ELSEVIER

European Journal of Pharmacology 344 (1998) 59–66

ejp

Effects of phosphodiesterase inhibitors and salbutamol on microvascular leakage in guinea-pig trachea

Jean-Michel Planquois [a,*], Geneviève Mottin [a], Myriam Artola [a], Vincent Lagente [b], Adrian Payne [a], Svein Dahl [a]

[a] *Department Allergie-Inflammation, Institut de Recherche Jouveinal, B.P. 100, 3-9 rue de la Loge, 94265 Fresnes Cedex, France*
[b] *INSERM U 456, Laboratoire de Pharmacodynamie et de Pharmacologie Moléculaire, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes 1, Rennes, France*

Received 28 October 1997; accepted 18 November 1997

Abstract

The aim of this study was to investigate the effects of selective phosphodiesterase inhibitors and their combination with salbutamol on antigen-induced microvascular leakage in the trachea. In actively sensitized anaesthetized guinea-pigs, the non-selective phosphodiesterase inhibitor theophylline (100 mg/kg p.o.) and the selective phosphodiesterase type 4 inhibitor Ro 20-1724 (30 mg/kg p.o.) inhibited antigen-induced microvascular leakage (−73.8% and −44.1%, respectively) as demonstrated by a reduced extravasation of plasma proteins measured by the use of Evans blue dye. No significant reduction in microvascular leakage was seen with (a) the selective phosphodiesterase type 4 inhibitor rolipram (10 mg/kg p.o.), (b) the selective phosphodiesterase type 3 inhibitors milrinone (30 mg/kg p.o.) and SK and F 94-836 (30 mg/kg p.o.) or (c) the selective phosphodiesterase type 1/5 inhibitor zaprinast (30 mg/kg p.o.). Neither Ro 20-1724 nor rolipram and theophylline inhibited microvascular leakage induced by either substance P or histamine. Pretreatment with aerosolized salbutamol (10 μg/ml) potentiated the inhibitory effects of theophylline (−49.8% at 30 mg/kg p.o.) and Ro 20-1724 (−52.6% at 10 mg/kg p.o.) versus antigen-induced microvascular leakage. Furthermore, a significant inhibitory effect of rolipram (10 mg/kg, p.o.) was obtained following pretreatment with this concentration of aerosolized salbutamol. Even at higher concentrations (0.3–2 mg/ml) salbutamol did not augment the corresponding inhibitory effects of rolipram and Ro 20-1724 versus microvascular leakage induced by either histamine or substance P. Theophylline had no effect versus substance P-induced microvascular leakage, but did inhibit it significantly ($P < 0.05$) after pretreatment with aerosolized salbutamol (0.3 mg/ml). The potentiation by salbutamol of the inhibitory effects of both non-selective and selective phosphodiesterase type 4 inhibitors versus antigen-induced microvascular leakage probably results from a synergistic reduction in the release of anaphylactic mediators. © 1998 Elsevier Science B.V.

Keywords: Phosphodiesterase inhibitor; Microvascular leakage; Trachea; (Guinea pig); β_2-adrenoceptor agonist

1. Introduction

Airway oedema is potentially an important contributing factor to the inflammatory component of asthma. This oedema results from an increase in the microvascular permeability of the tracheobronchial circulation, leading to plasma exudation and infiltration of inflammatory cells into the airway lumen (Djukanovic et al., 1990). The tracheobronchial microcirculation consists of a subepithelial capillary network. In some species, it consists of deeper capacitance vessels and an adventitial network. Postcapillary venules are the main site of plasma extravasation in inflammatory conditions. Although the capacitance system in humans has not been studied extensively, it is probably present with muscular walls. It is absent in rats but present in guinea pigs (Widdicombe, 1996). At present, the mechanism that provokes plasma protein extravasation is incompletely understood. There is evidence that it involves several mediators, such as histamine, platelet-activating factor (PAF) and substance P, all of which induce direct plasma extravasation from the postcapillary venules of the pulmonary microcirculation (Person, 1986). In asthma, airway oedema can contribute not only to airway narrowing but also to bronchial hyperresponsiveness (Boschetto et al., 1989; Erjefält and Persson, 1991). Hence inhibition of microvascular permeability

* Corresponding author. Tel.: +33-1-40967493; fax: +33-1-40967595.

could improve airway calibre and also reduce airway inflammation, thereby providing both therapeutic and prophylactic benefit.

The cyclic nucleotides cyclic adenosine monophosphate (cyclic AMP) and cyclic guanosine monophosphate (cyclic GMP) are important second messengers of cellular function. Increased intracellular levels of either cyclic AMP or cyclic GMP in airway smooth muscle results in bronchodilation. Similarly, increased intracellular levels of either nucleotide in inflammatory cells reduces mediator release (Nicholson and Shahid, 1994). The intracellular level of cyclic nucleotides is mainly determined by their breakdown by phosphodiesterases. Phosphodiesterases are divided currently into seven families (types), each with distinct substrate specificities and regulatory characteristics (Beavo et al., 1994). For example, phosphodiesterase type 3 and phosphodiesterase type 4 are specifically responsible for cyclic AMP hydrolysis, whilst phosphodiesterase type 5 is cyclic GMP specific. The availability of selective inhibitors allows the study of the influence of the enzymatic activity of various subtypes on airway oedema. In guinea-pig trachea, the selective phosphodiesterase type 4 inhibitor rolipram, reduces PAF, histamine and bradykinin-induced airway microvascular leakage (Ortiz et al., 1992a,b; Raeburn and Karlsson, 1993), an effect not shared by selective phosphodiesterase type 3 inhibitors. These findings suggest that selective phosphodiesterase type 4 inhibitors may provide clinical benefit in asthma by virtue of their potential to reduce airway oedema. Furthermore, such inhibitors may enhance both the bronchodilator and potential anti-inflammatory effects of β_2-adrenoceptor agonists, which raise cyclic AMP directly through stimulation of adenylate cyclase activity.

In the present study, we investigated the effect of pretreatment with selective inhibitors of various phosphodiesterase subtypes on antigen, substance P and histamine-induced microvascular leakage in guinea-pig trachea. We compared their effects with those of the non-selective phosphodiesterase inhibitor theophylline and the β_2-adrenoceptor agonist salbutamol. We further investigated the possible synergistic action of combined pretreatment with oral phosphodiesterase type 4 inhibitors and aerosolized salbutamol.

2. Materials and methods

2.1. Sensitization procedure and challenge

Male Hartley guinea pigs (250–300 g) were purchased from Charles River (Saint Aubin-Lès-Elbeuf, France). Animals were fed a standard pellet diet (UAR, Villemoisson-sur-Orge, France) and allowed water ad libitum. Animals were actively sensitized by i.p. injection of 20 μg ovalbumin and 100 mg aluminium hydroxide ($AL(OH)_3$) in 0.5 ml saline solution on day 1 and 2 of the experiment (Hui et al., 1991). 14–21 days later, the sensitized animals were anaesthetized (urethane, 1.5 g/kg, i.p.) and challenged by bolus injection of OA (0.007 to 1 mg/kg) together with Evans blue dye (20 mg/kg), administered through a jugular vein. 10 min after ovalbumin challenge, the trachea (from larynx to bronchial bifurcation) was removed (Section 2.3) and immediately afterwards animals were euthanased by exsanguination.

2.2. Administration of substance P and histamine

In another set of experiments, either substance P (0.3 to 3 μg/kg) or histamine (10 to 140 μg/kg) were administered intravenously together with Evans blue dye (20 mg/kg) in non-sensitized guinea pigs.

2.3. Measurement of tracheal microvascular leakage

Vascular permeability was measured by the extravasation of Evans blue dye, which correlates well with the extravasation of radiolabelled albumin in the airways (Rogers et al., 1989). 10 min after administration of antigen, substance P or histamine, the trachea was removed and carefully dissected. The trachea was cut into two portions approximatively equal in length which were then weighed. One portion was placed in formamide (6 ml/g wet weight tissue) at 20°C for 24 h to extract Evans blue dye in the tissue. The other portion was dried at 60°C for 24 h in order to calculate the ratio between dry weight and wet weight. The amount of Evans blue dye extracted was evaluated by spectrophotometry at 620 nm wavelength, using 96 well microplates. The results were plotted against a standard curve of Evans blue dye (0.5 to 50 μg/ml) in formamide and the Evans blue dye content of each sample calculated and expressed as Evans blue dye μg/g dry weight of tissue.

In this experimental procedure, perfusion with saline to remove the intravascular marker was not carried out. However, in a preliminary study we showed that there was no significant influence on Evans blue dye extravasation following histamine-induced microvascular leakage in guinea-pig trachea of vascular lavage (149.7 ± 22.8 and 162.7 ± 43.5 μg E.B./g dry weight tissue, with/without lavage, respectively) (Planquois et al., unpublished data).

2.4. Protocol for administration of phosphodiesterase inhibitors and salbutamol

Guinea-pigs were treated orally 60 min before administering ovalbumin (0.3 mg/kg i.v.), substance P (1 μg/kg i.v.) or histamine (100 μg/kg i.v.), with the selective phosphodiesterase type 4 inhibitors: rolipram (5 mg/kg p.o. or 0.3, 1, 5 mg/kg p.o.) or Ro 20-1724 (10, 30 mg/kg p.o. or 3, 10 mg/kg p.o.), or the non-selective

phosphodiesterase inhibitor: theophylline (100 mg/kg p.o. or 10, 30 mg/kg p.o.). Control groups were treated with vehicle (methyl cellulose 1%) alone. In all cases the dose volume was 1 ml/kg.

In experiments where salbutamol was administered, the phosphodiesterase inhibitors were given immediately beforehand. Salbutamol (1 μg/ml to 3 mg/ml) or saline solution (control group) was administered by aerosol for 30 min while the animals were conscious. For this purpose, the animals were placed in a plexiglass chamber ($30 \times 50 \times 30$ cm) and the aerosol was generated by a DeVilbiss™ ultrasonic nebulizer (ULTRA-NEB 99, Somerset, PA). In this case, ovalbumin challenge or administration of either histamine or substance P was carried out 30 min after the end of the administration of aerosolized salbutamol.

2.5. Drugs and solutions

Ethyl carbamate (urethane) was obtained from Prolabo (Paris, France). Theophylline, ovalbumin (albumin chicken egg, Grade V), substance P and histamine were purchased from Sigma (St Louis, MO). Salbutamol, dissolved in sterile saline (NaCl 0.9%), was purchased from Sigma (St Louis, MO). Ro 20-1724 was purchased from RBI, Natick (MA, USA). Rolipram (racemate) was synthesized at the Institut de Recherche Jouveinal. Previous studies (Coleman et al., 1995; Lagente et al., 1994, 1995) have shown that the pharmacological activity of the rolipram synthesized at the Institut de Recherche Jouveinal is equivalent to that described for rolipram manufactured by the others company. All phosphodiesterase inhibitors were suspended in methyl cellulose 1%, immediately before their oral administration. Doses used were selected on the basis of preliminary experiments and/or literature precedent (Howell et al., 1992, 1995; Underwood et al., 1993; Lagente et al., 1994, 1995).

2.6. Data analysis

Results are expressed in μg of Evans blue dye per g of dry weight of tissue (mean ± S.E.M.). As the data was distributed normally, statistical analysis was carried out by Student's *t*-test for unpaired samples, a *P*-value of < 0.05 being considered statistically significant.

3. Results

3.1. Effects of ovalbumin challenge and administration of substance P or histamine on microvascular leakage

Single bolus administration of ovalbumin (0.07 to 1 mg/kg i.v.) to sensitized animals elicited a dose-dependent increase in the amount of Evans blue dye recovered from the trachea ex situ. (Fig. 1). An ovalbumin dose of 0.3 mg/kg was used for subsequent experiments, since this produced an effect that was just submaximal.

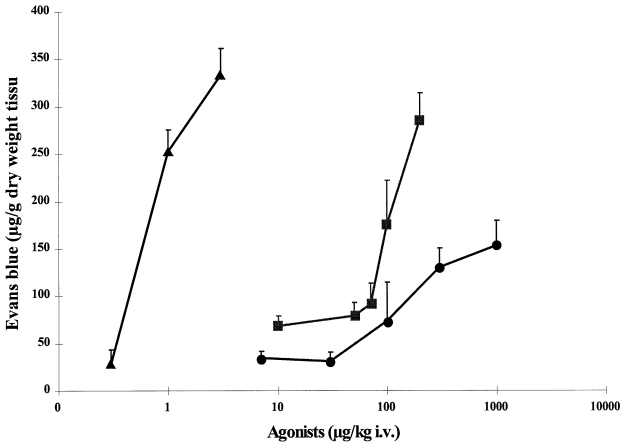

Fig. 1. Dose–response curve for substance P- (▲, $n = 3$), histamine- (■, $n = 6$) and ovalbumin- (●, $n = 3$) induced microvascular leakage in guinea-pig trachea. Abscissa: dose of agonist (mg/kg i.v.), ordinate: microvascular leakage in μg of Evans blue dye per g of dry weight tissue. Each value represents the mean ± S.E.M. from 3 to 6 observations.

Substance P (0.3 to 3 μg/kg i.v.) and histamine (10 to 140 μg/kg i.v.) induced a dose-dependent microvascular leakage in guinea-pig trachea (Fig. 1). Doses of 1 μg/kg substance P and 100 μg/kg histamine were used for subsequent experiments, since at these doses, they induced a sub-maximal microvascular leakage.

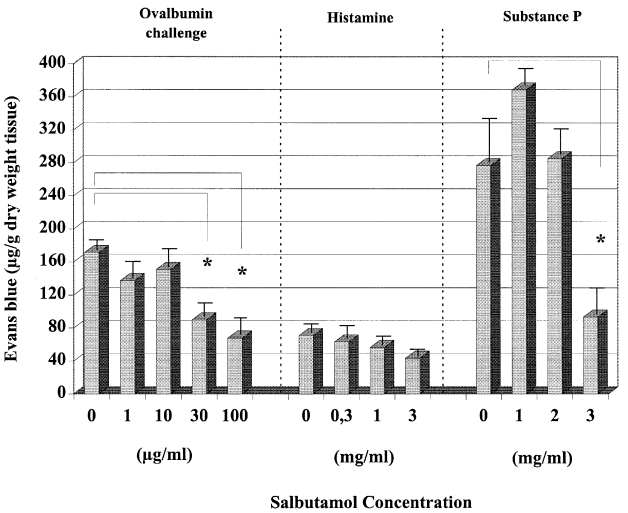

Fig. 2. Effects of the β_2-adrenoceptor agonist, salbutamol, on ovalbumin- (0.3 mg/kg i.v.), substance P- (1 μg/kg i.v.) or histamine- (100 μg/kg i.v.) induced microvascular leakage in guinea-pig trachea. Animals were exposed to salbutamol (1 to 3000 μg/ml) by aerosol for a period of 30 min, ending 30 min before ovalbumin administration. Abscissa: dose of drugs (mg/kg p.o.), ordinate: microvascular leakage in μg of Evans blue dye per g of dry weight tissue. Each column represents the mean ± S.E.M. from 6 to 10 observations. Not: $P > 0.05$; $^{*}P < 0.05$.

3.2. Effects of salbutamol on microvascular leakage

Aerosol administration of salbutamol induced a dose-dependent inhibition of antigen-induced microvascular leakage which was significant ($P < 0.05$) at concentrations of 30 and 100 μg/ml (Fig. 2). Salbutamol (0.3 to 3 mg/ml aerosol) failed to inhibit histamine-induced microvascular leakage, but at the highest concentration it did significantly ($P < 0.05$) inhibit substance P-induced microvascular leakage (Fig. 2).

3.3. Effects of phosphodiesterase inhibitors on ovalbumin-induced microvascular leakage

The non-selective phosphodiesterase inhibitor theophylline (100 mg/kg p.o.) and the phosphodiesterase type 4 inhibitor Ro 20-1724 (30 mg/kg p.o.) significantly reduced ovalbumin-induced microvascular leakage in guinea-pig trachea (Fig. 3). On the other hand, rolipram (5 mg/kg p.o.), a selective phosphodiesterase 4 inhibitor, did not inhibit microvascular leakage (Fig. 3). While, the higher dose (10 mg/kg p.o.) of rolipram reduced of ovalbumin-induced microvascular leakage by 11.5 ± 19.9% (data not shown), this effect did not attain statistical significance. Similarly, the two selective phosphodiesterase type 3 inhibitors: milrinone (30 mg/kg p.o.) and SK&F 94-836 (30 mg/kg p.o.), and the phosphodiesterase type 1/5 inhibitor: zaprinast (30 mg/kg p.o.), also failed to significantly reduce microvascular leakage induced by antigen (data not shown).

3.4. Effects of phosphodiesterase type 4 inhibitors on microvascular leakage induced by substance P and histamine

The selective phosphodiesterase type 4 inhibitors rolipram (5 mg/kg p.o.) and Ro 20-1724 (30 mg/kg p.o.), and the non-selective phosphodiesterase inhibitor theophylline did not reduce significantly the microvascular leakage induced by either substance P or histamine (Table 1).

3.5. Effects of combined pretreatment with phosphodiesterase type 4 inhibitors and salbutamol on microvascular leakage induced by ovalbumin, histamine and substance P

To investigate the possible synergistic interaction of salbutamol and phosphodiesterase type 4 inhibitors versus microvascular leakage, we selected the highest concentra-

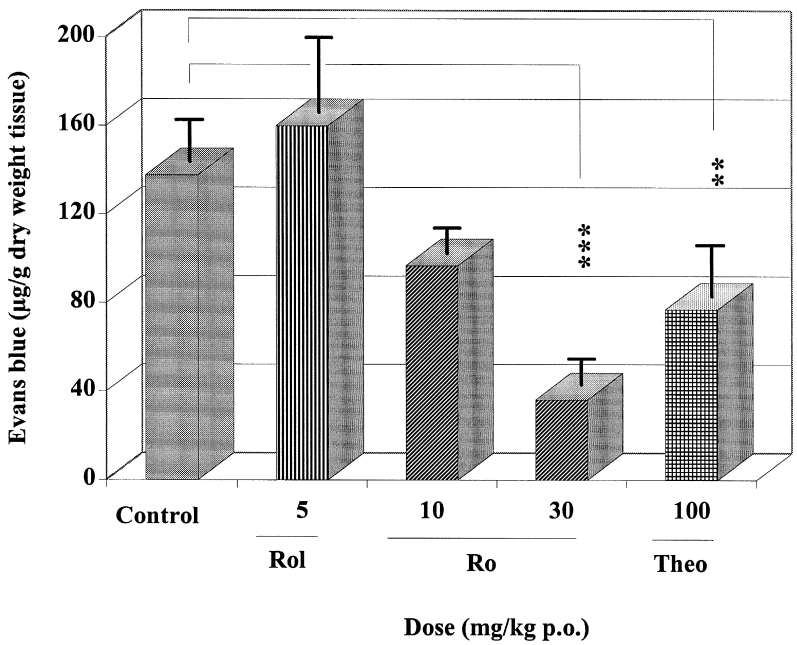

Fig. 3. Effects of selective phosphodiesterase inhibitors on ovalbumin (0.3 mg/kg i.v.)-induced microvascular leakage in guinea-pig trachea. Animals were treated with selective phosphodiesterase type 4 inhibitors: rolipram (Rol, 5 mg/kg) or Ro 20-1724 (Ro, 10 and 30 mg/kg) or with the non-selective phosphodiesterase inhibitor: theophylline (Theo, 100 mg/kg), orally 1 h before administration of ovalbumin. Results are expressed in μg of Evans blue dye per g of dry weight tissue. Each column represents mean ± S.E.M. from 5 to 8 observations. Not: $P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

tion of salbutamol (10 μg/ml or 3 or 2 mg/ml), which when administered as an aerosol, did not in themselves elicit a significant reduction of the microvascular leakage provoked by ovalbumin, histamine and substance P, respectively. When combined with aerosolized salbutamol (10 μg/ml aerosol), Ro 20-1724 (10 mg/kg p.o.) and theophylline (30 mg/kg p.o.) significantly ($P < 0.05$) reduced ovalbumin-induced microvascular leakage in trachea at doses previously found to be ineffective in animals that had not received salbutamol (Fig. 4). Furthermore, rolipram, previously found to be ineffective at 10 mg/kg p.o. versus ovalbumin-induced microvascular leakage, now produced significant ($P < 0.05$) inhibition of microvascular leakage at 5 mg/kg (Fig. 4). In contrast however, co-administered salbutamol (3 or 2 mg/ml) did not modify the lack of inhibitory effect of rolipram (5 mg/kg p.o.) or Ro 20-1724 (30 mg/kg p.o.) versus either histamine or substance P induced microvascular leakage (Table 1). In addition, it did not modify the lack of inhibition of histamine-induced microvascular leakage by theophylline (100 mg/kg p.o.). It did however increase significantly ($P < 0.01$) the slight inhibition of substance P-induced leakage by theophylline alone (Table 1).

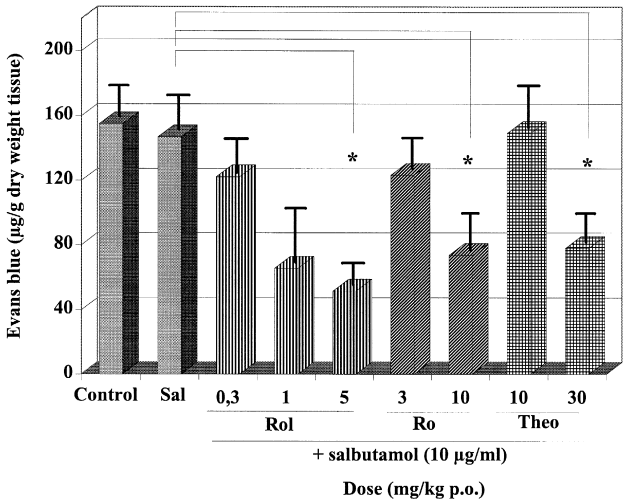

Fig. 4. Effects of selective phosphodiesterase inhibitors on ovalbumin (0.3 mg/kg i.v.)-induced microvascular leakage in guinea-pig trachea of animals, exposed to salbutamol (Sal, 10 μg/ml) for 30 min by aerosol. Animals were treated with selective phosphodiesterase type 4 inhibitors: rolipram (Rol, 0.3 to 5 mg/kg) or Ro 20-1724 (Ro, 3 to 10 mg/kg), or with the non-selective inhibitor: theophylline (Theo, 10 to 30 mg/kg), orally 1 h before administration of ovalbumin. The results are expressed in μg of Evans blue dye per g of dry weight tissue. Each column represents the mean ± S.E.M. from 7 to 8 observations. Not: $P > 0.05$; * $P < 0.05$.

Table 1
Effect of rolipram, Ro 20-1724 and theophylline on histamine (100 μg/kg) and substance P (1 μg/kg)-induced microvascular leakage in guinea-pig trachea

Treatment (N)	Dose	Evans blue (μg/g dry weight tissue)	
		histamine	substance P
Control (6)		164.5 ± 23.8	279.9 ± 50.0
Rolipram (6)	5	161.1 ± 11.7	317.7 ± 64.2
Control (6)		148.0 ± 38.2	280.1 ± 43.3
Salbutamol (6)		135.6 ± 31.4	285.5 ± 44.1
Rolipram + (salbutamol) (6)	5	118.5 ± 30.9	341.2 ± 27.6
Control (6)		166.9 ± 35.5	279.9 ± 50.0
Ro 20-1724 (6)	30	126.5 ± 16.5	300.9 ± 52.1
Control (6)		148.0 ± 38.2	280.1 ± 43.3
Salbutamol (6)		135.6 ± 31.4	285.5 ± 44.1
Ro 20-1724 + (salbutamol) (6)	30	117.4 ± 19.2	234.7 ± 23.3
Control (6)		153.6 ± 43.7	279.9 ± 50.0
Theophylline (6)	100	77.4 ± 19.0	198.5 ± 41.5
Control (6)		148.0 ± 38.2	280.1 ± 43.3
Salbutamol (5)		135.6 ± 31.4	285.5 ± 44.1
Theophylline + (salbutamol) (5)	100	102.0 ± 22.4	118.4 ± 7.3 * *

Animals were exposed to aerosolized salbutamol (0.3 or 2 mg/ml prior to histamine and substance P, respectively) or saline (control) for 30 min by aerosol. Animals were treated with the selective phosphodiesterase type 4 inhibitors rolipram (5 mg/kg) or Ro 20-1724 (30 mg/kg), or with the non-selective phosphodiesterase inhibitor: theophylline (100 mg/kg), orally 1 h before either histamine or substance P. Each result represents the mean ± S.E.M. from 5 to 6 observations.
Not significant, $P \geq 0.05$.
* * $P < 0.01$ versus salbutamol.

4. Discussion

The present data show that both the non-selective phosphodiesterase inhibitor, theophylline, and the selective phosphodiesterase type 4 inhibitor, Ro 20-1724, significantly reduced ovalbumin-induced microvascular leakage in guinea-pig trachea. Ovalbumin-induced microvascular leakage was also reduced by the selective phosphodiesterase type 4 inhibitor rolipram, although this failed to achieve statistical significance. Although rolipram may have been more effective in this respect had we used a higher dose, we were unable to do so because of the toxicity profile of this compound. The selective phosphodiesterase type 1/5 inhibitor zaprinast and the selective type 3 inhibitors milrinone and SK&F 94-836 did not reduce ovalbumin-induced microvascular leakage. Neither theophylline, Ro 20-1724, or rolipram reduced either histamine- or substance P-induced microvascular leakage. Co-administration of aerosolized salbutamol potentiated the inhibitory effect of theophylline, Ro 20-1724 and rolipram versus ovalbumin-induced microvascular leakage and the inhibitory effect of theophylline versus substance P-induced microvascular leakage. In the case of rolipram and Ro 20-1724 no augmentation of their effect versus either histamine- or substance P-induced microvascular

leakage was observed following co-administration of aerosolized salbutamol.

The actual physiological mechanism of airway microvascular leakage remain to be fully clarified. Plasma extravasation involves the uncoupling of the tight junctions between the endothelial cells of subepithelial microvessels in postcapillary venules. The way in which plasma is extravasated is thought to involve direct stimulation of receptors on the endothelial cells by inflammatory mediators (Barnes et al., 1990). Large gaps are formed between the endothelial cells lining the microvessels, possibly through a contractile effect or breaking of complex junction, which permits the movement of large plasma proteins such as albumin, fibrinogen and other quite large molecules out of the circulation and into the extravascular space (Barnes et al., 1990). The magnitude of plasma exudation may be modulated by blood flow. Importantly, the selective phosphodiesterase 4 inhibitors used in the present study have been shown not to influence the tone of pulmonary vascular smooth muscle (Fullerton et al., 1994) and furthermore, milrinone which is known to have a hypotensive effect at the doses used, did not modify extravasation in the tracheal (see Section 3.3).

In order to analyze the mechanism of action of theophylline and the phosphodiesterase type 4 inhibitors further, we investigated their effects versus microvascular leakage provoked by histamine and substance P. These two substances are released during antigen challenge in the guinea pig (Bertrand et al., 1993; Kelly et al., 1993) and their ability to induce plasma protein extravasation is well documented (Erjefält et al., 1985, 1993; Persson et al., 1986). Theophylline was ineffective at inhibiting either histamine- or substance P-induced microvascular leakage. In the same series of experiments, neither rolipram nor Ro 20-1724 inhibited either histamine- or substance P-induced microvascular leakage. Nevertheless, it has previously been demonstrated that rolipram reduces PAF, histamine and bradykinin-induced microvascular leakage in guinea pig trachea (Ortiz et al., 1992a,b; Raeburn and Karlsson, 1993), suggestive of a potential direct effect at the endothelial cell level. More recently, the selective phosphodiesterase type 4 inhibitor RP 73401 has been reported to inhibit histamine-induced microvascular leakage (Raeburn et al., 1994). However, the failure of rolipram and Ro 20-1724 to inhibit substance P and histamine-induced microvascular leakage do not support a direct mechanism of action for these drugs at the endothelium level. The discrepancy between the lack of effect of selective phosphodiesterase type 4 inhibitors versus histamine-induced microvascular leakage in the present study, and those reported above may have been due to the use of different experimental protocols.

Certainly phosphodiesterase type 4 is present in endothelial cells of large arteries (Lugnier and Schini, 1990) and an intracellular elevation of cyclic nucleotides (primarily cyclic GMP) induces relaxation of vascular tissue (Lincoln and Cornwell, 1991). Such a mechanism would tend to counteract the pro-microvascular leakage effect of vascular smooth muscle contraction. However, the inhibitory effect of theophylline, Ro 20-1724 and (to a lesser extent) rolipram on antigen-induced microvascular leakage is probably not the consequence of a vasodilatory effect, since these compounds do not have a marked vasodilator potential (Heaslip et al., 1991). Moreover, milrinone which is a more potent vasodilatory agent than rolipram, Ro 20-1724 and theophylline (Komas et al., 1991) did not significantly reduce ovalbumin-induced microvascular leakage. It is more likely therefore that the reduction of antigen-induced microvascular leakage by theophylline and selective phosphodiesterase type 4 inhibition relates to their inhibition of the release of anaphylactic mediators rather than antagonism of the actions of such mediators on the endothelial cells of the pulmonary microvasculature.

In the present study, we also found that aerosolized salbutamol moderately inhibited ovalbumin and substance P, but not histamine-induced microvascular leakage. It has also been shown that high doses of salbutamol do not significantly reduce the effects of PAF on microvascular permeability (Boschetto et al., 1989). In contrast, Advenier et al. (1992) reported a significant inhibition of salbutamol on histamine-induced microvascular permeability. The potentiation of the inhibition of ovalbumin-induced microvascular leakage by theophylline and phosphodiesterase type 4 inhibitors following co-administration of aerosolized salbutamol is analogous to the synergistic effect of β_2-adrenoceptor stimulation and phosphodiesterase type 4 inhibition on airway smooth muscle relaxation (Tomkinson et al., 1993; Quian et al., 1993; Planquois et al., 1996). The observation that β_2-adrenoceptor agonists and selective phosphodiesterase type 4 inhibitors increase cyclic AMP content in airway smooth muscle in a concentration- and time-dependent manner in parallel with smooth muscle relaxation (Zhou and Torphy, 1991), suggests that there is a close correlation between these two events in modulating the rise in intracellular cyclic AMP resulting from adenylate cyclase stimulation. This is conceivably also true for the inhibition of the release of anaphylactic mediators, although the actual mechanism of this interaction remains to be defined. Stimulation of β_2-adrenoceptors, present on vascular smooth muscle cells, produces vasorelaxation and elevates cyclic GMP levels to a greater extent than those of cyclic AMP (Gray and Marshall, 1992). This results suggests that β_2-adrenoceptor agonists have no direct effect on either isoform of guanylyl cyclase and that the cyclic AMP/cyclic GMP interaction might occur at the level of phosphodiesterase (Wright et al., 1994). On the other hand, there is a clear association between events in vascular endothelium and the underlying smooth muscle which also involves a close relationship between levels of cyclic GMP and cyclic AMP. It has been reported that an elevation of cyclic GMP by nitrovasodilators, potentiates the increase in cyclic AMP induced by stimulation of

adenylate cyclase (Maurice and Haslam, 1990; Maurice et al., 1991). The precise mechanisms involved are not known, but it has been proposed that cyclic GMP increases cyclic AMP by inhibition of phosphodiesterase type 3 in platelets (Maurice and Haslam, 1990) and vascular tissue (Komas et al., 1991; Jang et al., 1993). The resulting increase in cyclic AMP may therefore allow the control of cyclic AMP level by phosphodiesterase type 4 (Muller et al., 1992; Lugnier and Komas, 1993).

In conclusion, we have shown that in actively sensitized guinea-pig trachea in situ, non-selective inhibition of phosphodiesterase inhibits antigen-induced microvascular leakage. This inhibition can be reproduced by selective inhibition of phosphodiesterase type 4, but not by inhibition of type 1/5 or phosphodiesterase type 3. We have further shown that the inhibitory effect of non-selective inhibition of phosphodiesterase and selective inhibition of phosphodiesterase type 4 on microvascular leakage is potentiated by co-administration of aerosolized salbutamol. Irrespective of whether or not salbutamol was co-administered, selective phosphodiesterase type 4 inhibitors did not inhibit either histamine- or substance P-induced vascular leakage. This suggests that the inhibition of antigen-induced tracheal microvascular leakage by selective phosphodiesterase type 4 inhibitors and the potentiation of this effect by salbutamol results primarily from the inhibition of anaphylactic mediator release rather than an opposing action at the level of the vascular endothelial cell. As airway oedema resulting from microvascular leakage is a potentially important contributor to the pathophysiology of asthma, a combination of selective phosphodiesterase type 4 inhibition and β_2 adrenoceptor stimulation might therefore have a synergistic anti-inflammatory effect which could be relevant to the treatment of asthma.

References

Advenier, C., Qian, Y., Law Koune, J.D., Molimard, M., Candenas, M.L., Naline, E., 1992. Formoterol and salbutamol inhibit bradykinin- and histamine-induced airway microvascular leakage in guinea-pig. Br. J. Pharmacol. 105, 792–798.

Barnes, P.J., Boschetto, P., Rogers, D.F., Belvisi, M., Roberts, N., Chung, K.F., Evans, T.W., 1990. Effects of treatment on airway microvascular leakage. Eur. Respir. J. 3 (Suppl. 12), 663s–670s.

Beavo, J.A., Conti, M., Heaslip, R.J., 1994. Multiple cyclic nucleotide phosphodiseterases. Mol. Pharmacol. 46, 399–405.

Bertrand, C., Geppetti, P., Graf, P.D., Foresi, A., Nadel, J.D., 1993. Involvement of neurogenic inflammation in antigen-induced bronchoconstriction in guinea-pigs. Am. J. Physiol. 265, L507–L511.

Boschetto, P., Roberts, N.M., Rogers, D.F., Barnes, P.J., 1989. Effects of anti-asthma drugs on microvascular leakage in guinea-pig airways. Am. Rev. Respir. Dis. 139, 416–421.

Coleman, R.A., Knowles, I.D., Ball, D.I., Nials, A.T., 1995. A comparison of the relaxant activity of phosphodiesterase inhibitors on human bronchus, ferret trachea and guinea-pig trachea and bronchus. Am. J. Respir. Crit. Care Med. 151, A289 (Absract).

Djukanovic, R., Roche, W.R., Wilson, J.W., Beasley, C.R.W., Twyman, O.P., Howarth, P.H., Holgate, S.T., 1990. Mucosal inflammation in asthma. Am. Rev. Respir. Dis. 142, 434–457.

Erjefält, I., Persson, C.G.A., 1991. Pharmacologic control of plasma exudation into tracheaobronchial airways. Am. Rev. Respir. Dis. 143, 1008–1014.

Erjefält, I., G-Wagner, Z., Strand, S.E., Persson, C.G.A., 1985. A method for studies of tracheobronchial microvascular permeability to macromolecules. J. Pharmacol. Methods 14, 275–284.

Erjefält, I., Greiff, L., Alkner, U., Persson, C.G.A., 1993. Allergen-induced biphasic plasma exudation responses in guinea pig large airways. Am. Rev. Respir. Dis. 148, 695–701.

Fullerton, D.A., Hahn, A.R., Banerjee, A., Harken, A.H., 1994. Pulmonary vascular smooth muscle relaxation by cGMP- versus cAMP-mediated mechanisms. J. Surg. Res. 57, 259–263.

Gray, D.W., Marshall, I., 1992. Novel signal transduction pathway mediating endothelium-dependent β-adrenoceptor vasorelaxation in rat thoracic aorta. Br. J. Pharmacol. 107, 684–690.

Heaslip, R.J., Buckley, S.K., Sickels, B.D., Grimes, D., 1991. Bronchial versus cardiovascular activities of selective phosphodiesterase inhibitors in the anaesthetized beta-blocked dog. J. Pharmacol. Exp. Ther. 257, 741–747.

Howell, R.E., Sickels, B.D., Woeppel, S.L., 1992. Pulmonary antiallergic and bronchodilator effects of isoenzyme-selective phosphodiesterase inhibitors in guinea-pigs. J. Pharmacol. Exp. Ther. 264, 609–615.

Howell, R.E., Jenkins, L.P., Howell, D.E., 1995. Inhibition of lipopolysaccharide-induced pulmonary edema by isozyme-selective phosphodiesterase inhibitors in guinea pigs. J. Pharmacol. Exp. Ther. 275, 703–709.

Hui, K.P., Lötvall, J., Chung, K.F., Barnes, P.J., 1991. Attenuation of allergen-induced airway microvascular leakage and airflow obstruction in guinea-pigs by a 5-lipoxygenase inhibitor (A-63162). Am. Rev. Respir. Dis. 143, 1015–1019.

Jang, E.K., Davidson, M.M.L., Crankshaw, D., Haslam, R.J., 1993. Synergistic inhibitory effects of atriopeptin II and isoproterenol on contraction of rat smooth muscle: Roles of cGMP and cAMP. Eur. J. Pharmacol. 250, 477–481.

Kelly, L.J., Undem, B.J., Adams, G.K., 1993. Antigen-induced contraction of guinea-pig isolated pulmonary arteries and lung parenchyma. J. Appl. Physiol. 74, 1563–1569.

Komas, N., Le Bec, A., Stoclet, J.C., Lugnier, C., 1991. Cardiac cGMP-stimulated cyclic nucleotide phosphodiesterases: Effects of cGMP analogues and drugs. Eur. J. Pharmacol. 206, 5–13.

Lagente, V., Moodley, I., Perrin, S., Mottin, G., Junien, J.-L., 1994. Effects of isoenzyme-selective phosphodiesterase inhibitors on eosinophil infiltration in the guinea-pig lung. Eur. J. Pharmacol. 255, 253–256.

Lagente, V., Pruniaux, M.-P., Junien, J.-L., Moodley, I., 1995. Modulation of cytokine-induced eosinophil infiltration in guinea-pig airways by selective phosphodiesterase inhibitors. Am. J. Respir. Crit. Care. Med. 151, 1720–1724.

Lincoln, T.M., Cornwell, T.L., 1991. Towards an understanding of the mechanism of action of cyclic AMP and cyclic GMP in smooth muscle relaxation. Blood Vessels 28, 129–256.

Lugnier, C., Komas, N., 1993. Modulation of vascular cyclic nucleotide phosphodiesterases by cyclic GMP: Role in vasodilatation. Eur. Heart J. 14, 141–148.

Lugnier, C., Schini, V.B., 1990. Characterization of cyclic nucleotide phosphodiesterases from cultured bovine aortic endothelial cells. Biochem. Pharmacol. 39, 75–84.

Maurice, D.H., Haslam, R.J., 1990. Molecular basis of the synergistic inhibition of platelet function by nitrovasodilators and activators of adenylate cyclase: Inhibition of cyclic AMP breakdown by cyclic GMP. Mol. Pharmacol. 37, 671–681.

Maurice, D.H., Crankshaw, D., Haslam, R.J., 1991. Synergistic actions of nitrovasodilators and isoprenaline on rat aortic smooth muscle. Eur. J. Pharmacol. 192, 235–242.

Muller, B., Stoclet, J.-C., Lugnier, C., 1992. Cytosolic and membrane-

bound cyclic nucleotide phosphodiesterases from guinea-pig cardiac ventricles. Eur. J. Pharmacol. 225, 263–272.

Nicholson, C.D., Shahid, M., 1994. Inhibitors of cyclic nucleotide phosphodiesterase isoenzymes: Their potential utility in the therapy of asthma. Pulm. Pharmacol. 7, 1–17.

Ortiz, J.L., Cortijo, J., Vallés, J.M., Bou, J., Morcillo, E.J., 1992a. Rolipram inhibits PAF-induced airway microvascular leakage in guinea-pig: A comparison with milrinone and theophylline. Fund. Clin. Pharmacol. 6, 247–249.

Ortiz, J.L., Cortijo, J., Vallés, J.M., Bou, J., Morcillo, E.J., 1992b. Rolipram inhibits airway microvascular leakage induced by platelet-activating factor, histamine and bradykinin in guinea-pigs. J. Pharm. Pharmacol. 45, 1090–1092.

Person, C.G.A., 1986. Role of plasma exudation in asthmatic airways. Lancet ii, 1126–1128.

Persson, C.G.A., Erjefält, I., Andersson, P., 1986. Leakage of macromolecules from guinea-pig tracheobronchial microcirculation. Effects of allergen, leukotrienes, tachykinins, and anti-asthma drugs. Acta Physiol. Scand. 127, 95–105.

Planquois, J.-M., Ruffin-Morin, Y., Lagente, V., Payne, A.N., Dahl, S.G., 1996. Salbutamol potentiates the relaxant effects of selective phosphodiesterase inhibitors on guinea-pig isolated trachea. Fund. Clin. Pharmacol. 10, 356–367.

Quian, Y., Naline, E., Karlsson, J.-A., Raeburn, D., Advenier, C., 1993. Effect of rolipram and siguazodan on the human isolated bronchus and their interaction with isoprenaline and sodium nitroprusside. Br. J. Pharmacol. 109, 774–778.

Raeburn, D., Karlsson, J.A., 1993. Effects of isozyme-selective inhibitors of cyclic nucleotide phosphodiesterase on microvascular leak in guinea-pig airways in vivo. J. Pharmacol. Exp. Ther. 267, 1147–1152.

Raeburn, D., Underwood, S.L., Lewis, S.A., Woodman, V.R., Battram, C.H., Tomkinson, A., Sharma, S., Jordan, R., Souness, J.E., Webber, S.E., Karlsson, J.A., 1994. Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. Br. J. Pharmacol. 113, 1423–1431.

Rogers, D.F., Boschetto, P., Barnes, P.J., 1989. Plasma exudation: Correlation between Evans blue dye and radiolabelled albumin in guinea-pig airways in vivo. J. Pharmacol. Methods 21, 309–315.

Tomkinson, A., Karlsson, J.-A., Raeburn, D., 1993. Comparison of the selective inhibitors of phosphodiesterase types III and IV in airway smooth muscle with different β-adrenoceptor subtypes. Br. J. Pharmacol. 108, 57–61.

Underwood, D.C., Osborn, R.R., Novak, L.B., Matthews, J.K., Newsholme, S.J., Undem, B.J., Hand, J.M., Torphy, T.J., 1993. Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. J. Pharmacol. Exp. Ther. 266, 306–313.

Widdicombe, J., 1996. The tracheobronchial vasculature: A possible role in asthma. Microcirculation 3, 129–141.

Wright, I.K., Amirchetty-Rao, S., Kendal, D.A., 1994. Potentiation by forskolin of both SNP- and ANP-stimulated cyclic GMP accumulation in porcine isolated palmar lateral vein. Br. J. Pharmacol. 112, 1146–1150.

Zhou, H.-L., Torphy, T.J., 1991. Relationship between cyclic guanosine monophosphate accumulation and relaxation of canine trachealis induced by nitrovasodilators. J. Pharmacol. Exp. Ther. 258, 972–978.