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Effects of RP 73401, a novel, potent and selective phosphodiesterase type 4 inhibitor, on contractility of human, isolated bronchial muscle

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1 The aim of this study was to investigate the smooth muscle relaxant effects of the novel, selective phosphodiesterase (PDE) type 4 inhibitor, RP 73401 in comparison with the classical PDE 4 inhibitor, rolipram, the non-selective PDE inhibitor, theophylline and the β-adrenoceptor agonist, isoprenaline on the human, isolated bronchus.

2 At resting tone, the rank order of potency (pD_2) for the relaxants was RP 73401 ≥ rolipram ≥ isoprenaline > > theophylline. In terms of maximum relaxation produced (E_{max}) the PDE 4-selective inhibitors were similar, but the maximal effects (70–75% of theophylline, 3 mM) were lower than that observed with isoprenaline (98% of theophylline, 3 mM) or theophylline itself (100%).

3 On the human isolated bronchus pre-contracted with acetylcholine (ACh, 0.1 or 1.0 mM), the rank order of potency remained the same. The maximal responses to RP 73401 and rolipram were however markedly reduced (E_{max} 39.9–46.6%) compared with isoprenaline (E_{max} 79–85%).

4 In tissues pre-contracted with ACh (0.1 mM), RP 73401 and rolipram (10^{-9}–10^{-7} M) significantly and concentration-dependently increased tissue sensitivity to isoprenaline. RP 73401 and rolipram were similar in potency. Both selective PDE 4 inhibitors also significantly increased the maximal relaxant effects of isoprenaline. These effects were not observed with the PDE 3 inhibitor, siguazodan.

5 In terms of retention by tissues (an index of duration of action), the onset of action of RP 73401 (2.11 ± 0.53 min) and rolipram (1.70 ± 0.45 min) was significantly slower than that of isoprenaline (0.33 ± 0.06 min) or theophylline (1.17 ± 0.25 min). The retention of RP 73401 (89.0 ± 21.9 min) on the human isolated bronchial tissues after washing was however dramatically longer than that of rolipram (18.3 ± 4.5 min), theophylline (3.43 ± 0.58 min) or isoprenaline (2.81 ± 0.31 min).

6 These data indicate that RP 73401 is a potent and long-acting relaxant of human bronchial muscle *in vitro*. RP 73401 is more potent than the classical PDE 4-selective inhibitor rolipram and the non-selective PDE inhibitor theophylline and is retained in bronchial tissue for a much longer period of time.

Keywords: Cyclic nucleotide phosphodiesterase inhibitors; RP 73401; human bronchus; rolipram; theophylline; isoprenaline; β-adrenoceptor agonists

Introduction

The biochemical and functional roles of the various cyclic nucleotide phosphodiesterases (PDE) and PDE inhibitors have now been examined in human isolated airways preparations. The presence of PDE types 1 to 5 have been demonstrated (de Boer *et al.*, 1992; Cortijo *et al.*, 1993; Rabe *et al.*, 1993; Torphy *et al.*, 1993). PDE 4 (de Boer *et al.*, 1992; Cortijo *et al.*, 1993; Torphy *et al.*, 1993) and PDE 3 (Torphy *et al.*, 1993) appear to be the main adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-hydrolysing enzymes with PDEs 1 and 5 showing guanosine 3′:5′-cyclic monophosphate (cyclic GMP) hydrolytic activity (Torphy *et al.*, 1993). Two forms of PDE 1 (PDE 1a and PDE 1b) may be present (de Boer *et al.*, 1992; Torphy *et al.*, 1993) and multiple forms of PDE 4 may also exist (de Boer *et al.*, 1992).

There is good general agreement that among the inhibitors of the various isoforms of PDE, inhibitors of PDE 3 and PDE 4 produce the greatest relaxant responses in human airways smooth muscle preparations under basal tone or when pre-contracted (see review by Raeburn & Advenier, 1995). Some discrepancies (possibly methodological) have been reported where the PDE 4 inhibitor, rolipram, produced greater than 70% relaxation of tissues under basal tone (Cortijo *et al.*, 1993; Qian *et al.*, 1993) yet was without apparent effect in two other studies (Belvisi *et al.*, 1992; Rabe *et al.*, 1993). While rolipram was ineffective, denbufylline, a structurally unrelated PDE 4 inhibitor, reduced basal tone in human isolated airways smooth muscle preparations (Belvisi *et al.*, 1992; Cortijo *et al.*, 1993). There is also some controversy over the potential interactions between selective PDE inhibitors and β-adrenoceptor agonists in airways smooth muscle. We have previously reported (Qian *et al.*, 1993) that rolipram but not siguazodan (PDE 3 inhibitor) augmented isoprenaline-induced relaxation of human isolated bronchi whereas Torphy *et al.* (1993) found an interaction between PDE 3 inhibition and β-adrenoceptor stimulation, the opposite of our findings.

RP 73401 (3-cyclopentyloxy-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide) is a novel, highly selective and very potent PDE type 4 inhibitor (Karlsson *et al.*, 1993; Ashton *et al.*, 1994). PDE type 4 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 against the other PDE isoenzymes (Ashton *et al.*, 1994; Souness *et al.*, 1994). We have previously characterized the airways smooth muscle relaxant effects of RP 73401 in the guinea-pig isolated trachea (Raeburn *et al.*, 1994) where it relaxed tissues under basal tone or when pre-contracted by histamine, methacholine and leukotriene D_4 (LTD_4) indicating that RP 73401 acts as a functional antagonist. Following topical administration, RP 73401 also inhibited bronchospasm induced by histamine, methacholine and LTD_4 in the anaesthetized guinea-pig (Raeburn *et al.*, 1994).

Based on the similarities between guinea-pig and human

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airway preparations *in vitro* regarding PDE isoenzyme profiles and relative functional importance, it seems likely that RP 73401 will also relax human isolated airway preparations.

The present study characterizes the bronchial smooth muscle relaxant effects of RP 73401 in human airways *in vitro* in comparison with the classical PDE 4 inhibitor, rolipram, the PDE 3 selective inhibitor, siguazodan, the non-selective PDE inhibitor, theophylline and the β-adrenoceptor agonist, isoprenaline. Both RP 73401 and rolipram potently enhanced β-adrenoceptor-induced relaxation but, interestingly, the relative duration of action was much longer with RP 73401 than with any of the other compounds studied.

Methods

Human isolated bronchial preparations

Human bronchial tissue (inner diameter 1 to 3 mm) was obtained from patients undergoing surgery for lung cancer. Tissues were taken from macroscopically normal areas at a distance from the malignancy. Bronchi were dissected free of parenchyma and transported to the laboratory in Krebs solution previously aerated with a mixture of 95% O_2 and 5% CO_2 (pH 7.40). The tissues were stored over night at 4°C and experiments performed the next day. Previous experience in this laboratory and other published data have demonstrated that overnight storage of tissue does not alter its reactivity. Bronchial rings from a segmental bronchus were prepared and suspended in parallel in Krebs solution under an initial load of 2.0 g. After 1 h equilibration with washing every 15 min, the resting load was constant at between 1 and 2 g. Under these conditions, the responses obtained were reproducible. Force of contraction was measured isometrically with strain gauges (UF1), amplifiers and an I.O.S.-Moise 3 recorder system (EMKA Technologies, Mitry Mory, France). The composition of the Krebs solution was (mmol l^{-1}): NaCl 118, KCl 5.4; $CaCl_2$ 2.5, KH_2PO_4 1.1; $MgSO_4$ 0.6, $NaHCO_3$ 25 and glucose 11.7. In all experiments, human bronchi were first contracted maximally with acetylcholine (ACh, 1 mM) and then relaxed with theophylline (3 mM). These concentrations did not alter subsequent responsiveness of the tissue. The tissues were equilibrated for 60 min before beginning the experimental procedure.

Relaxation of human isolated bronchi under resting tone or pre-contracted with ACh

Following the equilibration period, the bronchial rings were left under resting load or were contracted with ACh (0.1 mM or 1 mM). After the contraction plateau was reached, concentration-response curves to the relaxants were established by their cumulative addition at intervals of 5–30 min to obtain a relaxation plateau. After the maximal effect of each drug was obtained, theophylline (3 mM) was added to the bath to determine the maximal relaxation achievable. Only one concentration-response curve was recorded for each bronchial ring.

Influence of PDE inhibitors on the concentration-response curves to isoprenaline in human isolated bronchial preparations

Following the equilibration period, bronchial rings were pretreated for 30 min with Krebs solution (control) or one concentration of RP 73401 (10^{-9}, 10^{-8}, 10^{-7} M), rolipram (10^{-9}, 10^{-8}, 10^{-7} M) or siguazodan (10^{-7}, 10^{-6}, 10^{-5} M). Each concentration-response curve for relaxation by isoprenaline was obtained with cumulative additions of the drug after the bronchial ring had been brought to a steady level of contraction with ACh (0.1 mM). At the end, theophylline (3 mM) was added in order to determine the maximal achievable relaxation. Only one concentration-response curve was recorded for each bronchial ring.

Kinetics of action and retention by tissues of RP 73401, rolipram, theophylline and isoprenaline

Following the equilibration period, drugs, at the concentration giving approximately 80% of their maximal responses of bronchus at basal tone, were added to the bath. When the maximal response had stabilized (15 min), bronchial rings were washed and allowed to return to basal tone. The half-time for the onset of relaxation ($t_{1/2}$ onset) was calculated as the time from drug administration to attainment of 50% maximal relaxation by the compound. The half-time for recovery ($t_{1/2}$ recovery) was defined as the time to return, after washing, from maximal relaxation to 50% of the maximum response. Responses were compared to theophylline and the ratios (compound/theophylline) were determined. The protocol is shown in Figure 1.

Statistical analysis of the results

Relaxation of human isolated bronchus is expressed as a percentage of the relaxation produced by theophylline (3 mM). pD_2 values represent the negative logarithm of the concentration of drug which induces a relaxation equal to 50% of its own maximal effect. E_{max} values represent the maximum relaxation achieved with each agent and is expressed as a percentage of the theophylline (3 mM) maximum.

Statistical analysis of the results was performed using Student's t test (two tailed, for paired sample). All values are expressed as mean ± standard error of the mean; $P<0.05$ was considered to be significant.

Compounds and solutions

The drugs used were: theophylline (Theophylline Bruneau, Paris, France), acetylcholine HCl (Pharmacie Centrale des Hôpitaux, Paris, France), isoprenaline HCl (Winthrop, Paris, France), siguazodan (SKB Pharmaceuticals), rolipram and RP 734401 (synthesised by Rhône-Poulenc Rorer, Dagenham, Essex).

All drugs were dissolved daily in distilled water or absolute ethanol and then diluted in Krebs solution.

Results

Relaxation of human isolated bronchial preparations

The concentration-response curves of the relaxant effects of RP 73401, rolipram, theophylline and isoprenaline on human bronchi at resting tone are shown in Figure 2. The PDE 4-selective inhibitors, RP 73401 and rolipram, relaxed tissues at basal tone by 72 ± 5 and 74 ± 7% respectively whereas theophylline and isoprenaline produced complete relaxation (Table 1). The rank order of potency (pD_2) for relaxation was RP 73401 ≥ rolipram ≥ isoprenaline > > theophylline (Figure 2a, Table 1). RP 73401 was significantly more potent than isoprenaline and theophylline ($P<0.01$).

In bronchi pre-contracted with ACh (0.1 or 1 mM), the concentration-response curves to RP 73401, rolipram and isoprenaline were displaced to the right and the maximum relaxation reduced when compared with results obtained at

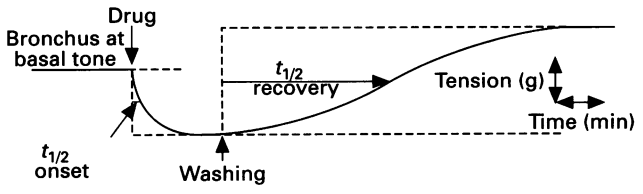

Figure 1 Protocol used to determine time of onset and duration of action of PDE 4 inhibitors, theophylline and isoprenaline.

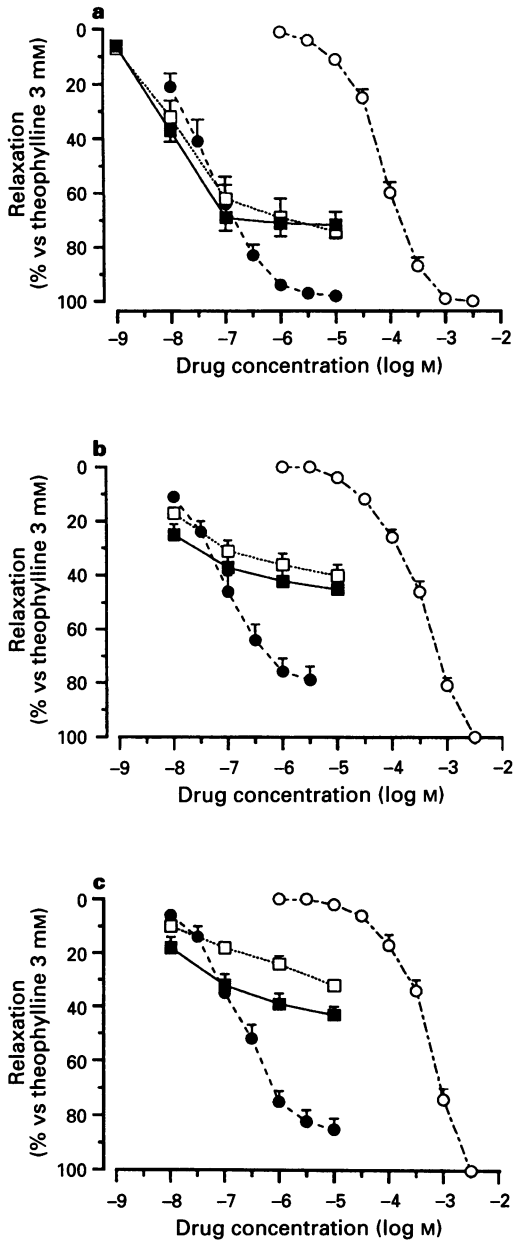

Figure 2 Comparison of the effects of: (a) RP 73401 (■, $n=7$), rolipram (□, $n=8$), isoprenaline (●, $n=12$) and theophylline (○, $n=13$) on human isolated bronchial rings under basal tone. (b) RP 73401 (■, $n=5$), rolipram (□, $n=5$), isoprenaline (●, $n=12$) and theophylline (○, $n=8$) on human isolated bronchial rings pre-contracted with ACh (0.1 mM) and (c) RP 73401 (■, $n=6$), rolipram (□, $n=8$), isoprenaline (●, $n=11$) and theophylline (○, $n=7$) on human isolated bronchial rings pre-contracted with ACh (1.0 mM). Responses represent the mean ± s.e.mean and are plotted as % theophylline maximum.

basal tone (Figure 2b,c, Table 1). During pre-contraction induced by ACh (0.1 mM), the order of potency (pD_2) of these substances remained the same as during basal tone, but they were, in terms of efficacy (E_{max}), less effective relaxants than isoprenaline. In tissues pre-contracted with ACh (1 mM) the maximal effects of RP 73401 and rolipram were similar to the effects seen at the lower concentration of ACh (0.1 mM) while the maximum response to isoprenaline was further reduced (Figure 2), when compared with tissues at resting tone.

Influence of PDE 4 inhibitors on concentration-response curves to isoprenaline

After 30 min incubation of ACh (0.1 mM)-pre-contracted human bronchial rings with RP 73401 or rolipram (10^{-8} and 10^{-7} M) which caused about a 20 to 30% relaxation, the concentration-response curves to isoprenaline were significantly shifted to the left and the magnitude of the relaxant effect of isoprenaline was increased (Figure 3c,d, Table 2). The PDE 4 inhibitors were about equipotent in this respect. A lower concentration (10^{-9} M) of RP 73401 or rolipram did not affect responses to isoprenaline (Table 2). In contrast, concentration-response curves to isoprenaline were not modified by the PDE 3 inhibitor, siguazodan (10^{-7} to 10^{-5} M) (Table 2).

Kinetics and retention by tissues of RP 73401, rolipram, theophylline and isoprenaline

When equieffective relaxant doses of the compound were compared, isoprenaline had the fastest onset followed by theophylline, rolipram and RP 73401. The speed of onset of isoprenaline was significantly greater than that of theophylline and the PDE 4-selective inhibitors ($P<0.01$). However, the absolute time differences for the fastest (0.33 min) and slowest (2.11 min) were small (Table 3). In contrast, the rank order for retention by bronchial tissues was RP 73401 > > rolipram > > theophylline > isoprenaline (Table 3). RP 73401 ($t_{1/2}$ recovery = 89.0 ± 21.9 min) had a duration of action approximately 32 times that of isoprenaline, 26 times that of theophylline and 5 times that of rolipram when assessed as the time to return to half-maximal response.

Discussion

RP 73401 is a potent and selective inhibitor of the cyclic AMP-specific PDE (PDE 4) (Karlsson *et al.*, 1993; Ashton *et al.*, 1994). We have previously demonstrated its ability to relax guinea-pig airways smooth muscle (under basal tone and when pre-contracted by several spasmogens) *in vitro* and *in vivo* (Raeburn *et al.*, 1994). In the present study we demonstrate that RP 73401 is also a potent relaxant of the human isolated bronchus *in vitro* under basal tone and when pre-contracted with ACh. RP 73401 was more potent than isoprenaline but, interestingly, its efficacy was more dependent on the degree of tone.

RP 73401 and rolipram relaxed human isolated bronchial

Table 1 pD_2 and E_{max} of RP 73401, rolipram, isoprenaline and theophylline on the human isolated bronchus at resting tone or contracted with acetylcholine (ACh, 0.1 and 1 mM)

	Basal tone			*ACh (0.1 mM)*			*ACh (1.0 mM)*		
	n	pD_2	E_{max}	n	pD_2	E_{max}	n	pD_2	E_{max}
RP 73401	7	8.03 ± 0.11**,††	72 + 5**,††	5	8.04 ± 0.13**,††	45 ± 4**,††	6	7.68 ± 0.16**,††	43 ± 3**,††
Rolipram	8	7.77 ± 0.14*,††	74 ± 7**,††	5	7.88 ± 0.28*,††	40 ± 4**,††	8	6.25 ± 0.23††	35 ± 5**,††
Isoprenaline	12	7.31 ± 0.12††	98 ± 1	12	7.12 ± 0.09††	79 ± 5††	11	6.56 ± 0.13††	85 ± 4††
Theophylline	13	4.13 ± 0.05**	100	8	3.46 ± 0.06**	100**	7	3.30 ± 0.04**	100**

n = number of experiments; pD_2: the negative logarithm of the concentration of drug which induces a relaxation equal to 50% maximal effect induced by itself. E_{max}: maximal effect as percentage of maximal effect induced by theophylline (3 mM). Significantly different from isoprenaline: *$P<0.05$, **$P<0.01$; Significantly different from theophylline: ††$P<0.01$.

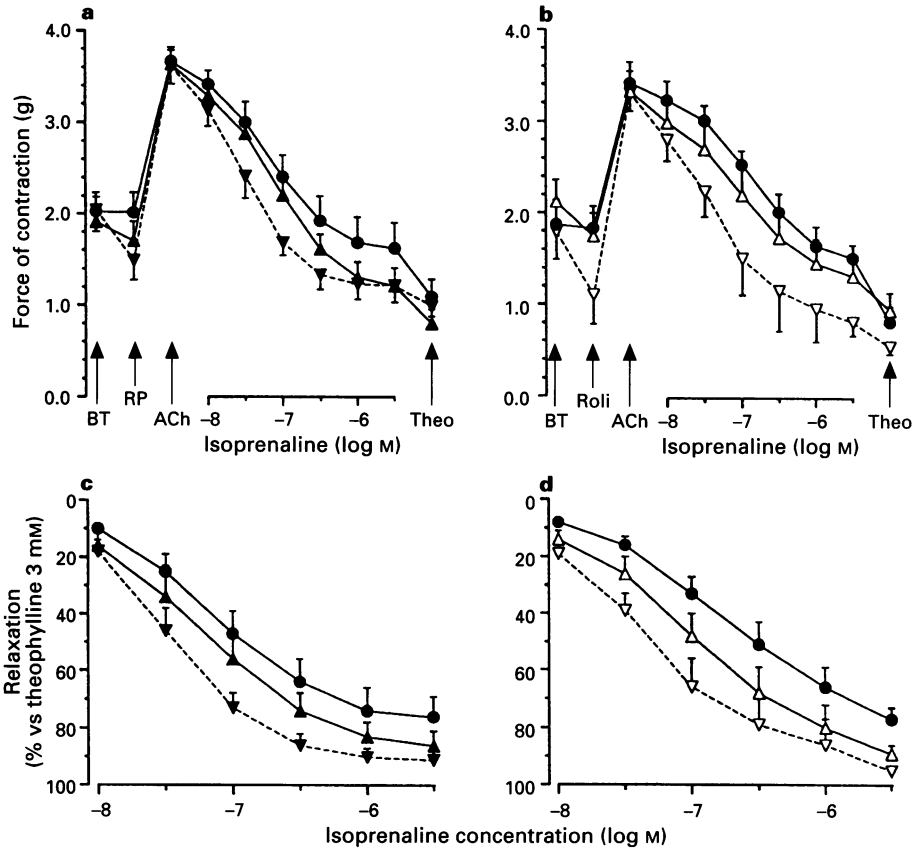

Figure 3 Effects of RP 73401 (RP) (10^{-8} M, ▲; 10^{-7} M, ▼, in a and c) and rolipram (Roli) (10^{-8} M, △; 10^{-7} M, ▽, in b and d) on concentration-response curves to isoprenaline in ACh (0.1 mM)-pre-contracted human, isolated bronchial rings. (●, isoprenaline plus vehicle). Results are expressed as force of contraction (g) (a, b) or as relaxation vs theophylline 3 mM (Theo) (c, d). BT is basal tone. Values are means ± s.e.mean of 7 to 8 experiments.

Table 2 Effect of RP 73401, rolipram and siguazodan on isoprenaline-induced relaxation of human isolated bronchial rings pre-contracted with acetylcholine (ACh, 0.1 mM)

	n	10^{-9} M Δcontrol	10^{-8} M Δcontrol	10^{-7} M Δcontrol	10^{-6} M Δcontrol	10^{-5} M Δcontrol
RP 73401	7–8	0.06±0.16	0.36±0.13*	0.56±0.15**	ND	ND
Rolipram	7	−0.03±0.11	0.33±0.16	0.68±0.14**	ND	ND
Siguazodan	5	ND	ND	0.02±0.35	0.04±0.30	0.24±0.23

	n	Control (% max relax)	10^{-9} M (% max relax)	10^{-8} M (% max relax)	10^{-7} M (% max relax)	10^{-6} M (% max relax)	10^{-5} M (% max relax)
RP 73401	7–8	76±7	80±6	86±5	91±4	ND	ND
Rolipram	7	77±4	78±5	89±3	95±2*	ND	ND
Siguazodan	5	83±8	ND	ND	84±3	83±8	92±3

Results are presented as shift to the left of $-\log EC_{50}$ and % maximum relaxation of isoprenaline after pretreatment with the PDE 4- or PDE 3-selective inhibitors. n = number of experiments; Δcontrol: shift to the left of $-\log EC_{50}$ of isoprenaline; % max relax: % maximal relaxation of isoprenaline; *$P<0.05$, **$P<0.01$ versus control. ND, not determined.

Table 3 Onset of action and duration of action of RP 73401, rolipram, isoprenaline and theophylline on the human isolated bronchial rings

	n	Onset of action: $t_{1/2}$ onset (min)	Onset of action: Ratio compound/theophylline	Offset of activation: $t_{1/2}$ recovery (min)	Offset of activation: Ratio compound/theophylline
RP 73401 (0.1 μM)	6	2.11±0.53**,†	1.77±0.31**,†	89.0±21.9**,††	28.4±7.7**,††
Rolipram (0.1 μM)	6	1.70±0.45**,†	1.50±0.20**,†	18.3±4.5**,††	5.15±0.93**,††
Isoprenaline (0.3 μM)	6	0.33±0.06†	0.28±0.05†	2.81±0.31	0.82±0.09
Theophylline (0.1 mM)	6	1.17±0.25**	1**	3.43±0.58	1

$t_{1/2}$ onset was calculated as the time from administration of compound to attainment of half maximal relaxation. $t_{1/2}$ recovery: was calculated as the time from washing the preparation to attainment of 50% recovery of basal tone. Values are mean ± s.e.mean. $n=6$, each compound. Significantly different from isoprenaline: *$P<0.05$; **$P<0.01$; Significantly different from theophylline †$P<0.05$; $P<0.01$.

rings at basal tone and when pre-contracted with ACh. At basal tone RP 73401 was more potent than rolipram and was significantly more potent than isoprenaline and theophylline in these experiments. In comparison with previous studies in the guinea-pig, RP 73401 was 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). The reduction in basal tone seen with RP 73401 supports previous findings with rolipram (Cortijo *et al.*, 1993; Qian *et al.*, 1993) and denbufylline (Belvisi *et al.*, 1992; Cortijo *et al.*, 1993) and suggests a role for PDE 4 in regulating basal cyclic AMP turnover. These data indicate that RP 73401 is an effective relaxant in human isolated airways smooth muscle preparations. The maximum relaxant effects of RP 73401, and rolipram, however were lower than those produced by theophylline or isoprenaline. In the presence of ACh, the maximum relaxant responses to the PDE 4 inhibitors and, to a lesser extent, isoprenaline were reduced compared to their effects in tissues under basal tone. It has been shown previously that PDE inhibitors are less effective in relaxing tissues at elevated tone, especially if tone has been elevated by stimulating muscarinic receptors (Nicholson & Shahid, 1994; Raeburn *et al.*, 1994). Stimulation of muscarinic (M_2) receptors reduces cyclic AMP production in guinea-pig and dog airways (Fernandes *et al.*, 1992; Watson & Eglen, 1994) and this would be expected to contribute to the reduced efficacy of PDE inhibitors in tissues with increased muscarinic tone. It is, however, unlikely to explain our findings in the ACh-contracted tissues since Watson *et al.* (1995) have shown that the effects of isoprenaline are unaffected by muscarinic (M_2) agonism in human airways smooth muscle.

Theophylline was at least 1000 fold less potent than RP 73401 and had a considerably shorter duration of action. While RP 73401 produced a smaller maximum relaxation than theophylline it has the potential, based on guinea-pig studies (Raeburn *et al.*, 1994) and the present *in vitro* studies to be topically active as a bronchodilator in man.

RP 73401 and rolipram significantly increased the potency of isoprenaline, seen as shifts to the left in the concentration-response curves to the β-adrenoceptor agonist. The potentiation effects may be specific for the type 4 inhibitors, since siguazodan (PDE 3 inhibitor) did not augment the effects of isoprenaline, confirming our earlier findings (Qian *et al.*, 1993). Recently, Man *et al.* (1994) have presented some preliminary data demonstrating that the PDE 4 inhibitor, rolipram but not the PDE 3 inhibitor, cilostamide, augments isoprenaline-induced cyclic AMP content in human bronchial smooth muscle cells in culture thus providing further support for the importance of PDE 4 in regulating cyclic AMP in human airways.

In tissues pre-contracted with ACh, isoprenaline had a reduced maximal relaxant effect. When examined in the presence of a PDE 4 inhibitor, the maximum relaxation was increased in a concentration-related fashion. This indicates that the combination of a β-adrenoceptor agonist and a PDE 4 inhibitor can antagonize a higher level of tone than is seen with either agent alone.

Perhaps the most interesting and significant finding of the present study was the duration of action of RP 73401 compared with the other smooth muscle relaxants tested. While the onset of the smooth muscle relaxant actions of RP 73401 and rolipram was slower than for isoprenaline or theophylline the difference was small. In contrast, the retention by the tissues of RP 73401 (a measure of duration of action) was dramatically longer than that of isoprenaline, theophylline or the other PDE 4-selective compound rolipram.

β_2-Adrenoceptor agonists are widely used bronchodilators in asthma therapy since they effectively antagonize constriction produced by various stimuli such as mediators, irritants or exercise. Their relatively short duration of action (generally <6 h) however, may be seen as a drawback to their usefulness. To overcome this, new, highly potent β_2-adrenoceptor agonists with a prolonged duration of action (formoterol, salmerterol) have been developed (Jepsson *et al.*, 1989; Lofdahl & Chung, 1991; Ullman *et al.*, 1992). Recently, Naline *et al.* (1994) demonstrated that formoterol and salmeterol respectively were approximately 8 and 25 fold (or more) longer-acting than isoprenaline or theophylline on human, isolated bronchus. The relaxation produced by salmeterol lasted for 100 to >150 min. Although formoterol was significantly slower in onset than the short-acting β-adrenoceptor agonist it was similar to theophylline and 3 times faster than salmeterol. In the present study we have shown that the effect of RP 73401 (89 min) is intermediate between formoterol (30 to 35 min) and salmeterol (100 to >150 min) but is similar in time of onset to formoterol, that is 3 times more rapid than salmeterol (see Naline *et al.*, 1994). Indeed RP 73401 was only slightly slower in onset than isoprenaline (this study) or salbutamol (Naline *et al.*, 1994). If these data can be extrapolated into the clinic, RP 73401 may have long duration coupled to a rapid onset of action.

There is now some evidence (see review by Barnes & Pauwels, 1994) that in patients dying of severe asthma, there is an uncoupling of the β-adrenoceptors in the airways smooth muscle reducing the benefit of this therapeutic approach. This suggests that alternative methods (such as phosphodiesterase inhibition) of inducing bronchodilatation may have an advantage in treating severe asthma. Theophylline is a weak bronchodilator at therapeutically useful doses and may not offer much improvement. However, potent PDE 4-selective inhibitors such as RP 73401 may theoretically be of benefit.

These data suggest that RP 73401 alone or in combination with a fast-acting, short duration β-adrenoceptor agonist may provide useful, long-lasting regulation of bronchospasm. This action, coupled to its anti-inflammatory actions as demonstrated in animal models (Karlsson *et al.*, 1993; Raeburn *et al.*, 1994) suggest a potentially useful role for RP 73401 in asthma therapy.

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