP 73401 7 ± 1 $\mu\text{g kg}^{-1}$, rolipram 5 ± 1 $\mu\text{g kg}^{-1}$.

In the anaesthetized rat bronchospasm induced by antigen (ovalbumin, 1 mg, i.v.) challenge was inhibited in a dose-dependent manner by RP 73401 (Figure 6). The calculated ID_{50} was 100 ± 25 $\mu\text{g kg}^{-1}$. Lactose alone had no significant effect on antigen-induced bronchospasm.

Antigen-induced cell influx

In guinea-pigs and rats, aerosolized antigen but not vehicle caused an increase in total cell and eosinophil numbers in

bronchoalveolar lavage 24 h later (Tables 3–5). In the guinea-pig, RP 73401 (4 and 40 $\mu\text{g kg}^{-1}$) significantly inhibited eosinophil influx (Table 3). Eosinophil numbers were also lower in guinea-pigs treated with rolipram (4 and 40 $\mu\text{g kg}^{-1}$) but the difference did not achieve statistical significance (Table 4). In the rat only the highest dose of RP 73401 (400 $\mu\text{g kg}^{-1}$) significantly inhibited ($41 \pm 16\%$) antigen-induced airway eosinophilia (Table 5). In each study the drug vehicle was without effect.

PAF-induced bronchial hyperreactivity

PAF infusion significantly increased bronchoconstrictor responses to bombesin from $18 \pm 3\%$ of maximum PIP (before PAF infusion) to $39 \pm 2\%$ of maximum PIP. This represented an increase of $179 \pm 5\%$ over the pre-infusion response. Infusion of PAF vehicle (0.1% BSA) had no significant effect. RP 73401 (0.02–100 $\mu\text{g kg}^{-1}$, i.v.) produced a dose-dependent and potent (ID_{50} : 0.09 ± 0.03 $\mu\text{g kg}^{-1}$) inhibition of PAF-induced bronchial hyperreactivity (Figure 7). RP 73401 vehicle (DMSO) had no significant effect.

Histamine-induced microvascular leakage

Histamine (60 nmol) significantly increased microvascular leakage of FITC-dextran in tracheal tissue from a basal level of 13.2 ± 1.6 ng mg^{-1} to 84 ± 12 ng mg^{-1} and leakage into the airway lumen (as measured in lavage fluid) from a basal level of 0.66 ± 0.24 $\mu\text{g ml}^{-1}$ to 3.2 ± 0.52 $\mu\text{g ml}^{-1}$. RP 73401 (0.4–40 $\mu\text{g kg}^{-1}$) significantly inhibited histamine-induced microvascular leakage into tracheal tissue (59–63% inhibition) and into the airway (87–95% inhibition) at each dose

($n = 5-11$) (Figure 8). Maximum inhibition was achieved with the smallest dose of RP 73401 which could be given by this technique. Lactose alone had no significant effect on leakage.

Effects on salbutamol-induced bronchodilatation

Salbutamol (2 or 10 $\mu\text{g kg}^{-1}$, i.t.) caused a dose-related inhibition of histamine-induced bronchospasm (Table 6). When given 2 min before salbutamol (2 mg kg^{-1}) RP 73401 (4 and 40 $\mu\text{g kg}^{-1}$) appeared to potentiate the effects of the β_2 -adrenoceptor agonist in terms of magnitude (Table 6). With the higher dose of RP 73401 (40 $\mu\text{g kg}^{-1}$) both the response magnitude and duration of action of salbutamol (2 mg kg^{-1}) were significantly increased. Combining the larger doses of salbutamol (10 $\mu\text{g kg}^{-1}$) and RP 73401 (40 $\mu\text{g kg}^{-1}$) completely inhibited responses to histamine with a duration of about 1 h.

Figure 5 The effects of RP 73401 (●) and rolipram (○) against antigen-induced bronchospasm in the conscious guinea pig. Results are expressed as mean $\pm$ s.e.mean, $n = 5-8$. * $P < 0.05$; ** $P < 0.01$ compared to vehicle control group.

Cardiovascular effects of RP 73401

Baseline heart rate and mean arterial blood pressure in the anaesthetized rat were 426 ± 16 beats min^{-1} and 120 ± 2 mmHg respectively, $n = 20$. RP 73401 up to the top dose (0.25 mg kg^{-1} , i.v.) was without effect on heart rate whereas a small ($14 \pm 1\%$) fall, which was not dose-related, in mean arterial blood pressure was seen. This compares with the vehicle-induced fall of $6 \pm 1\%$. The effect of RP 73401 was maximal within 5 min of dosing.

In the anaesthetized guinea-pig, baseline mean arterial blood pressure was 46 ± 3 mmHg. Baseline heart rate was 258 ± 6 beats min^{-1} . When administered as a dry powder formulation into the airways at the ID_{50} against histamine-induced bronchospasm (ID_{50} : 34 ± 6 $\mu\text{g kg}^{-1}$), RP 73401 ($n = 6$) had no significant effect on mean arterial blood pressure (% change: $+2 \pm 2\%$) or heart rate (% change: $+3 \pm 2\%$).

Figure 6 The effects of RP 73401 against antigen-induced bronchospasm in the anaesthetized rat. Results are expressed as mean $\pm$ s.e.mean, $n = 5-8$. * $P < 0.05$; ** $P < 0.01$ compared to vehicle control group.

Table 3 Effect of RP 73401 on inflammatory cell influx into BAL fluid in the anaesthetized, antigen-challenged, guinea-pig

	Total cell numbers (10^6 ml^{-1})	% inhibition of increase	Eosinophil numbers (10^6 ml^{-1})	% inhibition of increase
Naive	10 ± 1	—	2 ± 1	—
Unchallenged	8 ± 2	—	2 ± 1	—
Challenged + vehicle	$28 \pm 2\#$	—	$13 \pm 1\#$	—
Challenged + RP73401 (0.4 $\mu\text{g kg}^{-1}$)	28 ± 3	0	14 ± 2	0
Challenged + RP73401 (4 $\mu\text{g kg}^{-1}$)	$18 \pm 2^*$	50 ± 6	$7 \pm 1^*$	55 ± 8
Challenged + RP73401 (40 $\mu\text{g kg}^{-1}$)	$14 \pm 2^*$	70 ± 10	$5 \pm 1^*$	73 ± 14

$P < 0.05$ versus unchallenged; * $P < 0.05$ versus challenged plus vehicle. Data are presented as mean $\pm$ s.e.mean, $n = 5-10$ animals per group.

Table 4 Effect of rolipram on inflammatory cell influx into BAL fluid in the anaesthetized, antigen-challenged, guinea-pig

	Total cell numbers (10^6 ml^{-1})	% inhibition of increase	Eosinophil numbers (10^6 ml^{-1})	% inhibition of increase
Naive	10 ± 1	—	2 ± 1	—
Unchallenged	8 ± 2	—	2 ± 1	—
Challenged + vehicle	$28 \pm 2\#$	—	$13 \pm 1\#$	—
Challenged + rolipram (0.4 $\mu\text{g kg}^{-1}$)	27 ± 2	5 ± 2	14 ± 2	0
Challenged + rolipram (4 $\mu\text{g kg}^{-1}$)	21 ± 4	35 ± 11	10 ± 2	27 ± 19
Challenged + rolipram (40 $\mu\text{g kg}^{-1}$)	18 ± 3	50 ± 10	9 ± 2	36 ± 18

$P < 0.05$ versus unchallenged; * $P < 0.05$ versus challenged plus vehicle. Data are presented as mean $\pm$ s.e.mean, $n = 5-10$ animals per group.

Table 5 Effect of RP 73401 on inflammatory cell influx into BAL fluid in the anaesthetized, antigen-challenged, rat

	Total cell numbers (10^6 ml^{-1})	Eosinophil numbers (10^3 ml^{-1})
Naive	171 $\pm$ 19	2 $\pm$ 2
Unchallenged	158 $\pm$ 6	3 $\pm$ 2
Challenged + vehicle	280 $\pm$ 26#	42 $\pm$ 19#
Challenged + RP 73401 (4 $\mu\text{g kg}^{-1}$)	302 $\pm$ 54	53 $\pm$ 23
Challenged + RP 73401 (40 $\mu\text{g kg}^{-1}$)	290 $\pm$ 35	72 $\pm$ 15
Challenged + RP 73401 (400 $\mu\text{g kg}^{-1}$)	338 $\pm$ 89	26 $\pm$ 10* (41 $\pm$ 16% reduction)

$P < 0.05$ versus unchallenged; * $P < 0.05$ versus challenged plus vehicle.

Data presented as mean $\pm$ s.e.mean, $n = 5$ animals per group.

Figure 7 The effects of RP 73401 on PAF-induced bronchial hyperreactivity (BHR) to bombesin in the anaesthetized guinea-pig. Results are expressed as mean $\pm$ s.e.mean, $n = 3-12$. * $P < 0.05$ compared to vehicle control group.

Discussion

RP 73401 is a very selective inhibitor of the PDE IV isoenzyme compared with its effects on other PDE isoenzymes. RP 73401 inhibits the activity of PDE IV isoenzyme isolated from pig aorta or guinea-pig eosinophils with IC_{50} values of about 1 nM (Karlsson *et al.*, 1993; Ashton *et al.*, 1994). It is at least 19,000 fold more selective for the type IV than for type I, II, III or V (Karlsson *et al.*, 1993; Ashton *et al.*, 1994; Souness *et al.*, 1994b). RP 73401 seems to interact with the PDE IV isoenzyme differently from rolipram since the potency of the latter depends on enzyme location, enzyme conformation and cell type (Karlsson *et al.*, 1993; Souness *et al.*, 1994b). The present study demonstrates potent anti-inflammatory and bronchodilator effects of RP 73401 in guinea-pigs and rats.

RP 73401 relaxed guinea-pig isolated tracheal preparations under basal tone or when precontracted by putative asthma mediators such as histamine, a muscarinic cholinergic agonist and LTD_4 indicating that RP 73401 acts as a functional antagonist. Comparison with previously reported data in this preparation (Souness *et al.*, 1994a) demonstrates that RP 73401 is approximately 16 fold more potent than rolipram as an inhibitor of histamine-induced contractions (RP 73401 IC_{50} : 2 nM; rolipram IC_{50} 32 nM), 3 fold more potent against methacholine (RP 73401 IC_{50} : 29 nM; rolipram IC_{50} 96 nM) and 5 fold more potent against LTD_4 (RP 73401 IC_{50} : 4 nM; rolipram IC_{50} 19 nM). Thus, both PDE inhibitors were more effective against contractions produced by histamine and LTD_4 than against those induced by methacholine. RP

Table 6 The effects of pretreatment with RP 73401 on salbutamol-induced inhibition of histamine-induced bronchospasm in the anaesthetized guinea pig

Treatment	% inhibition of histamine-induced bronchospasm	Duration of action (min)
RP 73401 4 $\mu\text{g kg}^{-1}$	25 $\pm$ 3	10 $\pm$ 2
RP 73401 40 $\mu\text{g kg}^{-1}$	56 $\pm$ 7	31 $\pm$ 6
Salbutamol 2 $\mu\text{g kg}^{-1}$	42 $\pm$ 4	18 $\pm$ 4
Salbutamol 10 $\mu\text{g kg}^{-1}$	71 $\pm$ 8	29 $\pm$ 6
RP 73401 4 $\mu\text{g kg}^{-1}$ + salbutamol 2 $\mu\text{g kg}^{-1}$	61 $\pm$ 8*	24 $\pm$ 3
RP 73401 40 $\mu\text{g kg}^{-1}$ + salbutamol 2 $\mu\text{g kg}^{-1}$	70 $\pm$ 14*	57 $\pm$ 3*
RP 73401 4 $\mu\text{g kg}^{-1}$ + salbutamol 10 $\mu\text{g kg}^{-1}$	76 $\pm$ 10	25 $\pm$ 6
RP 73401 40 $\mu\text{g kg}^{-1}$ + salbutamol 10 $\mu\text{g kg}^{-1}$	100 $\pm$ 0*	54 $\pm$ 6*

Results are expressed as mean $\pm$ s.e.mean of 4-6 experiments. * $P < 0.05$ compared with salbutamol alone.

Figure 8 The effects of RP 73401 on histamine-induced microvascular leakage in the anaesthetized guinea pig lung. (A) Sham treated; (B) saline; (C) lactose + saline; (D) lactose + histamine (60 nmol); (E) RP 73401 (0.4 mg kg^{-1} , i.t.); (F) RP 73401 (4 mg kg^{-1} , i.t.); (G) RP 73401 (40 mg kg^{-1} , i.t.). Results are expressed as mean $\pm$ s.e.mean, $n = 5-8$. * $P < 0.05$ compared to vehicle control group.

73401 is less potent than the β_2 -adrenoceptor agonist salbutamol (Tomkinson *et al.*, 1993) but considerably more potent than the non-selective PDE inhibitor theophylline (Karlsson & Persson, 1981). All agents produced complete relaxations of the induced tone.

Consistent with the results *in vitro*, RP 73401 and rolipram each potently and dose-dependently inhibited bronchoconstriction induced by histamine, methacholine and LTD₄ in anaesthetized guinea-pigs, the contractions produced by LTD₄ being particularly sensitive (about 10 times more than the other spasmogens) to each of the PDE IV inhibitors. Furthermore, the duration of the inhibition of LTD₄ with RP 73401 or rolipram was significantly longer than with the other spasmogens. The prolonged duration was not dependent on the magnitude of the inhibition indicating that it is the mechanism of LTD₄-induced bronchospasm that is particularly sensitive to inhibition by the PDE inhibitors. Additionally in conscious animals, the bronchospasm produced by antigen aerosol (in the presence of a histamine (H₁) receptor antagonist) was suppressed by prior treatment with RP 73401 or rolipram. Interestingly, RP 73401 was 5 to 9 times more potent as an inhibitor of this allergic contraction, than of those produced by histamine and methacholine, supporting the view that leukotrienes indeed are major mediators of this immediate response. It is possible that suppression of mediator release from inflammatory cells (see below), in addition to a direct smooth muscle relaxant effect, also contributed to inhibition of this allergic response. Acute, antigen-induced bronchospasm in the rat was similarly inhibited by pretreatment with RP 73401.

RP 73401 and rolipram were equipotent inhibitors of bronchospasm in the guinea-pig, despite a 1000 fold difference in potency on the type IV enzyme isolated from pig aortic smooth muscle or bovine trachea (Karlsson *et al.*, 1993; Souness *et al.*, 1994b). This suggests that the *in vitro* data does not necessarily predict the *in vivo* potency against smooth muscle contraction. RP 73401 is more potent than rolipram in human cells such as monocytes (e.g. > 100 fold more potent as an inhibitor of TNF α release, Souness *et al.*, unpublished data) perhaps suggesting that the potency difference between the two compounds may be more marked in man.

β_2 -Adrenoceptor agonists are the most effective bronchodilators currently available for symptomatic relief. These agents act by raising intracellular levels of cyclic AMP, so the observation that pretreatment with RP 73401 increased the bronchodilator effects (potency and, particularly, duration) of salbutamol is to be expected. However, previously reported studies on the interaction between β_2 agonists and non-selective PDE inhibitors are contradictory (Karlsson & Persson, 1981).

The present data on the functional importance of the type IV PDE in regulating airways tone is in general agreement with earlier studies by us and others (see review by Raeburn *et al.*, 1993). It is worth noting, though, that selective inhibitors of the type III PDE relax guinea-pig trachealis *in vitro*, but with a lower potency (Tomkinson *et al.*, 1993). Similarities between guinea-pig and human airways preparations *in vitro* regarding PDE isoenzyme profiles and relative functional importance (Qian *et al.*, 1993; DeBoer *et al.*, 1993) predict that RP 73401 will also relax human isolated airways preparations.

RP 73401 significantly reduced the influx of eosinophils into the airways following antigen exposure in sensitized guinea-pigs and rats. RP 73401 was however twice as potent as rolipram (Karlsson *et al.*, 1993). PDE inhibitors may act in several ways to reduce cell trafficking into the airways (see review by Karlsson *et al.*, 1993). They may suppress antigen-induced release of chemotactic mediators from pro-inflammatory cells, inhibit endothelial cell contractility (thereby maintaining the integrity of the microvenular endothelium) or inhibit the expression of selectin and integrin adhesion molecules on either the endothelium or the leukocyte itself. It is not clear why RP 73401 was more effective in the guinea-pig than in the rat. This may have been a consequence of different challenge protocols, necessitated by the different sensitivities of the species to antigen challenge. In addition to this reduction in BAL fluid inflammatory cell numbers,

preliminary data indicate that RP 73401 also reduces inflammatory cell numbers and their degree of activation in lung tissues (unpublished observations). The reduced cell influx would further reduce granulocyte-derived chemotactic and inflammatory mediator content in the lung thereby reducing inflammatory changes. This is likely to occur since RP 73401 potently inhibits superoxide generation and the release of cytotoxic proteins from human granulocytes and guinea-pig eosinophils *in vitro* (Karlsson *et al.*, 1993; Ashton *et al.*, 1994).

Plasma extravasation from postcapillary venules of the tracheobronchial microcirculation induced by histamine and other pro-inflammatory mediators, has been reported as a potent pro-inflammatory mechanism in human asthmatic airways (Persson, 1988). These mediators may act directly to produce contraction of endothelial cells, a prerequisite for plasma extravasation. RP 73401 inhibited microvascular leakage in the guinea-pig which is in agreement with our earlier findings that PDE IV (rolipram) and PDE V (zaprinast) inhibition prevents PAF-induced MVL in the guinea-pig lung (Raeburn & Karlsson, 1993). Interestingly, RP 73401 was more potent in inhibiting leakage (this study) than rolipram (no effect at 50 μ g, i.t., Raeburn & Karlsson, 1993) although PAF was used as the stimulus in the latter study. MVL into the airway lumen (lavage fluid) was more readily inhibited than into the tracheobronchial tissues which suggests that RP 73401 more effectively inhibits epithelial than endothelial membrane permeability, possibly as a consequence of PDE isoenzyme distribution. The PDE isoenzyme profile in the endothelium of the airway microvasculature is not known but, in bovine and porcine aorta, PDE II and IV predominate whereas PDE III may be important in the pulmonary artery (see Raeburn & Karlsson, 1993 and references therein). Our data with RP 73401 indicate that PDE IV is of major importance in inhibiting endothelial cell contractility in the airway microcirculation.

In addition to the anti-inflammatory effects reported above, low doses of RP 73401 prevented PAF-induced BHR in the guinea-pig. PAF, a product of several inflammatory cell types, is a potent chemoattractant and activator of a range of inflammatory cells including eosinophils. It seems likely that the suppression of BHR reflects the potent inhibitory effects of RP 73401 on mediator release from granulocytes and monocytes (Karlsson *et al.*, 1993; Ashton *et al.*, 1994) rather than a direct antagonism of PAF itself. Even though PAF may not be an important mediator in human asthma, airway hyperresponsiveness is a characteristic feature and drugs such as RP 73401 with widespread anti-inflammatory actions may reduce BHR in asthmatic subjects.

Since RP 73401 is a selective inhibitor of type IV PDE, our results confirm that this isoenzyme is functionally important in modulating inflammation and bronchoconstriction in the guinea-pig and rat. Type III PDE also has a significant role in modulating airways smooth muscle contractility. However, PDE type III inhibitors have been shown to induce positive inotropism and vasodilatation whereas PDE type IV inhibitors are not often associated with such cardiovascular effects (Nicholson & Shahid, 1994). Indeed, whereas both type III and type IV PDE inhibitors have been shown to induce bronchodilatation in the dog, only the latter did not produce cardiovascular side-effects at doses which produced bronchodilatation (Heaslip *et al.*, 1991). In our study, RP 73401 did not cause significant changes in arterial blood pressure or heart rate when administered to anaesthetized rats or guinea-pigs at doses producing significant inhibition of bronchospasm. We have demonstrated that, following i.t. administration of RP 73401 (500 μ g kg⁻¹) to rats, peak plasma levels are reached after 5 min (97 $\pm$ 44 nM), remain high after 15 min (79 $\pm$ 40 nM) and thereafter decrease to 3 $\pm$ 1 nM after 6 h (data on file with Rhône-Poulenc Rorer Ltd.). This data demonstrates that, following i.t. administration, RP 73401 achieves a concentration in the systemic circulation which is greater than the IC₅₀ for inhibition of

PDE type IV. The lack of cardiovascular effects in our studies would therefore seem to be due to tissue PDE subtype selectivity rather than low systemic availability. Thus, specific type IV PDE inhibitors such as RP 73401 may be more selective and useful for the treatment of asthma than specific type III inhibitors or mixed type III/IV inhibitors. Furthermore, PDE III inhibitors such as siguazodan have no effect on airways MVL (Raeburn & Karlsson, 1993) and only poorly inhibit antigen-induced cell influx into BAL fluid (Karlsson *et al.*, 1993).

Asthma is an inflammatory disease of the airways where the prominent symptoms are a reversible airflow obstruction combined with an increased responsiveness to non-specific bronchoconstrictor stimuli. Inflammatory changes in the asthmatic airway include microvascular plasma protein leakage and oedema, mucous gland hyperplasia, smooth muscle hypertrophy, sub-epithelial fibrosis, luminal mucus and cellular debris, leukocyte (predominantly eosinophils) infiltration of the bronchial mucosa and epithelial cell damage and/or sloughing (Djukanovic *et al.*, 1990; Alexander *et al.*, 1991). Release of mast cell mediators such as histamine and possibly leukotrienes plays an important role in immediate bronchoconstriction; eosinophils, macrophages, lymphocytes and other inflammatory cells are more usually associated with late phase events and chronic disease (Djukanovic *et al.*, 1990; Alexander *et al.*, 1991).

Glucocorticosteroids are currently the most effective drugs

available to treat airway inflammation. The number and activity of infiltrating inflammatory cells, particularly eosinophils, are reduced and microvascular leakage (and thereby oedema) and other signs of the inflammatory response are dampened (Barnes & Pedersen, 1993). Glucocorticosteroids do not however inhibit the release of mast cell mediators, have no direct bronchodilator activity and, when given for prolonged periods, are associated with significant systemic adverse effects (Barnes & Pedersen, 1993). A drug with combined anti-inflammatory and bronchodilator actions would therefore provide additional benefits in the treatment of asthma. The *in vitro* and *in vivo* studies described here demonstrate RP 73401 to possess many of these desirable actions at least in animal models of allergic inflammation.

In conclusion, RP 73401 has bronchodilator activity and is a functional antagonist of the bronchoconstriction produced by asthma mediators. More importantly, its potent anti-inflammatory actions reduce those facets of allergic pulmonary inflammation in the guinea-pig and rat which are characteristic of human asthma. Its potency and selectivity make RP 73401 an ideal tool to investigate the functional importance of the PDE IV isoenzyme in biological systems. RP 73401 is a promising new agent for the treatment of asthma and other inflammatory disease of human airways.

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