Effects of rolipram and siguazodan on the human isolated bronchus and their interaction with isoprenaline and sodium nitroprusside

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1 The effects of the selective inhibitors of cyclic AMP phosphodiesterase type IV (rolipram) and type III (siguazodan) and their interactions with isoprenaline and sodium nitroprusside have been studied in the human isolated bronchus.

2 On bronchi under resting tone rolipram was, in terms of potency ($pD_2 = 7.77 \pm 0.14$, $n = 8$), very similar to isoprenaline ($pD_2 = 7.31 \pm 0.12$, $n = 12$) and salbutamol ($pD_2 = 7.12 \pm 0.17$, $n = 10$) and approximately 10 fold more potent than siguazodan ($pD_2 = 6.80 \pm 0.12$, $n = 6$). In terms of efficacy (E_{max} , expressed as percentage of maximal effect induced by theophylline 3 mM), both rolipram and siguazodan were less efficient ($E_{max} = 74 \pm 6.7\%$, $n = 8$ and $66 \pm 7.5\%$, $n = 6$, respectively) than isoprenaline ($E_{max} = 98 \pm 0.4\%$, $n = 12$) and salbutamol ($E_{max} = 83 \pm 2.4\%$, $n = 10$).

3 During precontraction induced by methacholine (3×10^{-7} M) or acetylcholine (10^{-3} M), concentration-response curves to rolipram and siguazodan were shifted to the right and maximal effects reduced. Rolipram was more potent than siguazodan and, in terms of efficacy, it was less active.

4 Rolipram 10^{-8} and 10^{-7} M but not siguazodan potentiated the effects of isoprenaline as shown by the shift to the left of the concentration-response curve to isoprenaline. Sodium nitroprusside-induced relaxation was not modified by either drug.

5 These results show that rolipram is a potent relaxant of the human isolated bronchus, potentiating the effects of β -adrenoceptor stimulation and suggest that, as previously demonstrated in other species (guinea-pig, cow) (Tomkinson *et al.*, 1993), there may be a connection between the β_2 -adrenoceptor subtype, which predominate in human airway smooth muscle, and the cyclic AMP phosphodiesterase type IV.

Keywords: Human airway smooth muscle; phosphodiesterase inhibitors; β -adrenoceptors; rolipram; siguazodan; isoprenaline; salbutamol; sodium nitroprusside

Introduction

Elevation of tissue adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels leads to relaxation of airway smooth muscle preparations. Amongst the potential mechanisms whereby this effect occurs are reduction in intracellular calcium levels, inhibition of inositol phospholipid hydrolysis, and alteration in the sensitivity of contractile proteins to calcium or activation of potassium channels (Giembycz & Raeburn, 1991). The breakdown of cyclic AMP to 5'AMP in tissues is dependent upon the activity of tissue cyclic nucleotide phosphodiesterases (PDE).

At least five distinct phosphodiesterase isoenzymes are present in mammalian airway smooth muscle, each having different selectivities and K_m values for cyclic AMP and guanosine 3':5'-cyclic monophosphate (cyclic GMP). Among these, type III (low K_m , cyclic GMP-inhibited) and type IV (high K_m , cyclic AMP-selective) isoenzymes appear to be important for the regulation of cyclic AMP breakdown in intact airway smooth muscle from canine, bovine or human airways (Torphy *et al.*, 1988; Silver *et al.*, 1988; Beavo & Reifsnnyder, 1990; Nicholson *et al.*, 1991; Torphy & Undem, 1991; Hall & Hill, 1992; Giembycz *et al.*, 1992).

Since β -adrenoceptor agonists act by increasing the tissue content of cyclic AMP, subsequent to stimulation of adenylyl cyclase, it was of interest to study the relationship between β -adrenoceptor activation and the type of PDE involved in the effects of β -adrenoceptor agonists. Tomkinson *et al.*

(1993) have recently hypothesized that there may be a connection between the β -adrenoceptor subtype (β_1 or β_2) which predominates in a tissue and the functional importance of the PDE (III or IV) isoenzyme in reducing smooth muscle contractility in various species (including guinea-pig, cow, pig, and mouse) and demonstrated that there was a relationship between β_2 -adrenoceptor and cyclic AMP PDE type IV on the one hand and β_1 -adrenoceptor and cyclic AMP PDE type III on the other hand.

The aim of this study was to extend these investigations to the human airway smooth muscle. We compared the effects of isoprenaline, salbutamol, rolipram, an inhibitor of PDE type IV, and siguazodan (SK&F 94836), an inhibitor of PDE type III, and their interactions on the human isolated bronchus.

Methods

Human bronchus preparation

Human bronchial tissue (usually with an inner diameter of 1 to 4 mm) was obtained from patients undergoing surgery for lung cancer but taken at a distance from malignancy. Bronchi were dissected free of parenchyma and transported to the laboratory in Krebs solution previously aerated with a mixture of 95% O₂ and 5% CO₂ (pH 7.40). The tissue was stored overnight at 4°C and the experiment was performed the next day. Previous experience in this laboratory and published data have demonstrated that overnight storage of tissue does not alter its reactivity.

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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 of equilibration with washing every 15 min, the resting load was 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 I. O. S.-Moise 3 recorder system (EMKA Technologies, Mitry Mory, France). The composition of the Krebs solution was (mM): 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 10^{-3} M and then relaxed with theophylline 3×10^{-3} M. These concentrations did not alter responsiveness of the tissue. A 1 h rest period was observed before the beginning of the experimental procedure.

Experimental procedures

Concentration-response curves obtained with isoprenaline, salbutamol, siguazodan and rolipram Following the resting period, the bronchial rings were either left under resting load or were contracted with methacholine 3×10^{-7} M or acetylcholine 10^{-3} M. After the contraction plateau was reached, concentration-response curves were established by cumulative addition of isoprenaline, salbutamol or the phosphodiesterase inhibitors siguazodan and rolipram at intervals of 5–30 min to obtain a relaxation plateau. After the maximal effect of each drug was obtained, theophylline 3×10^{-3} M was added to the bath in order to determine the maximal relaxation. Only one concentration-response curve was recorded in each ring.

Influence of siguazodan and rolipram on concentration-response curves to isoprenaline and sodium nitroprusside (SNP) Following the resting period, bronchial rings were pretreated for 30 min with Krebs solution (control) or one concentration of siguazodan (10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} M) or rolipram (10^{-9} , 10^{-8} , and 10^{-7} M). Each concentration-response curve for relaxation by isoprenaline or SNP was obtained with cumulative additions of the drug after the bronchial ring had been brought to a steady level of contraction with acetylcholine 10^{-4} M. Finally, theophylline 3×10^{-3} M was added in order to determine the maximal relaxation. Only one concentration-response curve was recorded in each ring.

Expression and statistical analysis of the results

The relaxant effects of isoprenaline, salbutamol, siguazodan or rolipram are expressed as a percentage of the relaxation produced by theophylline 3×10^{-3} M. pD_2 values represent the negative logarithm of the concentration of isoprenaline, salbutamol, siguazodan or rolipram which induces a relaxation equal to 50% of maximal effect induced by itself. E_{max} values represent the maximal relaxation induced by isoprenaline, salbutamol, siguazodan or rolipram and are expressed as percentage of that induced by theophylline 3×10^{-3} M.

Statistical analysis of the results was performed by analysis of variance and Student's *t* test. All values are expressed as mean $\pm$ standard error of the mean; *P* values lower than 0.05 were considered to be significant.

Drugs

The drugs used were: theophylline (Theophylline Bruneau, Paris, France), acetylcholine HCl (Pharmacie Central des Hôpitaux, Paris, France), isoprenaline HCl (Laboratoires Winthrop, Paris, France), methacholine HCl, salbutamol sulphate, sodium nitroprusside (Sigma, St Louis, U.S.A), siguazodan, rolipram (synthesized by Rhône-Poulenc Rorer, Dagenham, Essex).

All drugs were dissolved daily in distilled water and diluted in Krebs solution.

Results

Activity of isoprenaline, salbutamol, siguazodan and rolipram on the human isolated bronchus

The concentration-response curves to the relaxant effects of isoprenaline, salbutamol and the selective phosphodiesterase inhibitors, rolipram and siguazodan, on human bronchi at resting tone or precontracted with methacholine 3×10^{-7} M or acetylcholine 10^{-3} M are shown in Figures 1 and 2. The potency and efficacy of these substances were calculated by determining the pD_2 values and the maximal effects (E_{max}) (Table 1).

Table 1 shows that on bronchi at resting tone, rolipram was, in terms of potency, very similar to isoprenaline and salbutamol and approximately 10 fold more potent than siguazodan. During precontraction induced by methacholine 3×10^{-7} M, the order of potency of these substances remained the same but siguazodan was, in terms of efficacy, more effective than rolipram.

In bronchi precontracted with acetylcholine 10^{-3} M, the concentration-response curves to rolipram and siguazodan were, as with methacholine, displaced to the right (Figure 2). The maximal effects of rolipram and siguazodan were reduced when compared with the resting tone situation. Rolipram was more potent than siguazodan and, in terms of efficacy, it was less effective.

Figure 1 Concentration-response (relaxation) curves to isoprenaline (○), salbutamol (●), rolipram (□) and siguazodan (■) of human isolated bronchi. Experiments were performed on preparations precontracted with methacholine 3×10^{-7} M. The number of experiments is indicated in Table 1. Values are mean $\pm$ s.e.mean.

Figure 2 Concentration-response (relaxation) curves to siguazodan and rolipram of human isolated bronchi. Experiments were performed on preparations at resting tone (○) or precontracted with acetylcholine 10^{-3} M (●). The number of experiments is indicated in Table 1. Values are mean $\pm$ s.e.mean.

Influence of rolipram and siguazodan on concentration-response curves to isoprenaline and sodium nitroprusside

Figure 3 shows that after 30 min incubation of human bronchi with rolipram 10^{-8} and 10^{-7} M, the concentration-response curves to isoprenaline were significantly shifted to the left and the maximal effect of isoprenaline was increased. In contrast, concentration-response curves to isoprenaline were not modified by siguazodan 10^{-8} to 10^{-5} M. Finally, concentration-response curves to sodium nitroprusside were not displaced after 30 min incubation with rolipram or siguazodan.

Discussion

In a previous paper, Tomkinson *et al.* (1993) compared the relaxant properties of a PDE III inhibitor, siguazodan, and a

PDE type IV inhibitor, rolipram, on the guinea-pig, bovine, murine and porcine airway smooth muscle. They observed that the potency of rolipram approached those of isoprenaline and salbutamol on the guinea-pig and bovine trachea, where β_2 -adrenoceptors predominate, but rolipram was significantly less active than isoprenaline on the porcine bronchus (1000 fold) and murine trachea (>2000 fold), where β_1 -adrenoceptors predominate. They also demonstrated that siguazodan was 100 fold more active than rolipram on porcine tissues but 15 times less potent on guinea-pig airways. On the basis of these results, Tomkinson *et al.* (1993) suggested that there might be a connection between the β -adrenoceptor subtype which predominates in a given tissue and the functional importance of the PDE isoenzyme in reducing smooth muscle contractility. They postulated a possible relationship between the β_2 -adrenoceptor subtype and the functional importance of the PDE type IV isoenzyme on the one hand, and between the β_1 -adrenoceptor and the PDE type III isoenzyme on the other. De Boer *et al.* (1992) have

Table 1 pD_2 and E_{max} of isoprenaline, salbutamol and the selective phosphodiesterase inhibitors on the human isolated bronchus at resting tone or contracted with methacholine (MCh) 3×10^{-7} M or acetylcholine (ACh) 10^{-3} M

	n	Resting tone		n	MCh (3×10^{-7} M)		n	ACh (10^{-3} M)	
		pD_2	E_{max}		pD_2	E_{max}		pD_2	E_{max}
Isoprenaline	12	7.31 ± 0.12	98 ± 0.4	5	7.28 ± 0.06	97 ± 1.0	11	6.56 ± 0.13	85 ± 3.7
Salbutamol	10	7.12 ± 0.17	83 ± 2.4	5	7.19 ± 0.04	76 ± 7.7	11	6.62 ± 0.15	55 ± 6.5
Rolipram	8	7.77 ± 0.14	74 ± 6.7	5	7.47 ± 0.04	53 ± 8.1	8	7.28 ± 0.21	32 ± 3.0
Siguazodan	6	6.80 ± 0.12	66 ± 7.5	5	6.51 ± 0.09	71 ± 6.9	6	6.92 ± 0.09	55 ± 9.6

n = number of experiments.

pD_2 : the negative logarithm of the concentration of isoprenaline, salbutamol, rolipram or siguazodan which induces a relaxation equal to 50% of maximal effect induced by itself.

E_{max} : maximal effect as percentage of maximal effect induced by theophylline 3×10^{-3} M.

Figure 3 Influence of rolipram (a,c) or siguazodan (b,d) on the concentration-response curves to isoprenaline (a,b) or sodium nitroprusside (SNP) (c,d) of human isolated bronchi, precontracted with acetylcholine 10^{-4} M. Concentration-response curves to isoprenaline or SNP were performed after 30 min incubation with saline (control, $\circ$), rolipram or siguazodan (10^{-9} M: Δ , 10^{-8} M: $\square$, 10^{-7} M: $\blacktriangle$, 10^{-6} M: $\blacksquare$, 10^{-5} M: $\bullet$). Values are mean $\pm$ s.e. mean of 5 to 7 experiments. Significant differences from control are: $\$P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$.

reported that PDE types I, II, IV and V can be isolated by ion-exchange chromatography from human, isolated bronchial muscle. PDE IV was the main cyclic AMP hydrolytic activity. A distinct PDE III activity was not obtained but it was suggested that small amounts of PDE III may have co-eluted with the PDE IV.

Our results on the isolated human bronchus precontracted with methacholine 3×10^{-7} M, corresponding to 30% of the maximal contraction induced by this substance – i.e. under conditions similar to those described by Tomkinson *et al.* (1993) on the airways of guinea-pig, cow, pig and mouse – tend to support previous observations and suggest that there may be a similar relationship between β_2 -adrenoceptors and PDE type IV in the human bronchus since rolipram seems to be as potent (or slightly more potent) than isoprenaline and salbutamol, and about 10 fold more potent than siguazodan. Since both rolipram and the selective PDE III inhibitor Org 9935 were potently active at concentrations required for selectivity (de Boer *et al.*, 1992), both PDE III and IV may be important in regulating contractility. Indeed de Boer *et al.* (1992) demonstrated that equally potent inhibition of methacholine-induced contractions was seen with selective type IV and type III cyclic AMP PDE inhibitors. This was not the case in our study where rolipram was 10 fold more potent than siguazodan. The reason for this discrepancy is not clear but may be due to different PDE III inhibitory potency between Org 9935 and siguazodan. It is interesting to note (see Table 1) that while rolipram was the more potent cyclic AMP PDE inhibitor, siguazodan produced the greater maximum relaxation in the precontracted (but not basal) tissues. This supports the view of de Boer *et al.* (1992) that the relative amount of a PDE isoenzyme may not reflect the functional importance of its inhibition. It is also possible that the functional importance of PDE III and IV may vary with the contractile state of the tissue.

The observations concerning the relationship between β_2 -adrenoceptors and the functional effects of PDE type IV inhibition in airways smooth muscle preparations are also supported by our finding that in smooth muscle relaxant concentrations, rolipram potentiates the bronchodilator effects of isoprenaline, whereas under the same conditions siguazodan does not. In contrast to zaprinast (Zhang *et al.*, 1992), neither rolipram nor siguazodan potentiate the effects of sodium nitroprusside. These data indicate that these substances do not modulate cyclic GMP hydrolysis. One may argue that we have used isoprenaline which is an agonist of both β_1 - and β_2 -adrenoceptors and, in consequence, the β_1 -adrenoceptor might also be related to PDE type IV. It has however been clearly shown that the stimulation of β_1 -adrenoceptors in the airways is without functional effect either in man (Löfdahl & Svedmyr, 1982; 1984) or in guinea-pig (Iakovidis *et al.*, 1980; Johansson & Waldeck, 1981). Our results are concordant with those of Hall *et al.* (1992) who have demonstrated that rolipram potentiated the increase in [3 H]-cyclic AMP induced by isoprenaline in primary cultures

of human tracheal smooth muscle cells whereas siguazodan did not. Our results are also in agreement with Zaagsma *et al.* (1992) who showed that rolipram or the PDE III/PDE IV inhibitor Org 30029 potentiated the inhibitory effect of fenoterol on the antigen-induced contraction of the passively sensitized human bronchial isolated smooth muscle, whereas the PDE III selective inhibitor milrinone was inactive. Zaagsma *et al.* (1992) also demonstrated that the β -adrenergic inhibition of antigen-induced histamine release from guinea-pig peripheral lung was potentiated in the presence of rolipram. Human bronchi seem to differ from the bronchi of dog or guinea-pig on which it has been demonstrated that the effects of isoprenaline were potentiated by either siguazodan (SK&F 94836) or Ro 20-1724, a PDE type IV inhibitor (Torphy *et al.*, 1988; Rhoden & Barnes, 1990).

Thus, in the airways at least, PDE type IV inhibition may result in a potentiation that is specific to the effects of β_2 -adrenoceptor stimulation. This is of interest for substances to be used in the treatment of asthma and goes side by side with other specific and potentially interesting effects of PDE type IV inhibitors, such as inhibition of the microvascular leakage induced by PAF in the guinea-pig (Raeburn & Karlsson, 1992; Ortiz *et al.*, 1992), of the N-formylmethionyl-leucyl-phenylalanine (fMLP)-stimulated superoxide release and fMLP/thiomersal elicited leukotriene biosynthesis by human polymorphonuclear leukocytes (Schudt *et al.*, 1991), as well as inhibition of mediator release from human basophils, mast cells, monocytes or neutrophils (Peachell *et al.*, 1990; Neilson *et al.*, 1990) or of superoxide formation in guinea-pig eosinophils (Dent *et al.*, 1990).

Our results also show that, contrary to those reported by Giembycz *et al.* (1992) on the human trachea, rolipram relaxes the human bronchus at resting tone. This discrepancy might be due to a difference in preparation (trachea *versus* bronchus), but this is unlikely since these authors have shown that PDE type IV is predominant in the human trachea and Hall *et al.* (1992) have demonstrated that rolipram elevated basal [3 H]-cyclic AMP levels in primary cultures of human tracheal smooth muscle cells. The discrepancy may also be due to differences in experimental conditions, since the dilatation reserve is perhaps greater distally, but in their experiments Giembycz *et al.* (1992) have shown that SK&F 94120, a PDE III inhibitor, relaxes the trachea irrespective of whether it is at resting tone or contracted with methacholine.

In any case, our results are similar to those of Hall & Hill (1992) who observed a relaxant effect of rolipram on the bovine isolated trachea at resting tone and a shift to the right of rolipram concentration-response curves by functional antagonism under the influence of methacholine. Hall & Hill (1992) also observed a relaxant effect of SK&F 94120 and siguazodan, both PDE type III inhibitors, as we did with siguazodan on the human isolated bronchus and as Giembycz *et al.* (1992) did with SK&F 94120 on the human isolated trachea.

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