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Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea-pig by CDP840, a novel phosphodiesterase type 4 inhibitor

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1 The activity of CDP840, a novel, potent and selective cyclic nucleotide phosphodiesterase type 4 (PDE 4) inhibitor, was evaluated in guinea-pig models (*in vitro* and *in vivo*) of bronchospasm, ozone-induced airway hyperresponsiveness (AHR) and non-cholinergic bronchoconstriction. Comparisons were made with (i) other PDE 4 inhibitors: CT1731 (S-enantiomer of CDP840), rolipram, RP73401 and (ii) the clinically used agents salbutamol and theophylline.

2 CDP840 relaxed isolated trachea, under basal tone (EC_{50} 4.5 ± 1.1 μM) being 17 fold less potent than rolipram (EC_{50} 0.26 ± 0.13 μM) but attaining the same E_{max} ($83 \pm 6\%$ of the response to 300 μM papaverine).

3 CDP840 relaxed tracheae pre-contracted with carbachol (IC_{25} 39 ± 9 μM) and histamine (IC_{25} 4 ± 1 μM) producing monophasic curves. Stereoselectivity was not observed with CT1731 against either carbachol (IC_{25} 33 ± 11 μM) or histamine (IC_{25} 17 ± 10 μM). Aminophylline was 1.6 fold (carbachol) and 11 fold (histamine) less potent than CDP840. Rolipram and RP73401 produced tri-phasic relaxation curves but were of similar potency (at the IC_{25} level) to CDP840 against carbachol (rolipram 18 ± 5 μM, RP73401 39 ± 1 μM) whereas against histamine they were approximately 20 fold more potent (rolipram 0.2 ± 0.1 μM, RP73401 0.2 ± 0.1 μM). In producing $>30\%$ (carbachol) and $>60\%$ (histamine) relaxation these inhibitors had similar potency and were poor compared to salbutamol.

4 Pre-incubation with CDP840 (10 μM) did not antagonize histamine-induced contraction of isolated trachea; however, it did cause a slight potentiation of the subsequent relaxation to salbutamol (IC_{50} 23 ± 1 to 15 ± 2 nM)

5 Pretreatment (1 h) with either CDP840 (1 mg kg^{-1}, i.p. or 3 mg kg^{-1}, i.v.) or rolipram (1 mg kg^{-1}, i.p.) did not bronchodilate or antagonize bronchospasm due to inhaled histamine in anaesthetized, ventilated guinea-pigs. Salbutamol (1 mg kg^{-1}, i.p.) did not bronchodilate but caused a parallel 7 fold rightward shift in the histamine dose-response curve.

6 Stimulation of the vagus nerve in the presence of atropine resulted in a frequency-related bronchoconstriction. CDP840 and rolipram (i.v.) inhibited the response being $\sim$equipotent (EC_{50} ~ 10 μg kg^{-1}). Neither drug inhibited bronchospasm to inhaled substance P.

7 CDP840 ($1-10$ μg kg^{-1} i.p.) dose relatedly inhibited ozone-induced bronchoconstriction. CT1731 (1 mg kg^{-1}), rolipram (1 mg kg^{-1}), RP73401 (10 μg kg^{-1}) and aminophylline (10 mg kg^{-1}) had no effect. Ozone-induced AHR to inhaled histamine was inhibited by CDP840 in a dose-related manner, 10 μg kg^{-1} abolishing the AHR. This effect was stereoselective as CT1731 was ~ 30 fold less potent than CDP840. Rolipram was ~ 100 fold less potent and RP73401 and aminophylline had no effect. CDP840 was orally active being ~ 10 fold less potent compared to i.p. administration.

8 CDP840 is a poor spasmolytic and anti-spasmogenic agent in response to exogenous mediators; however, it potently inhibits vagally mediated non-cholinergic bronchoconstriction and ozone-induced AHR to histamine. It is possible that regulation of cyclic AMP by PDE 4 contributes to neuronal sensitivity in the airways. Furthermore, CDP840 may suppress AHR without being an overt bronchodilator. Such a profile of activity may have therapeutic benefit in airways diseases such as asthma.

Keywords: CDP840; phosphodiesterase inhibitor; bronchospasm; ozone; airway hyperresponsiveness; rolipram; non-cholinergic bronchoconstriction; vagal stimulation; tachykinins

Introduction

Asthma is a disease characterized by airway inflammation, reversible airways obstruction and airway hyperresponsiveness (AHR) to various stimuli. To account for these conditions multi-component mechanisms involving sensory nerves, inflammatory leukocytes such as T lymphocytes and eosinophils and their derived mediators have been proposed (Barnes, 1989). Successful therapy may therefore require multiple sites of action.

Much interest is currently being directed at inhibitors of cyclic nucleotide phosphodiesterase (PDE) for the treatment of asthma (see Nicholson & Shahid, 1994, for review). In particular, the PDE type 4 (PDE 4, Beavo & Reifsnyder, 1990) inhibitors appear to have great potential with reports of; bronchodilator activity (Harris *et al.*, 1989; Cortijo *et al.*, 1993), inhibition of pulmonary eosinophilia (Underwood *et al.*, 1994), inhibition of allergic bronchoconstriction (Underwood *et al.*, 1993), modulation of non-adrenergic non-cholinergic airway tone (Qian *et al.*, 1994; Holbrook *et al.*, 1995) and inhibition of AHR (Holbrook & Hughes, 1992; Turner *et al.*, 1994; Gozzard *et al.*, 1996a,b) in animal models.

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We now describe the activity of the novel, potent (IC_{50} = 4 nM vs PDE 4A) and selective (> 10,000 fold vs other PDE families) PDE 4 inhibitor, CDP840 (**R**-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenylethyl] pyridine) (Hughes *et al.*, 1995; 1996) in several models in the guinea-pig. CDP840 was evaluated for its ability to relax airway smooth muscle, inhibit bronchoconstriction and relax bronchospasm in response to exogenous spasmogens. To investigate neuronal mechanisms, activity against non-cholinergic bronchoconstriction was assessed. Finally, its activity in a model of ozone-induced AHR was studied. In these experiments CDP840 was compared with its S-enantiomer, CT1731, which is 10–50 times less active against PDE 4 isoforms, in order to investigate the correlation between enzyme inhibition and suppression of functional activity. The archetypal PDE 4 inhibitor, rolipram (Schwabe *et al.*, 1976) and the recently described highly potent PDE 4 inhibitor RP73401 (Raeburn *et al.*, 1994) were also used for comparison. Some of these studies have been presented to the British Pharmacological Society (Hughes *et al.*, 1995)

Methods

Male Dunkin Hartley guinea-pigs (400–500 g from Harlan Olac) were used for all experiments.

Isolated trachea

Animals were killed by cervical dislocation and exsanguinated by cutting the cervical blood vessels. Tracheae were removed and dissected transversely into sections 5–6 cartilage rings in length. These were suspended between two metal hooks which passed through the lumen of the trachea, in 10 ml organ baths containing Krebs solution at 37°C, pH 7 and gassed with 5% CO_2 in O_2. The composition of the Krebs solution was (mM): NaCl 118, $NaHCO_2$ 25, KH_2PO_4 1.18, KCl 4.83, $MgSO_4$ 1.17, $CaCl_2$ 2.54, D-glucose 11. A resting tension of 2 g was applied and changes in isometric force of contraction measured with force displacement transducers (Grass FT03), amplified and displayed on a chart recorder (Gould 3800). Tissues were allowed to equilibrate with frequent washing for 1 h.

To examine the effect of drugs on basal tone tissues were first contracted with a supra-maximal concentration of carbachol (10 μM, determined from preliminary experiments) which was subsequently washed from the tissues and tension allowed to return to basal tone. CDP840, rolipram or their respective solvents were then added in a cumulative manner. At the end of the experiment papaverine (300 μM) was added to elicit a maximal relaxation and results expressed as a percentage of this maximum.

Spasmolytic activity was assessed in tracheae precontracted with either histamine (10 μM) or carbachol (0.3 μM). These concentrations were determined from preliminary experiments to elicit 70% of their respective maximum contraction (EC_{70}) resulting in a developed tension of 1.6±0.3 g (n=10) and 3.4±0.6 g (n=10) for histamine and carbachol, respectively. Developed tension was allowed to stabilize (15–20 min) before the cumulative addition of CDP840, CT1731, rolipram, RP73401, aminophylline or salbutamol. At the end of the experiment papaverine (300 μM) was added to elicit maximum relaxation. Results are expressed as a percentage of this maximum.

Anti-spasmogenic activity was investigated by performing a dose-response curve to histamine and then washing to regain resting tension before incubating tissues with CDP840 (10 μM), or solvent for 60 min before the cumulative addition of histamine so as to construct a full concentration-response curve (CRC). Data are expressed as a percentage of the pretreatment maximum response.

To determine if CDP840 could interact with a β_2-sympathomimetic, tissues were incubated for 1 h with CDP840 (10 μM) or its solvent before constriction with EC_{70} concentrations of either carbachol or histamine. Salbutamol was then added in a cumulative manner (1 to 1,000 nM). At the end of the experiment papaverine (300 μM) was added to elicit maximal relaxation; data are expressed as a percentage of this maximum.

Measurement of lung function in anaesthetized guinea-pigs

Anaesthesia was induced with sodium pentobarbitone (40 mg kg^{-1}, i.p.) and maintained with further doses (2–5 mg kg^{-1}, i.v.) when required. A tracheostomy was performed between the 2nd and 3rd cartilage rings from the larynx, a polythene cannula (i.d. 4 mm) inserted and animals ventilated with room air (10 ml kg^{-1}, 54 breaths min^{-1}, Harvard small animal ventilator). A heated (37°C) pneumotachograph (Fleish 000) was positioned in the ventilator circuit to measure inspiratory and expiratory flow rate. The inspiratory limb also contained a nebulizer (DeVilbiss 8500U) to allow the generation of aerosols which could be carried into the airways with inspired air. A side arm from the tracheal cannula was attached to the positive port of a differential pressure transducer (Validyne MP45) and used to measure pulmonary inflation pressure (PIP). The negative port of the transducer was attached to a cannula (venflon 2, 2 mm, 14G) inserted through the 4th intercostal space in the right side of the thorax, such that its tip lay in the intra-pleural space. This was used to measure intra-thoracic pressure. The difference between these two pressures was transpulmonary pressure (TPP).

A jugular vein was cannulated (Portex 3FG) to allow anaesthetic and drug administration. Arterial blood pressure was measured by a transducer (Viggo-spectromed P10EZ) connected to a saline-filled cannula (Portex 4FG) inserted into the left carotid artery. Body temperature was monitored via a rectal probe and maintained at 38±0.5°C by a heated blanket. Signals for airflow, TPP and arterial blood pressure were amplified (PMS 800, Mumed Ltd) and fed via an analogue to digital converter to a personal computer. Lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated according to the method of Amdur & Mead (1958) by use of PMS dual software. For some experiments PIP was used as an index of lung function. In these instances signals were recorded by a chart recorder (Gould 3800).

Ozone-induced airway hyperresponsiveness to inhaled histamine

Guinea-pigs received drug or solvent (i.p. or p.o.) 60 min before being placed in a perspex chamber (50 × 30 × 30 cm) through which room air enriched to 3.0±0.5 p.p.m. ozone was pumped (15 l min^{-1}). For air challenge, normal room air was pumped through the chamber. After 30 min animals were removed from the chamber and prepared for analysis of lung function. Animals were allowed to stabilize (15 min) before their lungs were hyperinflated by occluding expired airflow for three breaths. Five minutes after hyperinflation a solution of histamine was nebulized for 10 s and the resulting aerosol carried into the airways with inspired air. After 10 min, the lungs were hyperinflated and after a further 5 min the spasmogen, at double the preceding concentration, was delivered. This was repeated with double the concentrations until R_L or C_{dyn} did not return to pre-histamine challenge values after hyperinflation.

Vagally mediated non-cholinergic bronchoconstriction

Guinea-pigs were prepared for the measurement of lung function as described above. In addition, both vagi were carefully isolated and tied at the level of the fifth cervical ganglion. The vagi were laid over two platinum electrodes and bathed in liquid paraffin to prevent dessication. Atropine (1 mg kg^{-1}, i.v.) was administered and 30 min later the vagi

stimulated (Digitimer D59A 5V square wave with a 5 ms pulse width) for 30 s at 1 Hz. This was repeated at 3 Hz and 6 Hz with 15 min between stimulations to produce a frequency-response curve. After a further 15 min drug or solvent was administered (1 ml kg^{-1}, i.v.) and 30 min later the frequency-response curve repeated. Data are expressed as a percentage of the maximum bronchoconstriction elicited with the pre-drug frequency-response curve.

Data analysis

Data are expressed as mean ± s.e.mean for n observations. For *in vitro* studies the concentrations of drug which inhibited the response by 25% (IC_{25}) or 50% (IC_{50}) were obtained by interpolation from concentration-response curves. For *in vivo* studies the $\log_{10}$ of the provocative dose of spasmogen to increase airways resistance (R_L) by 200% or decrease dynamic compliance (C_{dyn}) by 50% was obtained by interpolation from dose-response curves. The slope of the R_L curve was obtained by linear regression. Statistical analysis was performed by analysis of variance followed by Student's t test for parametric analysis and Wilcoxon ranked test for non-parametric analysis as appropriate. Significance was accepted if $P<0.05$.

Materials

Histamine, carbachol, substance P, acetylcholine, aminophylline, salbutamol, papaverine and atropine were all obtained from Sigma. Sodium pentobarbitone (Sagatal) was obtained from Rhone Merieux. CDP840, CT1731, (3-cyclopentyloxy -*N*-(3,5-dichloro-4-pyridyl)-4-methoxyhenzamide) (RP743401) and rolipram were all synthesized by the Department of Medicinal Chemistry, Celltech Therapeutics Ltd.

Results

Relaxation of basal tone

Both CDP840 and rolipram relaxed tracheae under basal tone, CDP840 being approximately 17 fold less potent ($P<0.01$) in exerting up to 50% relaxation with an EC_{50} of 4.5 ± 1.1 μM compared to 0.26 ± 0.13 μM for rolipram. Rolipram, however, displayed a very shallow CRC and both compounds were approximately equipotent in eliciting >60% relaxation attaining 83 ± 6% (rolipram) and 83 ± 5% (CDP840) at the highest concentration tested (100 μM) (Figure 1).

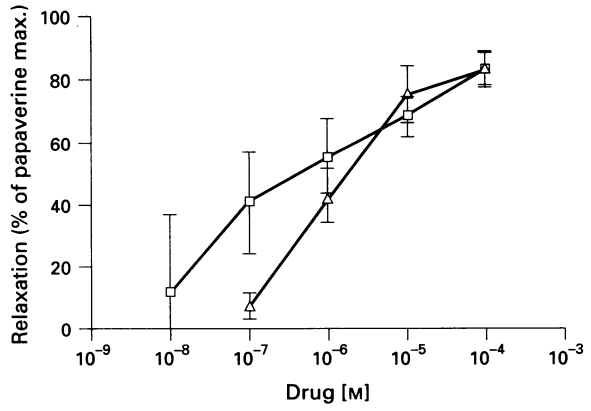

Figure 1 The effect of rolipram (□) and CDP840 (△) on guinea-pig isolated tracheae under basal tone. Data are the mean ± s.e.mean, $n=6$.

Spasmolytic activity

The ability of CDP840 compared to CT1731, RP73401, rolipram, aminophylline and salbutamol to relax carbachol or histamine pre-contracted tracheae is displayed in Figure 2a and b. Rolipram and RP73401 exhibited a tri-phasic concentration-response curve against both spasmogens with a plateau occurring between 0.1 and 10 μM, a relevant point before this plateau (EC_{25}) was therefore chosen for comparison. In contrast, CDP840, CT1731 and aminophylline produced shallow monophasic curves lacking the initial phase of relaxation exhibited by rolipram and RP73401. Against carbachol, rolipram and RP73401 were almost equipotent as were CDP840 and CT1731 which were 10 to 100 fold less potent in achieving up to 25% relaxation. All four compounds were comparable in eliciting a greater relaxation. All compounds were more potent against a histamine- than a carbachol-induced contraction with the first phase of the rolipram and RP73401 curves attaining greater than 40% relaxation; rolipram being 25 fold more potent than CDP840 in attaining 25% relaxation. Although CDP840 tended to be more potent than its enantiomer, CT1731, no significant difference was observed. In this model aminophylline was the least potent whereas none of the compounds approached the activity of salbutamol against either spasmogen (Table 1).

Anti-spasmogenic activity

Pre-incubation of tissues with CDP840 (10 μM) did not antagonize histamine-mediated contraction. Histamine developed a maximum tension of 2.0 ± 0.2 g ($n=6$) for solvent and 2.1 ± 0.6 g ($n=6$) for CDP840-treated tissues resulting in a

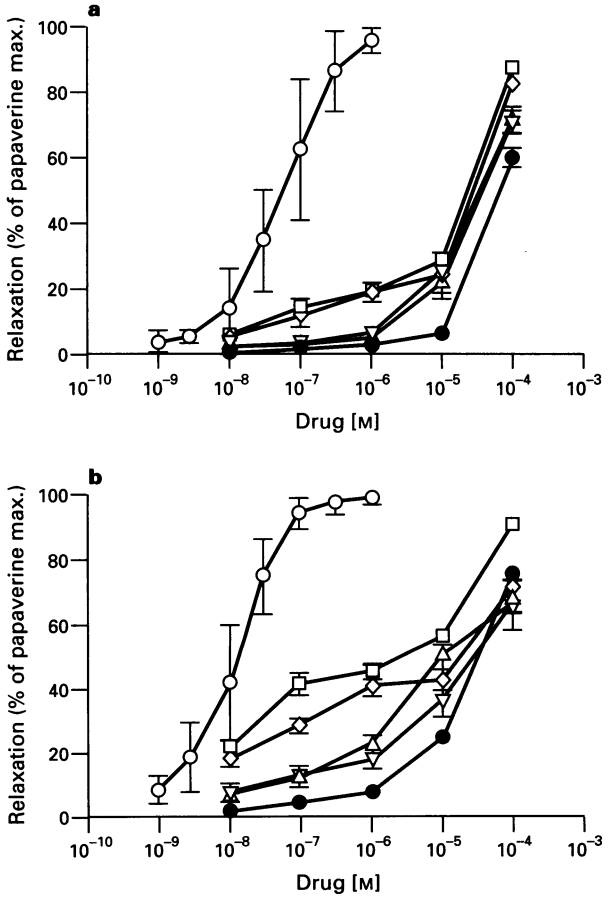

Figure 2 The relaxant effects of CDP840 (△), CT1371 (▽), rolipram (□), RP73401 (◇), aminophylline (●) and salbutamol (○) in guinea-pig tracheae precontracted with (a) carbachol or (b) histamine. Data are the mean ± s.e.mean, $n=6$.

105±6% and a 114±8% increase, respectively. The potency of histamine was also unaltered with IC_{50} values of 14.1±3.2 μM in the absence and 13.3±2.5 μM in the presence of CDP840.

Interaction with salbutamol

Pre-incubation of tissues with CDP840 (10 μM) before constriction with either histamine or carbachol (EC_{70}) potentiated the subsequent relaxant activity of salbutamol. This was evident by a slight, yet significant, reduction in the IC_{50} from 132.2±27.9 nM to 80.1±21.7 nM ($P<0.05$) for carbachol and from 22.9±1.3 nM to 15.5±2.0 nM ($P<0.01$) for histamine. Since salbutamol itself produced 100% relaxation no increase in E_{max} was possible (Figure 3).

In vivo *anti-spasmogenic studies*

None of the drug pretreatments had any effect on haemodynamics or basal airways tone when measured 1 h after i.p. administration (Table 2). In addition CDP840 had no effect on these parameters either acutely or over the following hour when administered at 3 mg kg^{-1}, i.v. (data not shown).

Inhalation of histamine induced a dose-related bronchoconstriction resulting in a 535±76% increase in R_L in control animals. This was unaffected by CDP840 (3 mg kg^{-1}) or rolipram (1 mg kg^{-1}) pretreatment, whereas salbutamol (1 mg kg^{-1}) caused a 7 fold parallel rightward shift in the dose-response curve and hence an increase in the $logPD_{200}$ from 2.17±0.07 (control) to 3.00±0.1 (salbutamol) (Figure 4). Inhalation of substance P also induced a dose-related bronchoconstriction resulting in a maximum increase in R_L of 498±58% and a $logPD_{200}$ of 3.3±0.1. Neither rolipram nor CDP840 (1 mg kg^{-1}, i.v.) administered 30 min before inhalation of substance P had any effect, with $logPD_{200}$ values of 3.5±0.1 and 3.4±0.1, respectively.

Non-cholinergic bronchoconstriction Stimulation of the vagus nerve elicted a frequency-related bronchoconstriction resulting in an increase in R_L from a basal level of 118±7 $cmH_2O\ l^{-1}\ s^{-1}$ to 185±16 $cmH_2O\ l^{-1}\ s^{-1}$ at 1 Hz, 406±52 $cmH_2O\ l^{-1}\ s^{-1}$ at 3 Hz and 703±85 $cmH_2O\ l^{-1}\ s^{-1}$ at 6 Hz ($n=22$). Following the administration of solvent for CDP840 this curve could be repeated being almost superimposable with

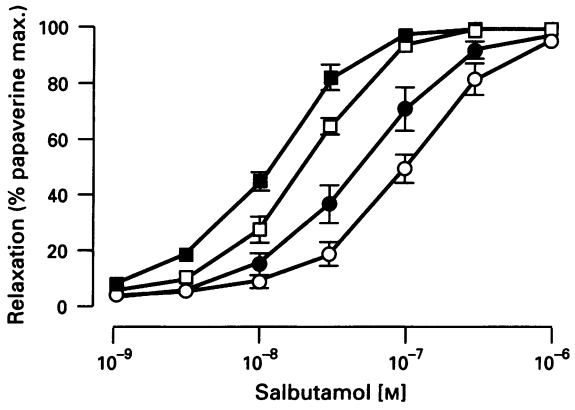

Figure 3 The relaxant activity of salbutamol in guinea-pig tracheae precontracted with either carbachol (○, ●) or histamine (□, ■) in the presence (solid symbols) or absence (open symbols) of 10 μM CDP840. Data are the mean±s.e.mean, $n=6$.

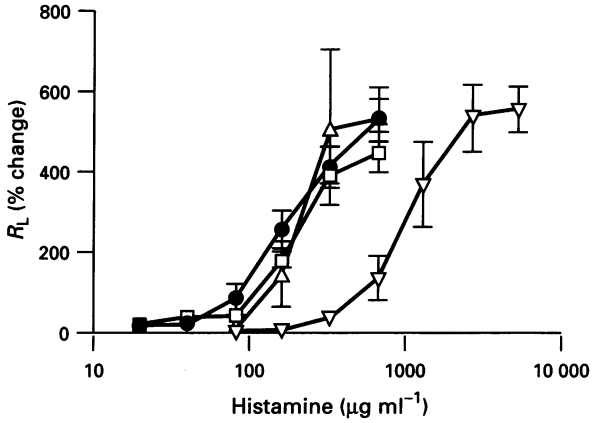

Figure 4 The effect of pretreatment with vehicle (●), 3 mg kg^{-1} CDP840 (△), 1 mg kg^{-1} rolipram (□) or 1 mg kg^{-1} salbutamol (▽) given i.p. 1 h before the inhalation of histamine, on lung resistance. Data are the mean±s.e.mean, $n=6-10$.

Table 1 Efficacy and potency of the compounds under study for relaxing carbachol or histamine precontracted guinea-pig isolated tracheae

	Carbachol		*Histamine*	
Treatment	E_{max} (%)	EC_{25} (μM)	E_{max} (%)	EC_{25} (μM)
CDP840	74.3±1.8	38.7±8.9	68.9±5.0	3.7±1.2
CT1731	71.9±3.4	33.3±10.8	65.6±6.4	16.6±9.6
Rolipram	88.2±1.6	17.8±5.4	91.0±1.5	0.15±0.1
RP73401	83.3±1.5	39.3±0.4	72.5±4.3	0.18±0.1
Aminophylline	61.2±2.5	62.9±0.6	76.4±1.4	40.2±9.0
Salbutamol	99.0±3.9	0.02±0.001	98.9±2.0	0.005±0.0003

Data are mean±s.e.mean, $n=6-8$. E_{max}: maximum relaxation as a percentage of that obtained with papaverine (300 μM); EC_{25}: the concentration of drug required to relax trachea to 25% of that obtained with papaverine (300 μM).

Table 2 The effect of drug pretreatment on cardiovascular and pulmonary parameters in anaesthetized guinea-pigs

Pretreatment (mg kg^{-1})	*HR* (beats min^{-1})	*MAP* (mmHg)	R_L ($cmH_2O\ l^{-1}\ s^{-1}$)	C_{dyn} (ml cmH_2O^{-1})
Solvent	287±4	58±4	102±6	0.93±0.19
CDP840 (3)	299±11	59±6	99±11	1.05±0.03
Rolipram (1)	301±6	65±5	97±10	0.96±0.18
Salbutamol (1)	270±8	59±3	93±9	1.11±0.05

Data are mean±s.e.mean, $n=6-10$, of measurements taken 60 min post-treatment. HR (heart rate), MAP (mean arterial pressure), R_L (lung resistance), C_{dyn} (dynamic compliance).

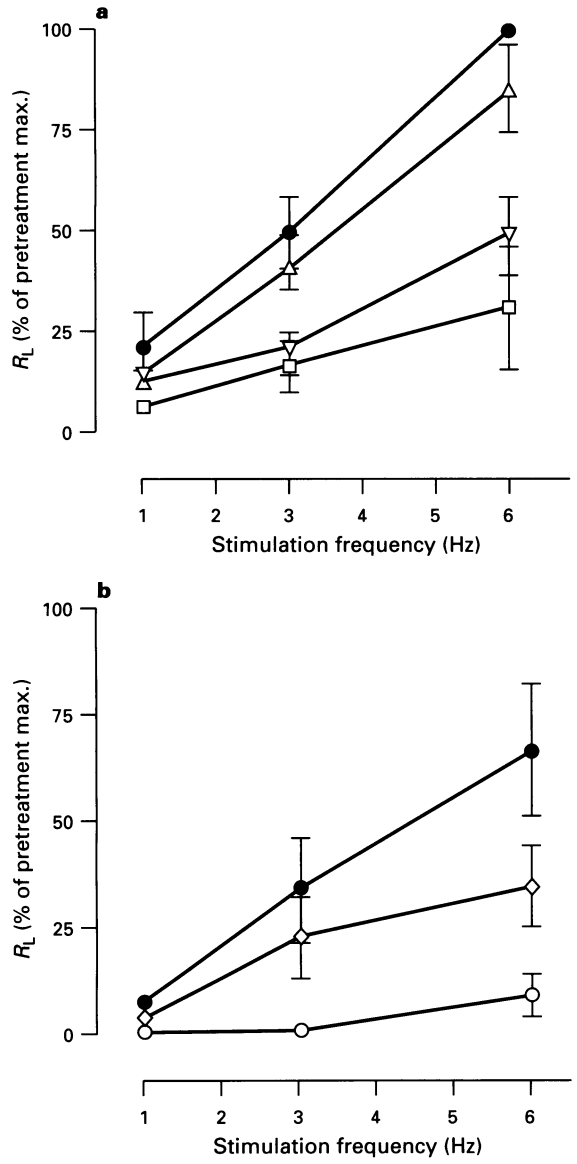

Figure 5 The effect of pretreatment (i.v.) with either: (a) vehicle (●) or CDP840 at (μg kg^{-1}) 1 (△), 10 (▽) and 100 (□) or (b) vehicle (●) or rolipram (μg kg^{-1}) at 10 (◇) and 100 (○) on bronchoconstriction due to vagal stimulation. Data are the mean ± s.e.mean, $n=6$, expressed as a percentage of the pretreatment increase in lung resistance at a stimulation frequency of 6 Hz.

the first, whereas treatment with CDP480 caused a dose-related flattening of the curve with 10 and 100 μg kg^{-1} shifting the curve to the right. Similar activity and potency were observed with rolipram, 100 μg kg^{-1} almost abolishing the response even at 6 Hz (Figure 5a and b).

Ozone-induced AHR

Ozone challenge precipitated a bronchoconstriction with an increase in R_L and a decrease in C_{dyn} of approximately 44% and 35%, respectively. Pretreatment with CDP840 potently attenuated this effect with 10 μg kg^{-1} maintaining R_L and 3 μg kg^{-1} C_{dyn} at sham levels. CT1731, rolipram, RP73401 and aminophylline had no such effect even at > 100 fold higher doses (Table 3).

Ozone challenge also resulted in a hypersensitivity to subsequent histamine inhalation indicated by a leftward shift in the R_L and C_{dyn} curves by approximately 8 fold and 10 fold, respectively. In addition hyperreactivity was evident by the

Table 3 The effect of ozone and drug pretreatment on airways tone in anaesthetized guinea-pigs

Pretreatment (μg kg^{-1})	*Air/O_3*	R_L ($cmH_2O\ l^{-1}\ s^{-1}$)	C_{dyn} ($ml\ cmH_2O^{-1}$)
Solvent	Air	102 ± 6	0.83 ± 0.09
Solvent	O_3	[a]147 ± 11	[a]0.54 ± 0.08
CDP840 (1)	O_3	146 ± 13	0.58 ± 0.12
CDP840 (3)	O_3	153 ± 10	**0.92 ± 0.10
CDP840 (10)	O_3	*119 ± 11	*0.87 ± 0.10
Rolipram (1,000)	O_3	164 ± 10	0.50 ± 0.20
CT1731 (1,000)	O_3	161 ± 15	0.47 ± 0.08
RP73401 (10)	O_3	166 ± 26	0.30 ± 0.05
Aminophylline (10,000)	O_3	192 ± 11	0.31 ± 0.03

Data are mean ± s.e.mean, $n=6-24$ of measurements taken post-ozone or air challenge. R_L (lung resistance), C_{dyn} (dynamic compliance).
*$P<0.05$, **$P<0.01$ compared to solvent + ozone,
[a]$P<0.01$ compared to solvent + air, Student's t test.

increase in the maximum response and by an 8 fold increase in the slope of the R_L curve. Taken together these constitute AHR.

CDP840 inhibited the AHR in a dose-related manner both with respect to R_L and C_{dyn}. A dose of 3 μg kg^{-1} caused a significant flattening and shifting of the R_L curve to the right while 10 μg kg^{-1} caused complete abolition (Figure 6a, Table 4). The S-enantiomer of CDP840, CT1731, was less potent in this model with 1,000 μg kg^{-1} causing similar protection as 3 μg kg^{-1} of CDP840 (Figure 6d, Table 4). Rolipram also reduced the AHR but was approximately 100 fold less potent, requiring 100 μg kg^{-1} for significant activity and 1,000 μg kg^{-1} to abolish the effects on both R_L and C_{dyn} (Figure 6c, Table 4). RP73401 (10 μg kg^{-1}) and the non-selective PDE inhibitor aminophylline (10 mg kg^{-1}) had no effect (Table 4).

CDP840 also had oral activity in this model being 10 to 30 fold less potent via this route with 100 μg kg^{-1} eliciting approximately 50% inhibition of the AHR (Figure 6b, Table 5).

Discussion

CDP840 is a potent inhibitor of ozone-induced AHR to inhaled histamine and vagally mediated non-cholinergic bronchoconstriction while being a relatively poor bronchodilator to exogenous spasmogens in the guinea-pig.

Experiments with isolated tracheae from the guinea-pig demonstrated CDP840 to be a poor spasmolytic, being 11 fold more potent than aminophylline but being 25 fold less potent than rolipram and 740 fold less potent than the β_2-sympathomimetic salbutamol against histamine-induced spasm. The data obtained with these reference agents are in agreement with that previously published with aminophylline and rolipram (Harris *et al.*, 1989; Cortijo *et al.*, 1993) and salbutamol (Ball *et al.*, 1991). In addition, in common with the data published with rolipram (Underwood *et al.*, 1994), CDP840 did not antagonize exogenously added histamine demonstrating that it is not a histamine antagonist. Although CDP840 is a relatively poor anti-spasmogenic or spasmolytic to exogenous histamine or carbachol, it is a potent inhibitor of antigen-induced bronchospasm (Hughes *et al.*, 1996; Gozzard *et al.*, 1996a).

Notably CDP840 and CT1731 produced shallow monophasic spasmolysis curves rather than tri-phasic curves as observed with rolipram and RP73401. This characteristic rolipram curve is well documented with the studies cited above describing a plateau in the 0.1 to 10 μM range and potency and activity similar to that described here. In the current study comparisons were made at points during the two exponential

phases of the rolipram curve to allow for multiple mechanisms of action. In producing greater than 30% (carbachol) and 60% (histamine) relaxation the PDE4 inhibitors were comparable but had poor potency relative to salbutamol. Clearly the first phase of rolipram- and RP73401-induced relaxation provides reasonable potency but with limited efficacy, although the relevance to the clinical situation is still to be evaluated.

We have previously shown in this model that the triphasic

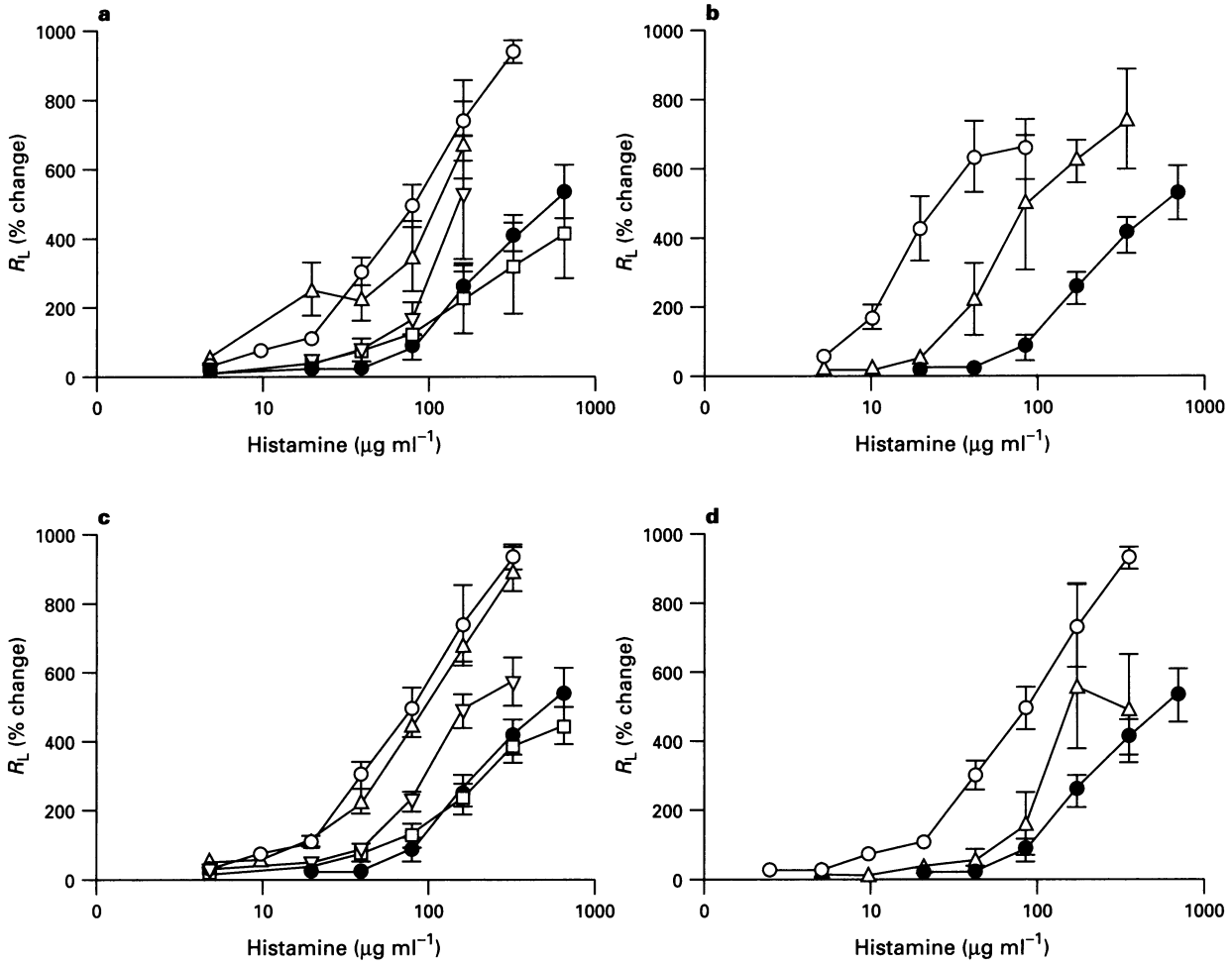

Figure 6 The effect of inhaled histamine on lung resistance (R_L) in anaesthetized guinea-pigs pretreated with: (a) solvent and air challenge (●) or CDP840 at (μg kg^{-1}, i.p.) 1 (△), 3 (▽), 10 (□) or solvent (○) and ozone challenge; (b) solvent and air challenge (●) or CDP840 (μg kg^{-1}, p.o.) 100 (△) or solvent (○) and ozone challenge; (c) solvent and air challenge (●) or rolipram (μg kg^{-1}, i.p.) 10 (△), 100 (▽), 1,000 (□) or solvent (○) and ozone challenge; (d) solvent and air challenge (●) or CT1731 (μg kg^{-1}, i.p.) 1,000 (△) or solvent (○) and ozone challenge. Data are the mean ± s.e.mean, $n = 6-24$.

Table 4 The effect of ozone and drug pretreatments (i.p.) on airway hyperresponsiveness to inhaled histamine in the anaesthetized guinea-pig

Pretreatment (μg kg^{-1}, i.p.)	*Air/O_3*	R_L *slope*	R_L *logPD_{200}*	C_{dyn} *logPD_{50}*
Solvent	Air	4.7 ± 0.9	2.17 ± 0.07	2.01 ± 0.10
Solvent	O_3	[a]36.4 ± 8.8	[a]1.26 ± 0.09	[a]0.99 ± 0.06
CDP840 (1)	O_3	19.5 ± 6.3	1.54 ± 0.2	1.22 ± 0.07
CDP840 (3)	O_3	**7.16 ± 1.8	***1.95 ± 0.09	**1.58 ± 0.14
CDP840 (10)	O_3	***2.97 ± 0.8	***2.25 ± 0.22	***1.98 ± 0.0
Rolipram (10)	O_3	30.0 ± 6.8	1.3 ± 0.10	1.11 ± 0.12
Rolipram (100)	O_3	*14.0 ± 7.4	**1.91 ± 0.11	**1.56 ± 0.12
Rolipram (1,000)	O_3	**5.2 ± 6.1	***2.23 ± 0.13	***1.99 ± 0.1
CT1731 (1,000)	O_3	**7.7 ± 1.7	***2.09 ± 0.16	**1.85 ± 0.17
RP73401 (10)	O_3	58.5 ± 19.7	1.05 ± 0.15	0.84 ± 0.15
Aminophylline (10,000)	O_3	18.3 ± 4.2	1.45 ± 0.08	1.12 ± 0.11

Data are mean ± s.e.mean, $n = 6-24$. R_L (lung resistance), C_{dyn} (dynamic compliance), R_L slope (maximum slope of the histamine dose-response curve), log PD_{200} (log_{10} of the provocative dose of histamine which increases R_L by 200%), log PD_{50} (log_{10} of the provocative dose of histamine which decreases C_{dyn} by 50%).
*$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to solvent + ozone;
[a] $P<0.001$ compared with solvent + air, Student's t test.

Table 5 The effect of ozone and pretreatments with CDP840 (p.o.) on airway hyperresponsiveness to inhaled histamine in anaesthetized guinea-pigs

Treatment ($\mu g\,kg^{-1}$, p.o.)	*Air/O_3*	R_L *slope*	R_L *logPD_{200}*	C_{dyn} *logPD_{50}*
Solvent	Air	4.2±0.8	2.20±0.06	2.00±0.10
Solvent	O_3	[a]39.1±6.3	[a]1.12±0.06	[a]0.87±0.06
CDP840 (100)	O_3	*19.1±6.3	**1.73±0.11	**1.58±0.1

Data are the mean±s.e.mean, $n=6$. R_L (lung resistance), C_{dyn} (dynamic compliance), R_L slope (maximum slope of the histamine dose-response curve), $logPD_{200}$ (log_{10} of the provocative dose of histamine which increases R_L by 200%), $logPD_{50}$ (log_{10} of the provocative dose of histamine which decreases C_{dyn} by 50%).
*$P<0.05$, **$P<0.01$, compared to solvent+ozone;
[a] $P<0.01$ compared with solvent+air, Student's t test.

curve of rolipram is due to the involvement of a cyclo-oxygenase product. When performed in the presence of indomethacin, a mono- rather than a triphasic curve was obtained and the potency decreased 132 fold against histamine and 3 fold against carbachol (Hughes *et al.*, 1992). This resulted in a potency for rolipram similar to that described here for CDP840. A possible explanation could be that histamine, and to a lesser extent carbachol, stimulate the release of a prostanoid which in turn, stimulates cyclic AMP turnover in a pool which is regulated by a rolipram-sensitive PDE4 subtype. Inhibition of this enzyme accounts for the early phase of the rolipram and RP73401 spasmolysis curves, whereas, the monophasic curves obtained with CDP840 and CT1731 suggests no such interaction and a single mechanism of action. It is possible that there is a specific PDE4 isoform, or conformation of the enzyme in smooth muscle which is relatively more sensitive to rolipram and RP73401 than to CDP840. Also, this activity may correlate with binding at the rolipram high affinity binding site (Hughes *et al.*, 1996). In addition a greater stimulation of prostanoid release by histamine would account for the greater potency observed for PDE4 inhibitors against a histamine rather than a carbachol induced spasm (Hughes *et al.*, 1992).

The activity observed with RP73401, however, is at odds with that previously published by Raeburn *et al.* (1994) who showed RP73401 to relax fully contracted guinea-pig tracheae with an EC_{50} of 2 nM for histamine and 29 nM for methacholine. This disparity is most likely due to methodological differences; Raeburn *et al.* used denuded tracheal strips and an EC_{30} contraction rather than intact rings; an EC_{70} contraction was used here. The bronchodilator activity of PDE4 inhibitors is well known to vary depending upon the species, spasmogen and experimental design (see Nicholson & Shahid, 1994, for review). In our hands for example rolipram was approximately 10 fold more potent in relaxing histamine contracted strips than rings (unpublished observations). Raeburn *et al.* did not compare RP73401 to rolipram in their *in vitro* preparation. In the anaesthetized guinea-pig, however, they found both compounds to be equipotent in inhibiting methacholine or histamine induced spasm which is similar to our *in vitro* data.

Regarding mechanism of action, the relative potency of these inhibitors for relaxing airway smooth muscle does not correlate with inhibition of PDE 4. Against human recombinant PDE 4A CDP840 is 40 fold more potent than its enantiomer, CT1731, and 75 fold more potent than rolipram whereas it is 20 fold less potent than RP73401 (Hughes *et al.*, 1996). This lack of correlation is in agreement with the work of Harris *et al.* (1989) who, using a greater range of inhibitors, also found no relationship. These workers did, however, obtain a correlation between bronchodilator potency and affinity at a non-catalytic rolipram binding site on the enzyme. A direct comparison cannot be made with the present study, however, since the bronchorelaxant studies of Harris *et al.* were performed in the presence of a PDE III inhibitor which resulted in a monophasic rather than a triphasic curve. In the present study such correlations are difficult to deduce due to the multi-phasic curves obtained. The relative order of affinity at the non-catalytic rolipram binding site is at odds, however, with any correlation since CDP840 has a 6 and 30 fold lower affinity than rolipram and RP73401, respectively, and a 7 fold greater affinity than CT1731 at this site. In addition, as has been demonstrated previously with the enantiomers of rolipram (Underwood *et al.*, 1993; Holbrook *et al.*, unpublished observations), no stereoselectivity was observed between CDP840 and CT1731 as bronchodilators.

To determine if CDP840 were causing bronchodilatation through a cyclic AMP mechanism its ability to potentiate the effects of the β_2-sympathomimetic salbutamol was assessed. CDP840 caused a slight yet significant leftward shift of the salbutamol curve suggesting that it was acting to inhibit the hydrolysis of cyclic AMP generated by salbutamol. There are, however, surprisingly few studies in which the bronchodilator effects of a combined PDE4 inhibitor and β_2-sympathomimetic treatment have been investigated. Qian *et al.* (1993) found an approximately 8 fold increase in potency and a 30% increase in efficacy of isoprenaline after incubation of human bronchus with 0.1 μM rolipram. Torphy *et al.* (1991) obtained similar results with RO20-1724 and canine trachealis. In both of these studies isoprenaline acted as a partial agonist and thus allowed for an increase in efficacy to be observed. In the present study salbutamol caused a maximum relaxation and therefore any potential increase in efficacy with CDP840 cannot be evaluated.

Experiments *in vivo* also demonstrated that CDP840 is a poor inhibitor of histamine or substance P induced bronchospasm, whereas, it is a potent antagonist of vagally mediated non-cholinergic bronchoconstriction, being equipotent to rolipram. This bronchoconstriction is mediated principally by the tachykinins neurokinin A and substance P (Stretton, 1991). It is likely, therefore, that CDP840 and rolipram inhibit tachykinin release from sensory nerve endings. Similar findings have been obtained *in vitro* (Qian *et al.*, 1994). Tachykinins released in the airways cause bronchoconstriction, vascular leakage, mucus secretion and they will facilitate cholinergic transmission (see Stretton, 1991 for review). Hence, they have been proposed to play a role in the development of airways disease such as asthma (Barnes, 1989). The inhibtion of tachykinin release by CDP840 may therefore be of therapeutic benefit.

CDP840 is also a highly potent and efficacious inhibitor of ozone-induced AHR to inhaled histamine having activity after both i.p. and p.o. administration. In this model CDP840 displayed stereoselectivity being 10 to 100 fold more potent than its S enantiomer, CT1731. This tends to equate with the relative potency against the PDE 4 catalytic site where CDP840 is 40 fold more potent than CT1731 (Hughes *et al.*, 1996). This, along with the anti-inflammatory activity of CDP840 described by Hughes *et al.* (1996) suggests that PDE4 inhibition is the mechanism of action in these models.

The potent PDE4 inhibitor RP73401 was without activity in the ozone model at a dose where CDP840 caused complete

abolition of the AHR. This is in contrast to the suppression of antigen-induced eosinophilia in the guinea-pig lungs, where CDP840 and RP73401 are comparable in potency (Hughes *et al.*, 1996). This difference may be explained by poor distribution of RP73401 in the airways whereas sufficient plasma levels of the compound are delivered to reduce eosinophil migration from the blood compartment. RP73401 has been shown to have activity when given i.v. in a guinea-pig model of platelet-activating factor (PAF) induced AHR to bombesin (Raeburn *et al.*, 1994). The lack of activity observed with RP73401 in the current study may indicate different mechanisms causing the AHR in the two models and thus different sites of action. Without knowledge of pharmacokinetics and possible active metabolites it is not possible to equate enzyme inhibitor potency with activity in an *in vivo* model.

The mechanism of ozone-induced AHR in the guinea-pig is unclear but it is dependent on the duration of exposure (Yeadon *et al.*, 1992). In our hands with a 30 min exposure a major component is peptidergic since prior depletion of tachykinins with capsaicin or pretreatment with neurokinin antagonists reduced the AHR to inhaled histamine by approximately 50% (Holbrook *et al.*, unpublished observations). The finding that CDP840 is not a histamine or substance P antagonist while potently inhibiting AHR to histamine suggests that CDP840 is acting to inhibit an ozone-induced lesion sensitive to histamine. One explanation is that histamine can release tachykinins from sensory nerves in the guinea-pig lung which upon binding to their receptors cause bronchoconstriction and inflammation. Exposure to ozone has been proposed to result in a dysfunction or loss of the enzyme neutral endopeptidase (NEP) in the airway lumen (Murlas *et al.*, 1990), which normally breaks down these tachykinins. Hence ozone may produce AHR to histamine due to epithelial damage leading to enhanced accessibility of the sensory nerves to inhaled spasmogen resulting in the enhanced release of tachykinins which in turn are less efficiently degraded due to loss of NEP. We have demonstrated that CDP840 is not a substance P antagonist but it does inhibit non-cholinergic vagally mediated bronchoconstriction, thus it is likely that it inhibits the release of tachykinins. Interestingly, no such activity has been obserevd with PDE III inhibitors either *in vitro* (Qian *et al.*, 1994) or *in vivo* (Holbrook *et al.*, 1995). Such a mechanism is consistent with our previous findings where we showed that rolipram but not the PDEIII inhibitor SK&F 94120 inhibits ozone-induced AHR to histamine (Holbrook & Hughes, 1992). This mechanism, however, cannot account for all of the activity observed for CDP840 and rolipram, since as previously stated only ~50% of the AHR can be attributed to histamine-mediated release of tachykinins. In addition, CDP840 is equipotent to rolipram in inhibiting non-cholinergic vagally mediated bronchoconstriction whereas it is 10 fold more potent than rolipram against ozone-induced AHR. Currently, it is unclear what mechanism(s) other than the inhibition of tachykinin release may contribute to the protective effect of CDP840 in the ozone model.

CDP840 is also a potent inhibitor of antigen-induced AHR in the rabbit (Gozzard *et al.*, 1996a). In this, as in the ozone model, rolipram appears to be less active and theophylline is inactive at the doses studied (Gozzard *et al.*, 1996b). Also in common with the ozone model AHR to inhaled histamine in the rabbit has a capsaicin-sensitive and hence a neuropeptide component (Herd *et al.*, 1995). It is unclear whether a common site of action accounts for efficacy in both models, since PDE 4 is present at multiple sites. It is clear, however, that CDP840 can abolish AHR in models predominatly driven by either oxidant damage or allergic inflammation.

In conclusion, CDP840 is a poor anti-spasmogenic or spasmolytic agent in response to exogenous mediators, whereas it potently inhibits the allergic (Hughes *et al.*, 1996; Gozzard *et al.*, 1996a) and neuronally mediated bronchoconstriction. CDP840 is also a potent inhibitor of ozone-induced AHR to inhaled histamine. The profile described here, and in the accompanying two papers, suggests that CDP840 has potential for the treatment of asthma. CDP840 is targeted at prophylactic treatment due to its anti-inflammatory activity and ability to inhibit AHR. With prophylactic treatment of asthma it may also be desirable for the drug not to possess potent intrinsic bronchodilator activity since the inappropriate use of bronchodilators may be detrimental and exacerbate the disease (Barnes & Chung, 1992). Thus a more appropriate treatment would be one which reduces the inflammation, maintains the patency and inhibits hyperresponsiveness of the airways hence reducing the requirement for bronchodilators. CDP840 is currently being evaulated in asthma.

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