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Comparison of the effects of selective inhibitors of phosphodiesterase types III and IV in airway smooth muscle with differing β-adrenoceptor subtypes

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1 The relaxant properties of the type IV adenosine 3',5'-cyclic monophosphate phosphodiesterase (cyclic AMP PDE) inhibitor, rolipram and the β_2-selective and non-selective β-adrenoceptor agonists salbutamol and isoprenaline, were compared on the guinea-pig, bovine, and mouse trachea and porcine bronchus all precontracted with methacholine (EC_{30}).

2 Rolipram and both β-agonists produced concentration-dependent reversal of the methacholine-induced tone in the four airway preparations.

3 Isoprenaline and salbutamol were similar in potency on the guinea-pig ($-\log_{10}IC_{50}$:8.43, 8.06) and bovine ($-\log_{10}$ IC_{50}:8.52, 8.40) airways. In contrast, salbutamol was much less potent than isoprenaline on the mouse trachea (>1000 fold) and the porcine bronchus (>100 000 fold).

4 The potency of rolipram approached that of isoprenaline on the guinea-pig and bovine trachea (β_2-adrenoceptors predominate). However, rolipram was significantly less active than isoprenaline on the porcine bronchus (1000 fold) and mouse trachea (>2000 fold) where β_2-adrenoceptors predominate.

5 Siguazodan, the type III cyclic AMP PDE inhibitor, produced concentration-dependent relaxations of the porcine bronchus and guinea-pig trachea contracted with methacholine. Siquazodan was 100 fold more active than rolipram in pig tissues indicating the type III isoenzyme may be of greater functional significance in this tissue. In contrast, siguazodan was 15 times less potent that rolipram in guinea-pig airways suggesting a greater role for the type IV PDE.

6 These findings may reflect a possible relationship between the β_2-adrenoceptor subtype and the functional importance of the type IV PDE isoenzyme. A similar relationship may exist between β_1-adrenoceptors and the PDE type III isoenzyme.

Keywords: Airway smooth muscle; phosphodiesterase inhibitors; β-adrenoceptors; rolipram; siguazodan

Introduction

β-adrenoceptor agonists are potent relaxants of smooth muscle, both *in vitro* and *in vivo*. The alteration in contractility may be mediated by selective stimulation of the β_1 or the β_2 adrenoceptor subtypes or by a non-selective stimulation of both depending on the receptor subtype(s) present (see review by Bülbring & Tomita, 1987).

In airway smooth muscle from several experimental animal species there are differences in the density and the relative proportions of the β-adrenoceptor subtypes present. The predominant subtype present in guinea-pig (O'Donnell, 1972; Zaagsma *et al.*, 1983) and bovine (Lemoine *et al.*, 1989) tissues is the β_2 whereas in the pig (Goldie *et al.*, 1983) and mouse (Henry & Goldie, 1990) the β_1 subtype predominate. In human airway smooth muscle only β_2-adrenoceptors have been demonstrated (Richardson & Ferguson, 1979; Carstairs *et al.*, 1985).

It has been shown that both the β_1 and β_2-adrenoceptors may coexist in cardiac muscle, although functionally the β_1-adrenoceptor subtype predominates (Heitz *et al.*, 1983; Molenaar & Summers, 1987). Thus, by the use of selective β_2-agonists it is possible to achieve a significant bronchodilatation without concomitant cardiovascular effects (Tattersfield & Britton, 1988).

It is widely accepted that β-adrenoceptor agonists produce their effects on airway and other smooth muscle by the elevation of tissue adenosine 3',5'-cyclic monophosphate (cyclic AMP) (see review by Giembycz & Raeburn 1991 and references therein), the level of which is regulated by the family of isoenzymes known as the phosphodiesterases (PDEs, Beavo & Reifsnyder, 1990; Torphy & Undem, 1991; Giembycz & Dent, 1992). Biochemical assay of the PDE isoenzyme profile has been performed in airway smooth muscle from a number of different species. In human airway smooth muscle PDE types I, II, III, IV and V have been resolved (Cieslinski *et al.*, 1988; Giembycz *et al.*, 1992; Shahid *et al.*, 1992). Types I, II, IV and V have been demonstrated in bovine trachealis (Giembycz & Barnes 1991; Shahid *et al.*, 1991) whereas only the type III and IV have been recognized in guinea-pig trachealis, although two additional fractions of PDE were not characterized (Silver *et al.*, 1988). All of these studies suggest that the type IV PDE may be the major isoenzyme present. In contrast to airways, in atrial and ventricular muscle and vascular smooth muscle the PDE type III isoenzyme appears to predominate (Silver *et al.*, 1988; Muller *et al.*, 1990 a, b).

In a preliminary study *in vitro* (Tomkinson *et al.*, 1992) we found that in the guinea-pig airways (predominantly β_2) the selective PDE type IV inhibitor rolipram approached the potency of salbutamol and isoprenaline (a selective and non-selective β-agonist respectively). On the other hand in porcine airways (predominantly β_1) isoprenaline was markedly more potent than either the selective β_2-adrenoceptor agonist or the type IV PDE inhibitor. Based on these findings we have hypothesized that there may be a relationship between β-adrenoceptor subtype and PDE isoenzyme in reducing airway smooth muscle contractility. In the present study, we have attempted to test this hypothesis by comparing the smooth muscle relaxing properties *in vitro* of salbutamol, isoprenaline, rolipram and siguazodan (SKF 94836, a PDE type III inhibitor) in airway preparations which have been shown to contain different β-adrenoceptor populations.

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Methods

Tissue preparation

Guinea-pigs (male, Dunkin-Hartley, 400–500 g. Interfauna U.K.) were killed by cervical dislocation followed by exsanguination and mice (male, 20–25 g, BALBc, Charles river, U.K.) were killed by exposure to a rising concentration of carbon dioxide. Tracheae were removed and placed in Krebs-Ringer Bicarbonate (KRB) solution and fat and connective tissue dissected away. Bovine and porcine tissues obtained from the local abattoir were placed immediately in KRB for transport. Bovine tracheae and porcine bronchi were cleaned as for the other tissues. All tissues were denuded, by mechanical abrasion, of their epithelium to remove the influence of epithelial-derived factors (Raeburn, 1990) and because of the high density of β-adrenoceptors present (Goldie et al., 1986a; Henry et al., 1990). Tracheal strips (guinea-pig, cow), tracheal rings (mouse) and bronchial strips (pig primary bronchus) were suspended under an applied load such that they were at their respective optimal length (Lo), derived from preliminary experiments, and equilibrated for 90 min washing at 15 min intervals. The KRB was maintained at 37°C and gassed with 5% CO_2 plus 95% O_2, pH was 7.4. The composition of the KRB (mM) was as follows: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, dextrose 10.1.

Contractility studies

Cumulative concentration-response curves were constructed to methacholine (10 nM–100 μM) in each tissue and the concentration producing 30% of maximum contraction (EC_{30}) was determined by computerized linear regression analysis. This concentration of spasmogen was chosen to reduce complications of functional antagonism seen at higher concentrations of the agonist. For investigation of their relaxant properties, tissues were contracted with methacholine (EC_{30}) and when the response had plateaued isoprenaline, salbutamol, rolipram, siguazodan (all from 10 nM to 100 μM) or vehicle control (saline, isoprenaline and salbutamol; DMSO, rolipram and siguazodan) were each added cumulatively. Ascorbic acid (0.57 mM) was added to the isoprenaline solution to prevent oxidative degradation. The concentration of relaxant producing 50% inhibition (IC_{50}) of the methacholine response was calculated in each tissue by linear regression. Results are expressed as the negative log of the IC_{50} ($-\log_{10} IC_{50}$).

Drugs and solutions

The following substances were used: (−)-salbutamol hemisulphate, (−)-isoprenaline (+)-bitartrate, histamine diphosphate, methacholine chloride (all Sigma); rolipram and siguazodan were synthesized by Rhône-Poulenc Rorer, Dagenham.

Statistical evaluation

Results are expressed as mean ± s.e.mean from n animals. EC_{30} values for methacholine and $-\log_{10} IC_{50}$ values for the relaxants were examined by one-way analysis of variance (ANOVA). Where a difference was found across the groups, Student's t test (unpaired, two-tailed) was performed to assess significance of difference. $P<0.05$ was accepted as significant. Least squares linear regression analysis was performed for comparison of tissue sensitivity (EC_{30}, IC_{50}) with contractility.

Results

Methacholine contractions

Cumulative concentration-response curves to methacholine in airway smooth muscle preparations from the various species are depicted in Figure 1. EC_{30} values and maximum force of contraction are listed in Table 1. The rank order of tissue sensitivity to methacholine was bovine trachealis = guinea-pig trachealis > mouse trachealis > pig bronchus. As expected, the maximum force of contraction was greater in the tissues with more muscle mass. There was, however, no correlation between maximum contractile force and tissue sensitivity to methacholine ($r = -0.29$).

Isoprenaline-induced relaxations

Isoprenaline produced concentration-dependent relaxations of the methacholine (EC_{30})-contracted airway smooth muscle

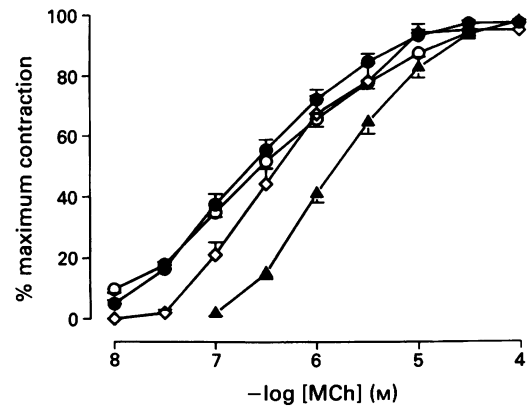

Figure 1 Comparison of the methacholine (MCh) cumulative concentration-response curves in airway smooth muscle preparations from four different species. (◇, $n = 6$) Mouse trachea, (●, $n = 10$) guinea-pig trachea, (○, $n = 5$) bovine trachea, (▲, $n = 6$) porcine bronchus. Abscissa scale: −log molar MCh concentration, ordinate scale: % maximum contraction. Values are the mean and vertical lines show s.e.mean.

Table 1 EC_{30} values and maximum force of contraction for methacholine in different airway smooth muscle preparations

Tissue	n	EC_{30} (nM)				Max. contraction (g)
Bovine trachea	5	81 ± 6	N.S.			10.74 ± 1.14
Guinea-pig trachea	10	93 ± 16		*		2.73 ± 0.30
Mouse trachea	6	210 ± 60			**	0.26 ± 0.03
Porcine bronchus	6	710 ± 60		*		3.04 ± 0.50

Data shown are means ± s.e.mean
N.S. $P>0.05$; *$P<0.05$; **$P<0.01$.

preparations from all four species (Figures 2–5). Relaxation of the methacholine-induced response was complete (>100%) in all tissues except the mouse trachea where maximum relaxation achieved was 86 ± 6% ($n = 3$). The potency of isoprenaline was similar in all tissues except the porcine bronchus (18 fold less potent) as indicated by the $-\log_{10} IC_{50}$ values quoted (Table 2).

Salbutamol-induced relaxations

Salbutamol caused concentration-dependent relaxations of the methacholine-contracted airway preparations (Figures 2–5). As with isoprenaline, relaxations to salbutamol were complete in all tissues except the mouse trachea where the maximum relaxation observed was 32 ± 14% ($n = 3$). The potency of salbutamol on the different airway preparations is indicated by the $-\log_{10} IC_{50}$ values shown in Table 2. Salbutamol was similar in potency to isoprenaline on bovine and guinea-pig tracheal preparations in which β_2-adrenoceptors predominate, the $-\log_{10} IC_{50}$ values for salbutamol being 8.40 ± 0.10 ($n = 5$) and 8.06 ± 0.10 ($n = 5$) respectively. In contrast, on mouse trachea and porcine bronchus, which have been shown to contain a predominance of β_1-receptors, salbutamol was much less potent (>1000 fold, >100,000 fold respectively) than isoprenaline.

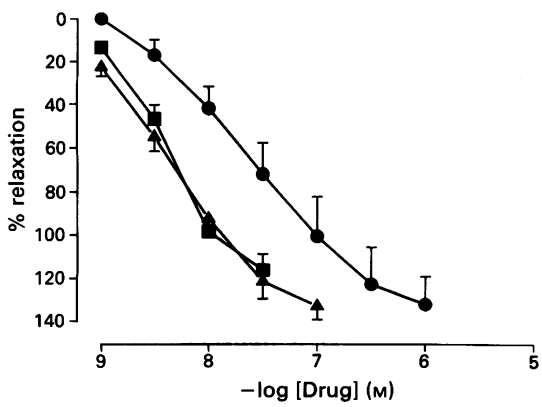

Figure 2 Relaxation of the methacholine (MCh, EC_{30})-contracted bovine tracheal smooth muscle preparation by isoprenaline (▲, $n = 5$), salbutamol (■, $n = 5$), and rolipram (●, $n = 5$). Abscissa scale: −log molar drug concentration, ordinate scale: % relaxation MCh contraction. Values are the mean and vertical lines show s.e.mean.

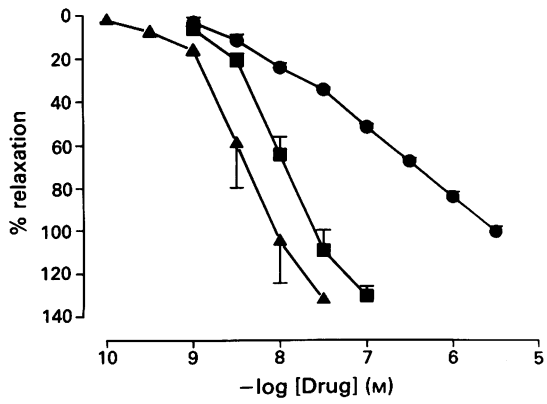

Figure 3 Relaxation of the methacholine (MCh, EC_{30})-contracted guinea-pig tracheal smooth muscle preparation by isoprenaline (▲, $n = 5$), salbutamol (■, $n = 5$), and rolipram (●, $n = 5$). Abscissa scale: −log molar drug concentration, ordinate scale: % relaxation MCh contraction. Values are the mean and vertical lines show s.e.mean.

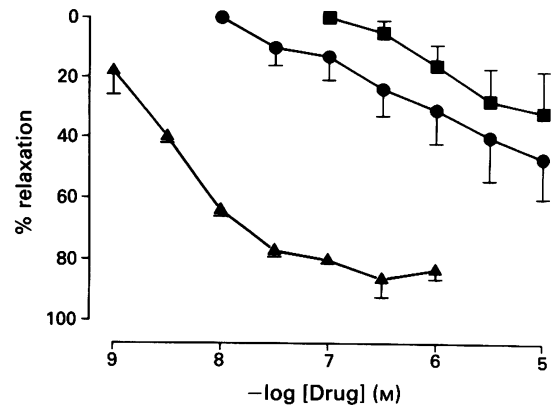

Figure 4 Relaxation of the methacholine (MCh, EC_{30})-contracted mouse tracheal smooth muscle preparation by isoprenaline (▲, $n = 3$), salbutamol (■, $n = 3$), and rolipram (●, $n = 5$). Abscissa scale: −log molar drug concentration, ordinate scale: % relaxation MCh contraction. Values are the mean and vertical lines show s.e.mean.

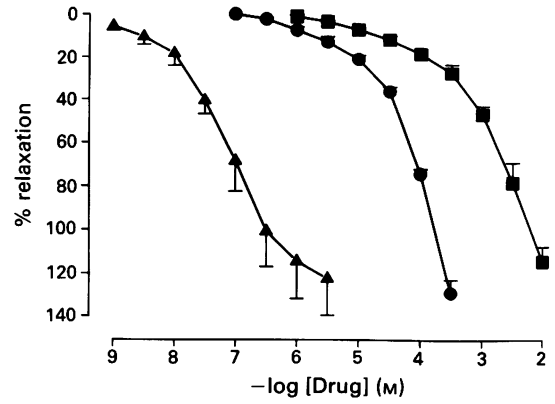

Figure 5 Relaxation of the methacholine (MCh, EC_{30})-contracted porcine bronchus smooth muscle preparation by isoprenaline (▲, $n = 5$), salbutamol (■, $n = 5$), and rolipram (●, $n = 5$). Abscissa scale: −log molar drug concentration, ordinate scale: % relaxation MCh contraction. Values are the mean and vertical lines show s.e.mean.

Rolipram-induced relaxations

Rolipram caused concentration-dependent relaxation of the methacholine-contracted airway preparations (Figures 2–5). Rolipram produced complete reversal of the methacholine-induced response in all tissues except the mouse trachea (47 ± 13%, $n = 5$). The $-\log_{10} IC_{50}$ values shown in Table 2 indicate the relative potency of rolipram on the preparations used. In bovine and guinea-pig tracheal preparations the potency of rolipram was similar and approached that of salbutamol and isoprenaline. In contrast, in porcine bronchus and mouse trachea rolipram was significantly less active than isoprenaline (1000 fold, >2000 fold respectively).

Siguazodan-induced relaxations

Siguazodan produced concentration-dependent relaxation of methacholine-contracted airway smooth muscle from pig bronchus and guinea-pig trachea. In each case reversal was complete (Figure 6). The $-\log_{10} IC_{50}$ for siguazodan in pig bronchus was 6.28 ± 0.13 ($n = 7$) indicating the type III PDE inhibitor to be 100 fold more potent than rolipram on this tissue (Figure 6a). Siguazodan-induced reversal of the methacholine-induced contraction of the guinea-pig trachea (Figure 6b) was considerably less potent (approximately 15 times) than that seen with rolipram ($-\log_{10} IC_{50}$: 5.87 ± 0.07, $n = 5$).

Table 2 $-\log_{10} IC_{50}$ values for isoprenaline, salbutamol and rolipram in methacholine (EC_{30})-contracted airway smooth muscle preparations

Tissue	n	Predominant β-receptor subtype	$-logo_{10} IC_{50}$ Isoprenaline	Salbutamol	Rolipram
Bovine trachea	5	β_2	8.52 ± 0.08	8.40 ± 0.10	7.71 ± 0.23
Guinea-pig trachea	5	β_2	8.43 ± 0.15	8.06 ± 0.10	7.01 ± 0.04
Mouse trachea	3-5	β_1	8.30 ± 0.03	< 5.00	< 5.00
Porcine bronchus	5	β_1	7.22 ± 0.20	2.94 ± 0.08	4.25 ± 0.02

Data shown are mean ± s.e.mean

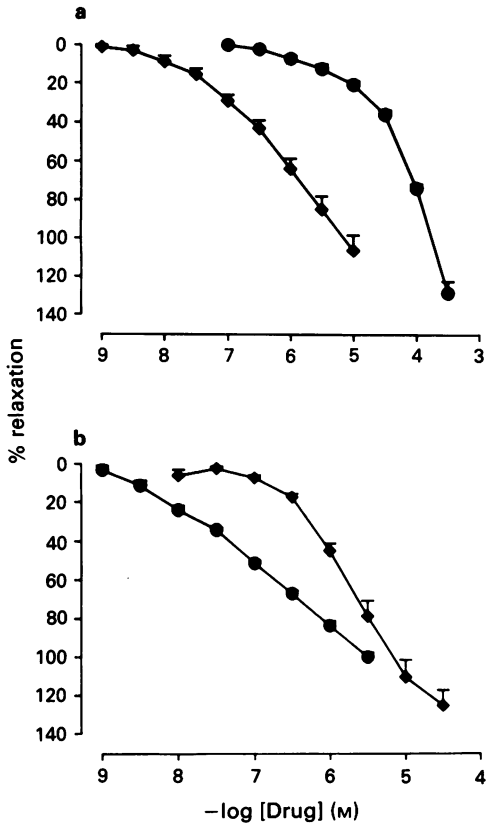

Figure 6 Comparison of the relaxant properties of rolipram (●) and siguazodan (◆) on the porcine bronchus (a) and guinea-pig trachea (b) contracted with methacholine (MCh, EC_{30}). Abscissa scale: −log molar drug concentration, ordinate scale: % relaxation MCh contraction. Values are the mean and vertical lines show s.e.mean ($n > 5$).

Discussion

In guinea-pig and bovine tracheal preparations the potency of salbutamol was similar to that of isoprenaline, confirming the predominance of the β_2-adrenoceptor subtype previously demonstrated functionally and by radioligand binding studies (Carswell & Nahorski 1983). In contrast to the guinea-pig and bovine trachea, salbutamol was significantly less potent than isoprenaline on the mouse trachea. Functional and autoradiography studies have recently demonstrated, quite comprehensively, the β_1-adrenoceptor subtype as being the predominant functional adrenoceptor in this tissue, supporting our observations (Henry & Goldie 1990; Henry *et al.*, 1990).

As with the mouse trachea, salbutamol was significantly less potent than isoprenaline on the porcine bronchus. The difference in potency of the β-agonists was particularly evident in this preparation where the potency of salbutamol was approximately 100 fold less than that seen on the mouse trachea. Surprisingly, given its low potency, salbutamol eventually produced complete reversal of the methacholine response. This probably reflects non-selectivity of salbutamol at these very high concentrations. Our findings support those of Goldie *et al.* (1983) who demonstrated an homogeneous population of β_1-adrenoceptors on the porcine bronchus.

The varied effects of the type IV PDE inhibitor, rolipram, revealed a marked difference between guinea-pig and bovine tracheal preparations on the one hand and the mouse tracheal and porcine bronchial preparations on the other, as observed for the selective β_2-adrenoceptor agonist salbutamol. Rolipram produced complete reversal of the methacholine-induced response on both the guinea-pig and bovine preparations. The potency approached that of isoprenaline and salbutamol indicating the type IV PDE isoenzyme to be of significant functional importance. These observations are consistent with previous profile and functional studies (Silver *et al.*, 1988; Harris *et al.*, 1989; Osborn *et al.*, 1990; Shahid *et al.*, 1991). In contrast, on the mouse trachea and porcine bronchus rolipram was significantly less potent than isoprenaline suggesting the type IV PDE to be of less functional significance in these tissues.

Although the PDE isoenzyme profile of the porcine bronchus and mouse trachea has yet to be described, the very low potency of rolipram on these tissues would suggest that a different PDE isoenzyme to the type IV isoenzyme may be of greater functional significance. In the present study the relaxant effect of siguazodan, a type III cyclic AMP PDE inhibitor, was determined on the porcine bronchus where it was approximately 100 fold more active than rolipram. In contrast, siguazodan was approximately 15 times less potent than rolipram on the guinea-pig trachea. These findings suggests that the type III isoenzyme may be the major functional PDE isoenzyme in pig airways whilst confirming the functional significance of the type IV isoenzyme in the guinea-pig airways. Based on these observations, we feel that as was the case with pig airways the type III isoenzyme may be the major functional isoenzyme in the mouse trachea but this has yet to be confirmed.

Collectively, these results may be interpreted as suggesting a possible functional relationship between the β_2-adrenoceptor subtype and the type IV cyclic AMP PDE isoenzyme, as proposed in our hypothesis. Furthermore, a similar relationship between β_1-adrenoceptors and the type III PDE may also occur. The existence of such a relationship would predict a low potency for rolipram on the mouse and pig tissues previously shown to contain a predominance of β_1-adrenoceptors.

In conclusion, the relatively high potency of rolipram in the guinea-pig and bovine trachea, in which β_2-adrenoceptors predominate over β_1-adrenoceptors, and the relatively low potency in the mouse trachea and porcine bronchus, in which β_1-adrenoceptors predominate over β_2-adrenoceptors, suggests a possible relationship between the β_2-adrenoceptor subtype and the functional importance of the type IV cyclic AMP PDE isoenzyme. A Similar relationship may exist between the β_1-adrenoceptor and the type III cyclic AMP PDE isoenzyme as suggested by the potency of siguazodan, relative to rolipram, on the guinea-pig and porcine airway preparations. However, since in every preparation examined rolipram and

siguazodan relaxed the tissues, irrespective of the predominant β-adrenoceptor subtype present, this suggests that both the type III and type IV PDE isoenzymes are involved in regulating airway smooth muscle contractility. It is possible that rolipram and siguazodan could be acting additionally at the type III and type IV isoenzymes respectively, that is to say that at the higher concentrations used we may be seeing some non-selective effects. However, given their respective IC_{50} values for inhibiting PDE type III and IV (Torphy & Undem, 1991; Giembycz & Dent, 1992) this seems less likely. Theophylline, a non-selective PDE inhibitor, is similar in potency on the porcine bronchus (Goldie *et al.*, 1986a), bovine trachea (Lohmann *et al.*, 1977; Grandordy *et al.*, 1987) and guinea-pig trachea (Goldie *et al.*, 1986b) suggesting that it is unlikely that the differences in potency of rolipram in these tissues represents a difference in phosphodiesterase activity *per se*.

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