Human bronchial cyclic nucleotide phosphodiesterase isoenzymes: biochemical and pharmacological analysis using selective inhibitors

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1 The aims of the present study were to characterize the cyclic nucleotide phosphodiesterase (PDE) isoenzyme activities present in human bronchi and to examine the ability of selective isoenzyme inhibitors to relax histamine and methacholine precontracted preparations of human bronchi.

2 Three separations of pooled human bronchial tissue samples were performed. Ion-exchange chromatography showed that the soluble fraction of human bronchial preparations contains PDE I, II, III, IV and V isoenzyme activities. Multiple forms of PDE I and PDE IV were observed and PDE IV was the main cyclic AMP hydrolytic activity.

3 3-Isobutyl-1-methylxanthine (IBMX) non-selectively inhibited all separated isoenzyme activities. Zaprinast selectively inhibited PDE V, but also effectively inhibited one of the two PDE I isoforms identified. The PDE IV selective inhibitors rolipram and RO-201724, inhibited the PDE IV activities as did the dual PDE III/IV inhibitor, Org 30029. Org 9935, a PDE III selective inhibitor, potently attenuated part of the PDE IV activity peak in one of three separations performed, indicating that some PDE III activity may co-elute with PDE IV under the experimental conditions employed.

4 PDE IV-selective (rolipram), PDE III-selective (Org 9935) and dual PDE III/IV (Org 30029) inhibitors were effective relaxants of human bronchial smooth muscle. The PDE V/PDE I inhibitor, zaprinast was relatively ineffective.

5 The present study demonstrates in human bronchi, as in animal airways smooth muscle, that inhibitors of PDE III, PDEIV and dual PDE III/IV have potentially useful bronchodilator activity and are worthy of further consideration as anti-asthma drugs.

Keywords: Human bronchi; cyclic nucleotide phosphodiesterase isoenzymes; isoenzyme selective inhibitors; rolipram; Org 30029; Org 9935; zaprinast; IBMX

Introduction

There are five main families of cyclic nucleotide phosphodiesterase isoenzymes which catalyse the hydrolysis of the intracellular second messengers, adenosine 3'5'-cyclic monophosphate (cyclic AMP) and guanosine 3'5'-cyclic monophosphate (cyclic GMP) to 5' nucleotides (Beavo & Reifsnyder, 1990). In addition to showing different regulatory properties, these phosphodiesterase isoenzymes can be distinguished by their sensitivity to a variety of isoenzyme selective inhibitors (Nicholson et al., 1991). Despite the ubiquitous presence of cyclic AMP and cyclic GMP, isoenzyme selective inhibitors do not invariably alter tissue function, possibly because isoenzyme distribution is markedly tissue-dependent (Nicholson et al., 1991). Thus, selective inhibitors may be potentially useful therapeutic agents for a variety of diseases including asthma (Nicholson et al., 1991). Indeed, selective inhibitors of particular phosphodiesterase isoenzymes have been shown both to produce bronchodilatation (Torphy, 1988; Torphy et al., 1988; Harris et al., 1989; Shahid et al., 1991b) and to inhibit mediator release from inflammatory cells (Dent et al., 1991; Souness et al., 1991; Robicsek et al., 1991; Torphy & Undem, 1991).

A combination of bronchodilator and anti-inflammatory activities makes phosphodiesterase isoenzyme inhibition a potentially attractive mechanism for the treatment of asthma. However, isoenzyme selective inhibitors show marked species variation in their airway relaxant effects. Selective inhibitors of the cyclic GMP inhibited phosphodiesterase isoenzyme

(PDE III) more effectively relax guinea-pig (Harris et al., 1989) and canine (Torphy et al., 1988) than bovine (Shahid et al., 1991b) tracheal smooth muscle preparations. In contrast, selective inhibitors of the cyclic GMP-insensitive phosphodiesterase isoenzyme (PDE IV) more potently relax bovine (Shahid et al., 1991b) than canine (Torphy et al., 1988) and guinea-pig (Harris et al., 1989) tissues. Although the reasons for the tissue and species variation are not known, they may arise from differences in abundance and/or intracellular localization of functionally important phosphodiesterase isoenzymes. Whether similar species variation exists for the anti-inflammatory effects of isoenzyme-selective phosphodiesterase inhibitors has not been reported.

It is clearly important to clarify the phosphodiesterase isoenzyme profile and the functional activity of selective isoenzyme inhibitors in human airway smooth muscle. Thus, the aims of the present study were to characterize the phosphodiesterase isoenzyme activities present in human bronchi and to examine the functional effects of a variety of known selective isoenzyme inhibitors. A preliminary account of this study has been presented to the British Pharmacological Society (Shahid et al., 1992).

Methods

Separation of cyclic nucleotide phosphodiesterase isoenzymes

Macroscopically normal, human lungs were obtained from lung cancer patients undergoing surgery and were transport-

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ed to the laboratory in ice cold Krebs-Henseleit solution previously well aerated with 95% O_2 and 5% CO_2. None of the patients had been receiving medication for airways disease, in particular no patient had recently received theophylline containing drugs. Premedication and anaesthetics were the same for all patients. All subsequent steps were performed on ice. Peripheral bronchi (diameter 2–3 mm) were isolated, according to the procedure described by van Amsterdam et al. (1990), and carefully trimmed free of vascular and parenchymal tissue before being frozen in liquid nitrogen and stored at − 70°C. Frozen bronchi (9–10 g) obtained from 3 to 4 lung biopsies were pooled, thawed, cut into small cubes and homogenized (Polytron: PT20 probe, 2 × 10 s bursts at speed setting 5 and 2 × 10 s bursts at speed setting 7) in three volumes of buffer A (pH 6.5) containing (mmol l^{-1}): Bistris 20, dithiothreitol 1, benzamidine 2, EDTA 2, NaCl 50 and phenylmethanesulphonyl fluoride (PMSF) 0.1. The extract was centrifuged (107,000 g × 60 min) and the supernatant fraction containing the soluble material was used as the source of phosphodiesterase isoenzymes. The lung tissue was obtained from smokers, hence the particulate material was contaminated and could, therefore, not be analysed. The supernatant fraction was filtered (0.22 μm) and the phosphodiesterase activities separated by ion exchange chromatography on a Mono Q column, pre-equilibrated in buffer A, attached to a fast protein liquid chromatography (FPLC) system essentially as described previously (Shahid et al., 1991b). Bound protein was eluted with a NaCl gradient and 1 ml fractions collected in test tubes kept on ice and containing 0.2 mg bovine serum albumin to protect enzyme activity. Routinely, samples were analysed within 3–4 h after homogenization. For long term storage, the fractions were kept at − 70°C. Three different phosphodiesterase separations were performed.

Cyclic nucleotide phosphodiesterase assay

Phosphodiesterase activity was measured at 37°C as described in detail by Shahid et al. (1991b). All assays were performed at 1 μmol l^{-1} substrate concentration and inhibitors were tested with cyclic AMP as substrate except for PDE V where cyclic GMP was used. Drug solutions were prepared as described previously (Shahid et al., 1991b). The drug concentration producing 50% inhibition of phosphodiesterase activity (IC_{50} value) was determined by use of a non-linear regression curve fitting programme. Inhibitors were tested on phosphodiesterase isoenzymes from all three separations.

Bronchial smooth muscle experiments

Human bronchial preparations were prepared as described by van Amsterdam et al. (1990). The preparations were mounted (300 mg load) for isotonic recording in 20 ml water jacketed organ baths and equilibrated for 30 min in Krebs-Henseleit solution (37°C) containing (mmol l^{-1}): NaCl 118, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.5, NaH_2PO_4 1.29, $NaHCO_3$ 25, and glucose 5.5 aerated with 95% O_2 and 5% CO_2 (pH 7.4). After equilibration, (−)-isoprenaline (0.1 μmol l^{-1}) was applied to establish basal tone. The tissue was washed immediately for 30 min and contracted by cumulative administration of methacholine 10 and 90 μmol l^{-1} followed by 60 min of repeated washings. Subsequently, contraction was induced with either histamine (1 μmol l^{-1}) or methacholine (1 μmol l^{-1}); 15 min later, cumulative relaxation curves were constructed with a phosphodiesterase inhibitor. After completion of the curve (−)-isoprenaline (10 μmol l^{-1}) was added to reestablish basal tone. Only one phosphodiesterase inhibitor was tested on each preparation. Contraction of bronchial muscle was expressed as a % of the first response to 0.1 mmol l^{-1} methacholine. The pD_2 (− log EC_{50}) values of all phosphodiesterase inhibitors were determined from the concentration-relaxation curves according to van Rossum (1963). Maximal relaxation (E_{max}) was expressed as percen-

tage of the full relaxation induced by 10 μmol l^{-1} (−)-isoprenaline given at the end of the experiment.

Chemicals

The following substances were used: 3-isobutyl-1-methyl xanthine (IBMX), calmodulin (Sigma, Poole, Dorset), RO-20-1724 (Gibco BRL, Paisley, Scotland, rolipram (a gift from Schering, Berlin, Germany), Org 30029 (N-hydroxy-5,6-dimethoxy-benzo[b]thiophene-2-carboximidamide HCl) and Org 9935 (4,5-dihydro-6-(5,6-dimethyoxybenzo[b]thien-2-yl-5-methyl-3(2H)pyridazinone) (Chemistry Department, Organon Laboratories Ltd., Newhouse, Lanarkshire), [³H]-cyclic AMP, [³H]-cyclic GMP (Amersham International, Bucks) and Mono Q (Pharmacia, Milton Keynes). All other chemicals were obtained either from Sigma Chemical Co. or BDH Chemicals (both of Poole, Dorset) and of Analar or equivalent grade. Water purified on a Milli-Q (Millipore, Herts.) system was used throughout.

Data analysis

All values are presented as mean ± s.e.mean.

Results

Separation of cyclic nucleotide phosphodiesterase isoenzymes

The cyclic AMP hydrolytic activity in the soluble extract was 4–5 fold greater than that of the cyclic GMP activity. Neither Ca^{2+}/calmodulin nor cyclic GMP significantly affected the cyclic AMP activity of the soluble fraction. Separation of phosphodiesterase isoenzymes revealed an elution profile with, at least, six distinct peaks of activity represented by fractions 12, 24, 29, 34, 45, and 62 (Figure 1). The majority of the cyclic nucleotide hydrolytic activity in the peaks corresponded to phosphodiesterase isoenzymes V, I, I, II, IV and IV, respectively, according to the nomenclature proposed by Beavo & Reifsnyder (1990). In agreement with the phosphodiesterase activity of the soluble extract, the total cyclic AMP activity in the fractions collected from the Mono Q column was much greater than the cyclic GMP hydrolytic activity. Approximately, 80–90% of the activity applied to the ion-exchange column was recovered.

Figure 1 shows the elution profile for phosphodiesterase activity in the cytosolic fraction of human bronchial smooth muscle. Cyclic GMP phosphodiesterase activity determined the position of PDE V which eluted first, fraction 12 (0.13 mol l^{-1} NaCl) represented the peak of activity (Figure 1a). This peak showed higher activity for cyclic GMP than for cyclic AMP, was not activated by Ca^{2+}/calmodulin (Figure 1b), but was potently inhibited by zaprinast (Table 1). The effects of the regulators Ca^{2+}/calmodulin and cyclic GMP on cyclic AMP hydrolysis were examined in an attempt to identify other phosphodiesterase isoenzyme activities. Ca^{2+}/calmodulin stimulated cyclic AMP hydrolysis in fraction 18 to 30 with hydrolytic activity peaks at fractions 24 (0.21 mol l^{-1} NaCl) and 29 (0.24 mol l^{-1} NaCl) (Figure 1b). The two Ca^{2+}/calmodulin stimulated peaks (PDE I) showed differential sensitivity to zaprinast (Table 1). In contrast, the phosphodiesterase activity peaks corresponding to fractions 34, 45 and 62 were not affected by Ca^{2+}/calmodulin. The cyclic AMP hydrolytic activity of the fourth peak, fraction 34 (0.28 mol l^{-1} NaCl) was stimulated 3 fold by 1 μmol l^{-1} cyclic GMP (Figure 1c), suggesting that it contains PDE II. The last two peaks of activity, fractions 45 (0.35 mol l^{-1} NaCl) and 62 (0.65 mol l^{-1} NaCl), eluting at high salt concentration, were not markedly inhibited by cyclic GMP (Figure 1c), suggesting these peaks contain mainly PDE IV. The fifth peak was the largest activity peak and represented 51 ± 4% of the total cyclic AMP hydrolytic activity. A distinct cyclic
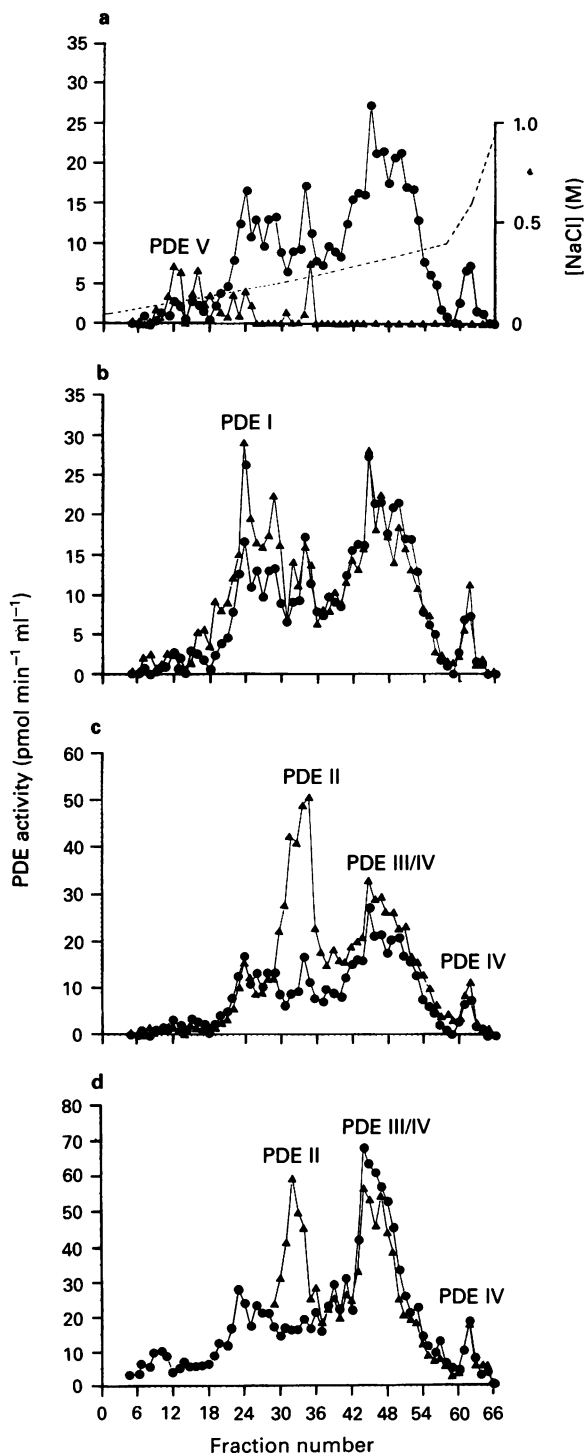
Figure 1 FPLC elution profiles, showing cyclic nucleotide phosphodiesterase activies in human bronchial smooth muscle.The results show the cyclic AMP (●; 1 μmol l⁻¹) and cyclic GMP (▲; 1 μmol l⁻¹) hydrolytic activity of fractions eluted using a NaCl gradient in the absence of any regulator (a) and cyclic AMP hydrolytic activities in the absence (●) presence (▲) of Ca^{2+}/calmodulin (b) and cyclic GMP (c) and (d). The results shown in (c) and (d) were obtained from two different FPLC elutions.

GMP-inhibited phosphodiesterase (PDE III) was not obtained. However, in one separation a small (15–20%) but constant level of inhibition of peak five was observed in the presence of cyclic GMP (Figure 1d). This suggested that PDE III, may be co-eluting with PDE IV in amounts which varied between separations. This suggestion was supported by further experiments using a potent and selective PDE III

inhibitor, Org 9935 (Nicholson & Shahid, 1992). Org 9935 produced a biphasic inhibition, with a clear plateau at 0.1 to 3 μmol l⁻¹, of peak five cyclic AMP hydrolytic activity in the separation in which cyclic GMP inhibited hydrolytic activity (Figure 2). As Org 9935 has little ability to inhibit PDE IV at concentrations below 1 μmol l⁻¹ (Shahid et al., 1991b), these data suggest the joint presence of both PDE IV and III activities in peak five. At concentrations below 100 μmol l⁻¹, rolipram did not completely inhibit all enzyme activity in those fractions in which Org 9935 displayed significant effects at low concentrations, further indicating the presence of PDE III activity (Figure 2). Kinetic evaluation of human bronchial smooth muscle phosphodiesterase isoenzymes was not possible due to low levels of enzyme activity.

Effects of phosphodiesterase inhibitors on phosphodiesterase isoenzyme activity

The effects of phosphodiesterase isoenzyme inhibitors on human bronchial phosphodiesterase isoenzyme are summarized in Table 1. IBMX was non-selective and inhibited almost equally all phosphodiesterase isoenzymes. Zaprinast selectively inhibited PDE V. This compound also showed differential inhibitory effects on the two PDE I isoforms present in activity peaks two and three, being seventeen times more potent against peak two than peak three. However, the IC_{50} value for peak two was still ten times greater than that for peak one (PDE V). Rolipram, RO-20-1724 and Org 30029 were all potent inhibitors of peak five with rolipram being the most potent. In a few experiments, RO-20-1724 was also tested on peak six; the compound had a slightly higher potency with an IC_{50} value of 7.4 μmol l⁻¹. Org 9935 inhibited peak one and peak five. As discussed above, the effects on the latter activity peak were biphasic in one of the three separations performed (Figure 2).

Relaxation of bronchial smooth muscle

The concentrations of histamine and methacholine employed produced $78.2 \pm 6.0\%$ (n = 11) and $68.6 \pm 4.9\%$ (n = 11), respectively, of the maximum response produced by methacholine (0.1 mmol l⁻¹). All phosphodiesterase inhibitors tested produced concentration-dependent relaxation of histamine or methacholine precontracted bronchial segments (Figures 3 and 4); the − log EC_{50} values are summarized in Table 2. IBMX, zaprinast and Org 9935 were more effective against histamine than methacholine wherease Org 30029 and rolipram equally relaxed contractions produced by these spasmogens. Rolipram and Org 9935 were most potent in causing relaxation. However, both of these drugs produced biphasic concentration-response curves (Figure 3). At concentrations below 1 μmol l⁻¹, Org 9935 invariably partially attenuated histamine-induced, but not always methacholine-induced, contractions. The concentration-response curves for IBMX, zaprinast and Org 30029 were essentially monophasic (Figure 4).

Discussion

The present study has demonstrated that the soluble fraction of human bronchial tissue contains PDE I, II, III, IV, and V isoenzyme activities; this is similar to the results reported for human trachea in a preliminary communication by Cieslinski et al. (1988). Bergstrand & Lindquist (1978) have previously examined phosphodiesterase subtypes in human bronchi and shown the presence of four phosphodiesterase activities. However, they did not characterize the regulatory properties of the isoenzymes. Studies on animal airways preparations have also shown the presence of representatives of all five phosphodiesterase isoenzyme families (Shahid et al., 1991b; Torphy & Cieslinski, 1990). However, there are significant species-dependent differences in the exact elution profile and

Table 1 Inhibition of human bronchial smooth muscle cyclic nucleotide phosphodiesterase (PDE) isoenzymes

Activity peak	PDE isoenzyme	IBMX	Zaprinast	Rolipram	RO-20-1724		Org 9935	Org 30029
1	V	4.72 ± 0.04	6.37 ± 0.08	<3.60	<3.60		4.91 ± 0.03	<3.60
2	I	4.38 (n = 1)	5.44 (n = 2)	ND	ND		ND	ND
3	I	4.54 ± 0.08	4.16 ± 0.21	ND	ND		ND	ND
4	II	4.64 ± 0.17	3.97 ± 0.06	<3.60 (n = 2)	<3.6 (n = 1)		<3.60 (n = 2)	<3.60 (n = 1)
5	III/IV	4.77 ± 0.09	4.17 ± 0.09	5.47 ± 0.07	4.73 ± 0.09	1st phase 2nd phase	7.7 ± 0.1* 4.2 ± 0.2*	4.78 ± 0.08

Column header top: − log IC₅₀ ($- \log IC_{50}$)

Values are mean ± s.e.mean
ND - Not determined.
*Data presented for Org 9935 are for the biphasic inhibition curves observed in one separation, values for all other compounds are averaged values for all separations.
All values $n \geqslant 3$ except when indicated.
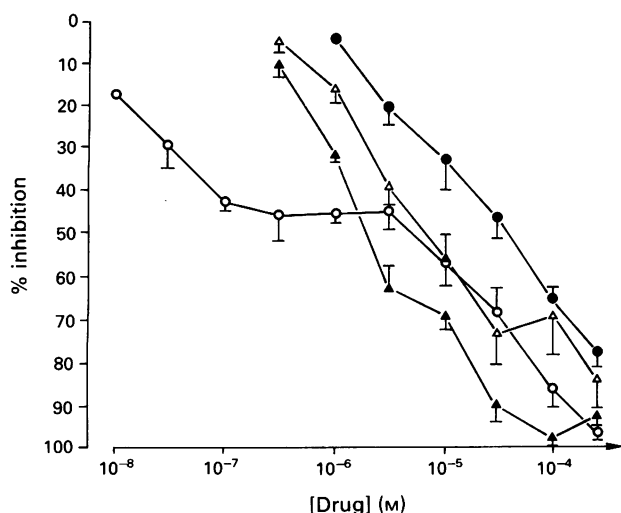IBMX: 3-isobutyl-1-methylxanthine.

Figure 2 The inhibition of human bronchial smooth muscle peak 5, cyclic AMP hydrolytic activity by Org 9935 (O) and rolipram (△). Open symbols indicate inhibition of peak enzymatic activity from a separation in which cyclic GMP inhibited cyclic AMP hydrolysis; closed symbols indicate pooled data from two further separations in which no cyclic GMP-induced inhibition was observed (see text for details).

Figure 3 Relaxation of histamine (▲; 1 μmol l⁻¹)and methacholine (O; 1 μmol l⁻¹) contracted human bronchial smooth muscle by rolipram (a) and Org 9935 (b).

relative activity levels of different phosphodiesterase isoenzymes.

In contrast to the present study and to human tracheal data (Cieslinski et al., 1988), only one form of PDE I has been detected in canine (Torphy & Cieslinski, 1990) and bovine (Shahid et al., 1991b) tracheal preparations. Human bronchial (the present study) and tracheal (Cieslinski et al., 1988) PDE I isoforms show differential sensitivity to zaprinast, suggesting that they represent distinct PDE I subtypes. Multiple forms of PDE I have been detected in other tissues such as testes (Purvis & Hallgier, 1988), brain (Sharma & Wang, 1986) and cardiac muscle (Shahid et al., 1991a). At least four distinct, but related, genes coding for the PDE I isoenzyme family have been described (Beavo & Reifsnyder, 1990). Human bronchial PDE V eluted well before PDE I; this is different from the elution profile in canine (Torphy & Cieslinski, 1990) and bovine (Shahid et al., 1991b) tracheal smooth muscle where PDE I and PDE V co-elute during ion-exchange chromatography. Furthermore, the relative activity levels of PDE I and PDE V were less than those of PDE IV in the present study, whereas in canine (Torphy & Cieslinski, 1990) and bovine (Shahid et al., 1991b) trachea both PDE I and PDE V activities are much higher than PDE IV.
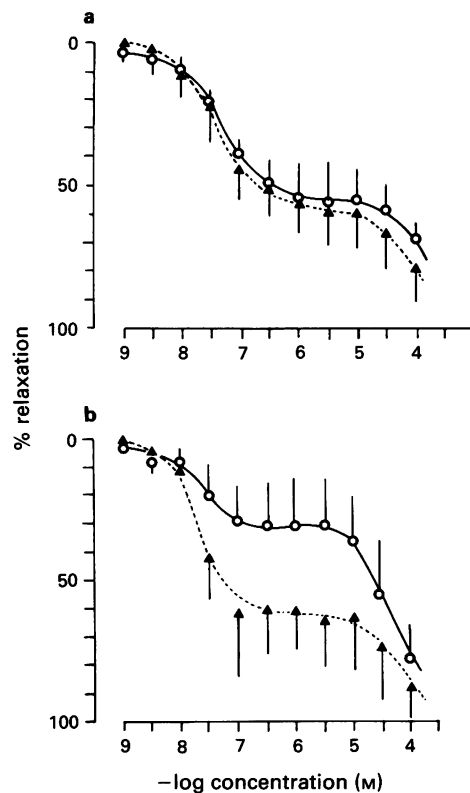
The main cyclic AMP hydrolytic activity, peak five, contained mainly PDE IV. However, using a highly potent and selective PDE III inhibitor, Org 9935 (Shahid et al., 1991b) a significant presence of PDE III was detected. Co-elution of PDE III, and PDE IV activities has previously been observed in guinea-pig (Harris et al., 1989), canine (Torphy & Cieslinski, 1990) and bovine (Shahid et al., 1991b) trachea. This is despite the fact that the separation techniques used in the present study successfully separate PDE III and PDE IV activities in other tissues such as cardiac muscle (Shahid & Nicholson, 1990). The ratio of PDE III/PDE IV activity level in airways tissue varies between species. As judged by the relative activity of isoenzyme, selective inhibitors the PDE III/IV activity ratio decreases in the order of guinea-pig ≥ man > dog > cow. A second relatively minor, PDE IV activity (peak six) was also insensitive to cyclic GMP and was inhibited by RO-20-1724. Multiple PDE IV activities

Table 2 Relaxation of human bronchial smooth muscle by cyclic nucleotide phosphodiesterase inhibitors in the presence of methacholine (1 μmol l^{-1}) or histamine (1 μmol l^{-1})

		Histamine			Methacholine		
		pD$_2$		E$_{max}$	pD$_2$		E$_{max}$
IBMX		5.58 ± 0.27	(3)	100	5.14 ± 0.13	(3)	> 98.9 ± 1.1
Rolipram	1st phase	7.14 ± 0.17	(3)	60.1 ± 12.2	7.48 ± 0.21	(3)	56.2 ± 13.5
	2nd phase	ca 4		> 79.3 ± 11.6	< 4		> 68.7 ± 5.0
Zaprinast		5.12 ± 0.26	(3)	> 84.0 ± 7.5	4.33 ± 0.36	(3)	> 54.1 ± 11.2
Org 9935	1st phase	7.47 ± 0.19	(3)	60.6 ± 15.6	7.49 ± 0.10	(3)	30.2 ± 17.5
	2nd phase	4.61 ± 0.35	(3)	> 88.1 ± 9.8	4.49 ± 0.26	(3)	> 77.9 ± 12.6
Org 30029		5.18 ± 0.54	(3)	> 84.0 ± 7.3	5.41 ± 7.3	(3)	> 84.0 ± 7.1

pD$_2$: − log EC$_{50}$ ± s.e.mean from 3 experiments.
E$_{max}$: % maximal relaxation.
IBMX: 3-isobutyl-1-methylxanthine.

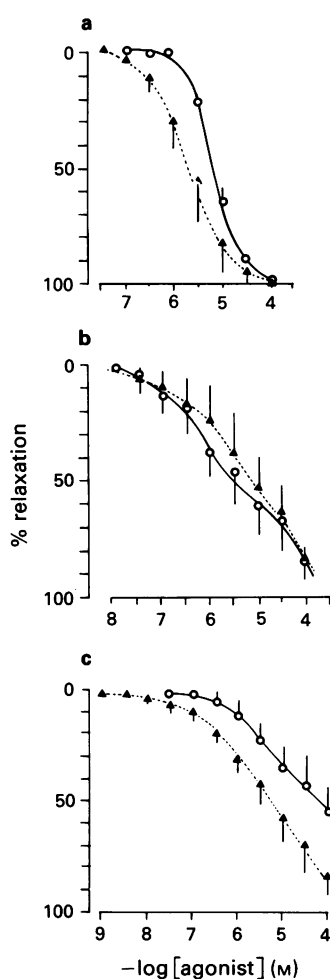**Figure 4** Concentration-relaxation curves of human bronchial smooth muscle contracted with histamine (▲; 1 μmol l^{-1}) and methacholine (○; 1 μmol l^{-1}) by the cyclic nucleotide phosphodiesterase inhibitors isobutyl-methylxanthine (a), Org 30029 (b) and zaprinast (c).

profiling in the present study does not consist solely of smooth muscle (Bloom & Fawcett, 1968; Bosken et al., 1990). Therefore, the relative amounts of differing isoenzymes in bronchial preparations may not precisely reflect their proportional activities in bronchial smooth muscle. Comparison of isoenzyme activities in preparations of cardiac tissue with those in cardiac myocytes has revealed differences in the relative activity levels in rats (Bode et al., 1991), whilst in guinea-pigs both preparations have similar isoenzyme profiles (Bethke et al., 1991). A further study in human airways smooth muscle cells is required to analyze the phosphodiesterase isoenzyme activities. However, the combination of the elucidation of the isoenzyme profile in human bronchi with the examination of the functional activity of isoenzyme selective inhibitors enables an estimation of the importance of individual isoenzymes for the control of smooth muscle function.

Although all phosphodiesterase inhibitors tested relaxed precontracted bronchi, there were marked differences in their potency and their ability to relax depended on the spasmogen used. Rolipram and Org 9935 were the most potent compounds tested and were active over concentrations maintaining selectivity for inhibition of PDE IV and PDE III isoenzymes, respectively (Reeves et al., 1987; Shahid et al., 1991b). This suggests that in human airway smooth muscle, both PDE III and PDE IV are important in regulating intracellular cyclic AMP and thus for modulation of function. The ability of Org 9935 to relax all the histamine contracted preparations indicates that PDE III is indeed present in human airways smooth muscle; why PDE III was not invariably demonstrated in the separation experiments requires further study. This may be due to the localization of a significant PDE III activity in a particular subcellular compartment or, alternatively, the heterogeneous nature of the tissue preparation used may have partially obscured the detection of PDE III. The ability of both PDE III and PDE IV inhibitors to relax the human airways preparation is more similar to data reported for guinea-pig (Harris et al., 1989) and canine (Torphy & Cieslinski, 1990) than for bovine (Shahid et al., 1991b) trachea. In the present study, both rolipram and Org 9935 produced biphasic concentration-relaxation curves, indicating that selective PDE IV and PDE III inhibition alone is insufficient to relax fully bronchial smooth muscle. However, the ability of phosphodiesterase inhibitors, like β-adrenoceptor agonists, to relax airways preparations is dependent upon the concentration of agonist employed (Torphy et al., 1988). Hence, selective inhibitors may be more effective if a lower concentration of spasmogen were employed. At high concentrations (> 10 μM), both Org 9935 and rolipram lose phosphodiesterase selectivity and also inhibit other isoenzymes (Shahid et al., 1991b). This loss of selectivity may explain the more complete relaxation of airways preparations observed at high concentrations. Alternatively, the biphasic nature of Org 9935 and rolipram relaxation curves could be due to the involvement of mechanisms

have been reported in airways preparation (Shahid et al., 1991b), and other tissues such as liver (Lavan et al., 1989) and skeletal muscle (Nicholson & Wilke, unpublished data). In liver, the two forms of PDE IV showed differential sensitivity to rolipram and RO-20-1724; one form being inhibited 5–9 fold more potently. A similar result was obtained with RO-20-1724 for peak six in the present study. Recent studies indicate that there may be four gene subfamilies for the PDE IV isoenzyme family (Beavo & Reifsnyder, 1990). As in previous studies using animal airways tissue, the preparation used for phosphodiesterase isoenzyme

other than phosphodiesterase inhibition. A binding site at which rolipram has a higher affinity than the K_i for PDE IV has been reported (Schneider *et al.*, 1986). The nature of this binding site is unknown and until it is clarified, caution should be exercised in assigning all the pharmacological activity of rolipram to phosphodiesterase inhibition. The IBMX and Org 30029 concentration-relaxation curves were monophasic, this is likely to be due to the fact that these compounds are not isoenzyme selective inhibitors. Org 30029 is an equipotent inhibitor of PDE III and PDE IV (Shahid & Nicholson, 1990). Dual inhibition of PDE III and PDE IV seems a more effective mechanism for relaxation of human bronchial smooth muscle than selective inhibition of either enzyme. Zaprinast was not an effective relaxant at concentrations showing selective inhibition of PDE V and PDE I, suggesting that these isoenzymes are relatively less important in the regulation of smooth muscle tone in this preparation.

It has been shown in a number of studies that phosphodiesterase inhibitors are more effective at relaxing histamine-induced contractions than muscarinic agonist-induced contractions (Torphy *et al.*, 1988; Harris *et al.*, 1989; Hall *et al.*, 1990; Shahid *et al.*, 1991). In agreement, the present study showed zaprinast, IBMX and Org 9935 to be less active at relaxing methacholine contractions, although in the case of Org 9935 only the maximum relaxation (first phase; Figure 3) was reduced with no change in the EC_{50} value. Whilst the mechanism(s) underlying the differential sensitivity to phosphodiesterase inhibitors against histamine or methacholine-induced spasms are not clear it may be related to differences in the ability of these agonists to stimulate phosphatidyl inositol metabolism (Hall & Hill, 1988; Madison & Brown, 1988; van Amsterdam *et al.*, 1989; 1990; Hall *et al.*, 1989; 1990). In human bronchial smooth muscle, the maximal increase in inositol phosphate production is smaller after challenge with histamine than after methacholine (van Amsterdam *et al.*, 1990). In contrast to the other compounds used in this study, Org 30029 and rolipram, did not show spasmogen-dependency in the functional experiments. It is conceivable that these compounds possess properties additional to phosphodiesterase inhibition, alternatively PDE IV inhibition may equally attenuate

smooth muscle tone generated by muscarinic or histamine receptor agonists in this preparation. Further experiments in intact preparations are required to confirm that the phosphodiesterase inhibitors tested act by increasing cellular cyclic AMP in human bronchial smooth muscle. This is important since a recent communication by Hall *et al.* (1992) has shown that in primary cultures of human tracheal smooth muscle, IBMX and rolipram increased cellular cyclic AMP whilst the PDE III inhibitor SKF 94120 had no effect. In animal tissues, increases in cyclic AMP produced by PDE IV (Hall *et al.*, 1989) and PDE III (Torphy *et al.*, 1988) inhibitors as well as activation of cyclic AMP-dependent protein kinase (Torphy *et al.*, 1988) are observed at concentrations producing tracheal relaxation.

In conclusion, the present study has shown human bronchi to contain representatives of all five presently identifiable phosphodiesterase isoenzyme families. Multiple isoforms of PDE I and PDE IV were observed and PDE IV was the main cyclic AMP hydrolytic activity. Based on inhibitor experiments, significant PDE III activity was identified the level of which was similar to that in guinea-pig airway smooth muscle. Both PDE III and PDE IV selective and dual PDE III/IV (Org 30029) inhibitors were effective relaxants of human bronchial smooth muscle, whereas the PDE V inhibitor, zaprinast was relatively ineffective. The present study confirms data, from experiments using animal tissue, that inhibitors of PDE III, PDE IV and PDE III/IV have potentially useful bronchodilator activity. Experiments with human inflammatory cells (Nielson *et al.*, 1990; Robicsek *et al.*, 1991; Torphy & Undem, 1991) indicate that PDE IV and PDE III/IV inhibitors possess greatest potential as anti-inflammatory agents. Consequently, inhibitors of PDE IV isoenzymes and possibly dual PDE III/IV inhibitors are worthy of further consideration as anti-asthma drugs.

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