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Prostaglandin E_2, but not prostacyclin inhibits histamine-induced contraction of human bronchial smooth muscle

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Abstract

The effects of exogenous prostaglandin E_2 and prostacyclin on the function of epithelium-intact and epithelium-denuded human bronchial smooth muscle and the role of these mediators in the inhibition of histamine-induced contraction was examined using bronchi obtained from 22 patients undergoing thoracotomy. Under resting tension, a variable biphasic contraction-relaxation or monophasic relaxation was observed following the cumulative addition of exogenous prostaglandin E_2 or prostacyclin. Cumulative addition of these mediators to pre-contracted bronchi produced incomplete relaxation, irrespective of the presence of epithelium. Addition of prostaglandin E_2, at a concentration equating to that produced after histamine stimulation (1.2×10^{-9} M), produced a reduction (24%) in the maximum contractile response (E_{max}) to a subsequent histamine challenge ($P < 0.03$). However, a similar response was not observed after the addition of prostacyclin at a concentration similar to that produced endogenously (6.5×10^{-10} M). The combined addition of both mediators resulted in a significant reduction (26%) in the E_{max} to histamine ($P < 0.02$) but this effect was not statistically different to that of prostaglandin E_2 alone. The addition of supramaximal concentrations (1 μM) of each prostanoid, either alone or in combination, did not inhibit responses to histamine. These data suggest that whilst prostaglandin E_2 does not act as a direct acting relaxant agonist, it may inhibit histamine-induced muscle contraction and thereby contribute to the observed tachyphylaxis to this mediator. In contrast, prostacyclin appears to be of little importance in modulating human bronchial smooth muscle responses to histamine either directly or by enhancing responses to prostaglandin E_2. The inhibitory effect of prostaglandin E_2 appears to be concentration-dependent and suggests a bimodal action of this mediator in human airways.

Keywords: Histamine; Prostanoid; Airway; (Human)

1. Introduction

Previously, it has been reported that the generation of prostaglandin E_2 and prostacyclin from the respiratory epithelium of human isolated bronchus is significantly enhanced following histamine exposure and this was associated with the induction of histamine tachyphylaxis (Knight et al., 1994). This hypothesis was further supported by data demonstrating that histamine tachyphylaxis was abolished in vitro by chronic exposure of donor patients to non-steroidal anti-inflammatory agents (NSAIDs) (Knight et al., 1994). These observations are consistent with previous data suggesting that the release of prostaglandins, such as prostaglandin E_2 and prostacyclin are involved in histamine tachyphylaxis in both animal (Shore et al., 1985; Watanabe et al., 1988) and human airways (Manning et al., 1987).

However, whilst prostaglandin E_2 and prostacyclin have been indirectly implicated in modulating human airway smooth muscle function, observations of the direct effects of these mediators on airway smooth muscle have been conflicting. For example, some investigators have reported little effect (Coleman and Sheldrick, 1989) whereas others indicate that complex biphasic contraction-relaxation responses may occur (Armour et al., 1989). These data suggest that prostaglandins may have multiple actions depending on the predominant receptor subtype and species studied (Coleman et al., 1984), the concentration of prostanoid (Coleman and Sheldrick, 1989), the airway region

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(Norel et al., 1991) or possibly the type of agonist used to induce tone.

In the current investigation, the direct effect of prostaglandin E_2 and prostacyclin on the modulation of human isolated bronchial smooth muscle function and the role of these mediators in the development of histamine tachyphylaxis was characterised.

2. Materials and methods

2.1. Tissue preparation and organ bath protocol

Lung tissue was obtained from 22 patients undergoing thoracotomy for removal of lung cancer. The patients had not received any medication known to alter airway responses to histamine as has been previously reported (Knight et al., 1994). Macroscopically normal bronchi (4–8 mm i.d.) were dissected free of all visible blood vessels and parenchyma and used either on the day of resection or stored overnight at 4°C.

2.2. The effect of exogenous prostaglandin E_2 and prostacyclin on human isolated bronchial smooth muscle responsiveness to histamine

In two separate sets of experiments, prostaglandin E_2 and prostacyclin were added to the organ bath to investigate the direct effects of these mediators on human isolated bronchial smooth muscle contractility. In the first set of experiments, bronchial preparations were treated with the non-steroidal anti-inflammatory drug flurbiprofen (5 μM) for 30 min to inhibit the generation of endogenous prostanoids. A cumulative concentration-effect curve was then constructed for both prostaglandin E_2 and prostacyclin, in the range of 10^{-10}– 3×10^{-6} M. In the second set of experiments, bronchial preparations were treated with flurbiprofen for 30 min and then maximally stimulated with histamine (10^{-3} M). Once a stable contraction was obtained, cumulative concentration-effect curves to either prostaglandin E_2 or prostacyclin were constructed.

In order to compare the effect of induced tone on the relaxant effects of prostaglandin E_2, separate muscle strips from the same airways were pre-contracted with an EC_{50} concentration of histamine and a cumulative concentration-effect curve to prostaglandin E_2 or prostacyclin then constructed.

2.3. The role of prostaglandin E_2 and prostacyclin in the modulation of histamine-induced contraction

Exogenous prostaglandin E_2 and prostacyclin, in concentrations similar to those previously measured from epithelium-intact bronchial preparations in organ bath effluents (Knight et al., 1994) were used to investigate their relative contribution to histamine tachyphylaxis. Expressed in molar terms, the mean histamine-induced generation of prostaglandin E_2 was 1.2×10^{-9} M and prostacyclin (measured as its stable metabolite 6-keto-PGF_{1a}), was 6.5×10^{-10} M.

In these experiments, a control histamine cumulative concentration-effect curve was constructed in the presence of flurbiprofen, followed by a recovery and equilibration period. All bronchial preparations were again treated with flurbiprofen for 30 min. Prostaglandin E_2 (1.2×10^{-9} M) or prostacyclin (6.5×10^{-10} M) were then added either in isolation or in combination to the organ bath, followed by a second exposure to histamine. For comparison, a supramaximal concentration of prostaglandin E_2 (1 μM) and prostacyclin (1 μM) were added in a similar manner and the responses to a subsequent histamine cumulative concentration-effect curve recorded.

2.4. Analysis of results

The maximal tension (E_{max}), the EC_{50} and agonist potency, pD_2 ($-\log_{10} EC_{50}$) were determined from the raw data for each cumulative concentration-effect curve. The tensions developed in the second histamine cumulative concentration-effect curve were expressed as a percentage of the maximum tension of the first cumulative concentration-effect curve. Simple descriptive statistics were performed on the data to determine the mean and standard error of the mean. Comparative statistics were performed using ANOVA followed by Tukey's test for multiple comparison or by Student's t-test using the SAS statistical program.

2.5. Drugs and chemicals

The drugs used were acetylcholine hydrochloride (Sigma); flurbiprofen, prostaglandin E_2 and prostacyclin (Cayman Chemical); histamine diphosphate (British Drug House). Solutions of acetylcholine and histamine were prepared fresh in 0.9% w/v NaCl. Stock solutions of flurbiprofen, prostaglandin E_2 and prostacyclin were freshly prepared in either dimethylsulfoxide or 100% ethanol. Subsequent dilutions were performed in Krebs-Henseleit solution. Vehicle solutions were shown not to influence responses of bronchial preparations.

3. Results

3.1. The effect of prostaglandin E_2 and prostacyclin on resting muscle tone

Exposure of both epithelium-intact and -denuded human isolated bronchial smooth muscle strips to flur-

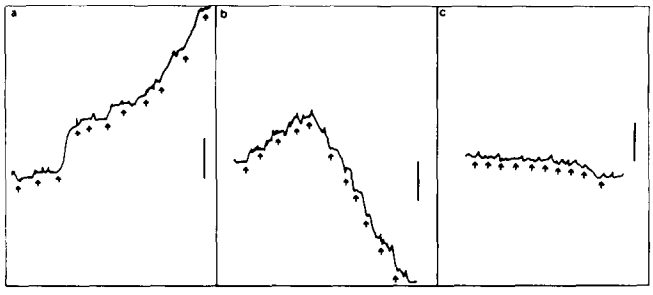

Fig. 1. An example of concentration-dependent responses of epithelium-denuded human isolated bronchial smooth muscle to prostaglandin E_2 (10^{-10}–10^{-6} M). Bronchial preparations were under resting tension and treated with flurbiprofen (5 μM) for 30 min prior to exposure to prostaglandin E_2. Vertical bars represent 200 mg tension. Tracings a, b and c represent responses of bronchi from three different donors.

Table 1
Comparison of the relaxant effect of prostaglandin E_2 and prostacyclin on human isolated bronchial smooth muscle pre-contracted with histamine (10^{-3} M)

	Epithelium-intact ($n = 8$)		Epithelium-denuded ($n = 8$)	
	pD_2 [a]	E_{max} [b]	pD_2	E_{max}
Prostaglandin E_2	8.00 ± 0.14	31.87 ± 2.81	8.18 ± 0.16	40.67 ± 7.82
Prostacyclin	7.80 ± 0.20	33.84 ± 5.37	7.74 ± 0.23	49.18 ± 9.49

Results are expressed as means ± S.E.M. n = number of patients from whom lung samples were obtained. [a] pD_2 is calculated as $-\log_{10}(EC_{50})$. [b] E_{max} represents the maximum relaxant effect of each prostanoid expressed as a percentage of the induced contraction.

biprofen did not significantly influence the tone of these preparations. However, the effects of prostaglandin E_2 on bronchial smooth muscle tone were variable despite the presence of the cyclooxygenase inhibitor. In some preparations, low concentrations of prostaglandin E_2 produced relaxation which was followed by contractile responses at higher concentrations. With other preparations, only single concentration-dependent contractions or concentration-dependent relaxations were observed (Fig. 1a-c). Similarly, the cumulative addition of prostacyclin to adjacent sections of the same airway also produced highly variable monophasic or biphasic responses (Fig. 2a-c). The magnitude of responses obtained following exposure to prostacyclin were always lower than those observed for prostaglandin E_2.

3.2. The relaxant effect of prostaglandin E_2 and prostacyclin following histamine-induced contraction

Prostaglandin E_2 induced a concentration-dependent relaxation of bronchial preparations pre-contracted with a maximum concentration of histamine. However, the mean maximum relaxation observed for epithelium-intact preparations was only 31.9 ± 2.8% of the induced contraction and for epithelium-denuded preparations the maximum relaxation was 40.7 ± 7.8% which were not significantly different ($P > 0.2$). Similarly, epithelium removal did not appear to alter the sensitivity of muscle preparations to the relaxant effects of prostaglandin E_2, ($P > 0.1$, Table 1). In a small number of epithelium-intact bronchi ($n = 4$) which were pre-contracted with an EC_{50} of histamine, the mean maximum relaxant response attained by prostaglandin E_2 was only 30.9 ± 10.1% of the induced contraction, a value which was not significantly different to the degree of relaxation observed when muscle preparations were contracted with a maximal concentration of histamine ($P > 0.375$). All muscle preparations examined elicited dose-dependent contractile responses when concentrations of prostaglandin E_2 exceeded 0.1 μM. However, the pD_2 of prostaglandin E_2 during the relaxant component of the biphasic response was 8.59 ± 0.15 which was significantly greater than that observed when bronchi were maximally contracted with histamine ($P < 0.05$).

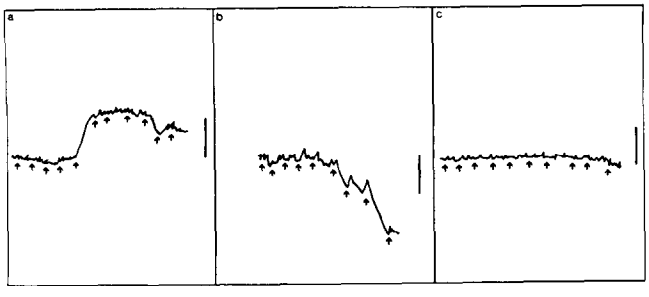

Fig. 2. An example of concentration-dependent responses of epithelium-denuded human isolated bronchial smooth muscle to prostacyclin (10^{-10}–3×10^{-6} M). Bronchial preparations were under resting tension and treated with flurbiprofen (5 μM) for 30 min prior to exposure to prostacyclin. Vertical bars represent 200 mg tension. Tracings a, b and c represent responses of the same bronchi as in Fig. 1.

Relaxation of both epithelium-intact and epithelium-denuded muscle preparations following exposure to prostacyclin was also observed. As observed for prostaglandin E_2, there were no significant differences in the maximum relaxant response for prostacyclin in epithelium-intact 33.8 ± 5.4% and denuded bronchi 49.2 ± 9.5% ($P > 0.1$). Although the relaxant potency of prostacyclin tended to be greater in epithelium-denuded muscle preparations compared to epithelium-intact preparations, there was no statistical difference observed ($P > 0.05$; Table 1). Similarly, although there was a tendency for the relaxant potency of prostacyclin to be lower when compared to prostaglandin E_2 this failed to reach statistical significance in either epithelium-intact ($P = 0.371$) or denuded ($P = 0.12$) preparations (Table 1).

3.3. The role of prostaglandin E_2 and prostacyclin in the modulation of histamine-induced contraction

The addition of prostaglandin E_2 (1.2×10^{-9} M) or prostacyclin (6.5×10^{-10} M) to epithelium-intact bronchial preparations, in the presence of flurbiprofen, did not produce significant effects on baseline tension.

In the presence of exogenously added prostaglandin E_2, the mean maximum contractile response to subsequent histamine exposure was reduced to $76.03 \pm 3.35\%$ of control values ($P < 0.03$) (Fig. 3a). However, the mean contractile potency of histamine was not significantly reduced by exposure to this concentration of prostaglandin E_2, the pD_2 values for histamine before and after exposure to prostaglandin E_2 being 5.23 ± 0.08 and 5.33 ± 0.15 respectively ($P > 0.1$). Following exposure to prostacyclin, the mean maximum contractile response to histamine was $96.9 \pm 14.7\%$ of initial values ($P > 0.375$) (Fig. 3b), whilst the contractile potency of histamine was also not significantly altered by exposure to prostacyclin ($P > 0.3$). When preparations were exposed to a combination of prostaglandin E_2 and prostacyclin, the mean maximum contraction in response to histamine was reduced to $73.7 \pm 10.6\%$ of the maximum contractile response in the control curve ($P < 0.02$) (Fig. 3c). This value, however, was not significantly different to that attained following exposure to prostaglandin E_2 alone ($P > 0.375$). Although, the mean pD_2 value of histamine was increased from 5.17 ± 0.1 to 5.23 ± 0.2 by exposure to the combination of prostaglandin E_2 and prostacyclin, this difference did not reach statistical significance ($P > 0.1$).

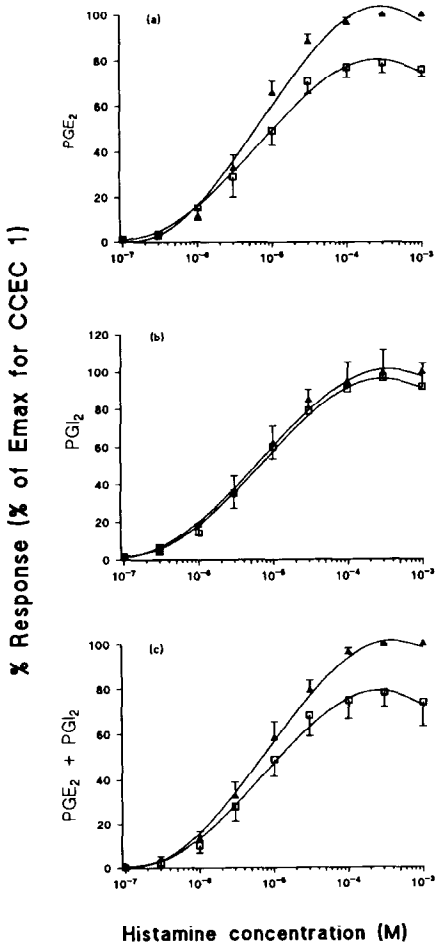

Fig. 3. Consecutive cumulative concentration-effect curve constructed for histamine in the absence (▲) and presence (□) of (a) prostaglandin E_2 (1.23×10^{-9} M), (b) prostacyclin (6.5×10^{-10} M) and (c) a combination of prostaglandin E_2 and prostacyclin. Results are expressed as means ± S.E.M. percentage of the maximum response to histamine in the absence of prostaglandin ($n = 8$).

The addition of a supramaximally effective concentration of prostaglandin E_2 (1 μM), did not significantly influence the subsequent contractile response to histamine. Consequently the mean maximum contraction was not significantly different to control values ($P > 0.375$) (Fig. 4a). Although prostaglandin E_2 (1 μM) reduced the contractile potency of histamine by 1.5-fold, this failed to reach statistical significance ($P > 0.1$). When bronchial preparations were exposed to a supramaximally effective concentration of prostacyclin (1 μM) under identical conditions, both the mean maximum contractile response and sensitivity to histamine were not significantly different to control values ($P > 0.375$ for both E_{max} and pD_2; Fig. 4b). The combined addition of supramaximally effective concentrations of both prostaglandin E_2 and prostacyclin had variable effects on baseline tension, with either slight contraction, slight relaxation or no effect being observed. In those preparations in which a response was observed, the magnitude was always less than 10% of resting tension. When both prostanoids were added simultaneously, a slight but insignificant increase in the E_{max} ($P > 0.3$) and contractile potency ($P > 0.2$) of the histamine response was observed when compared to that obtained in the absence of prostaglandins (Fig. 4c).

4. Discussion

In a previous study from this laboratory it was demonstrated that the development of histamine tachyphylaxis in human isolated bronchial smooth muscle was an epithelium-dependent phenomenon (Knight et al., 1992). More recently, these results have been confirmed and in addition, data have indicated that the generation of prostaglandin E_2 and prostacyclin, primarily from the respiratory epithelium also participate in modulating histamine-induced contraction of human isolated bronchial smooth muscle (Knight et al.,1994). In the current investigation, exogenously added prostaglandin E_2 and prostacyclin were used to assess the

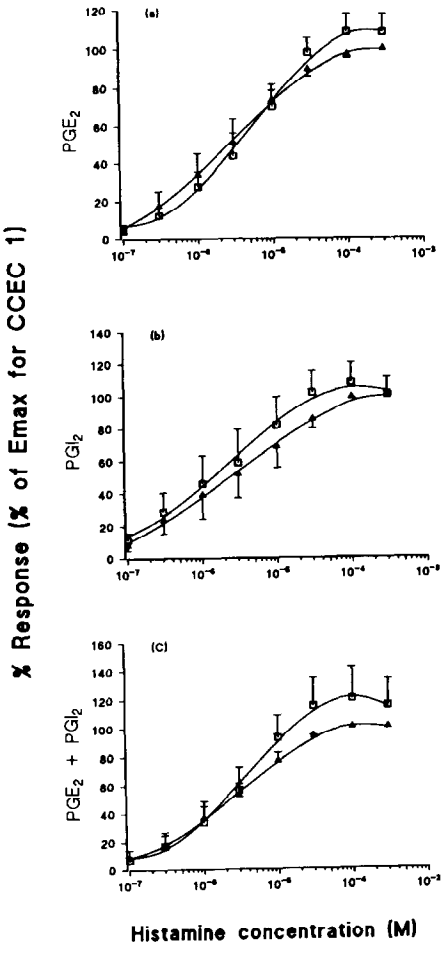

Fig. 4. Consecutive cumulative concentration-effect curves constructed for histamine in the absence (▲) and presence (□) of (a) prostaglandin E_2 (1 μM), (b) prostacyclin (1 μM) and (c) a combination of prostaglandin E_2 and prostacyclin. Results are expressed as means ± S.E.M. percentage of the maximum response to histamine in the absence of prostaglandin ($n = 8$).

relative role of each of these mediators on human bronchial smooth muscle at rest, following contraction with histamine, and in the development of histamine tachyphylaxis.

Both prostaglandin E_2 and prostacyclin produced highly variable responses on bronchial preparations under resting tension. For example, concentration-dependent relaxation, contraction or biphasic contraction-relaxation responses were observed. Similar variability in responses to these and other prostanoids has also been demonstrated by other investigators using human smooth muscle isolated from airways (Coleman and Sheldrick, 1989; Armour et al., 1989) and myometrium (Senior et al., 1992), but the mechanisms involved are presently unclear. However, given that all preparations studied were pretreated with flurbiprofen, it is unlikely that these secondary effects were due to the release of endogenous prostaglandins.

Coleman and Sheldrick (1989) suggested that prostanoid-induced contraction of human bronchial smooth muscle is mediated by a single receptor subtype (TP) and thus the comparatively weak contractions induced by prostaglandin E_2 and prostacyclin may reflect non-specific interactions between high concentrations of prostanoids at this receptor. However, in the present study, the majority of contractions induced by either prostanoid occurred at low concentration, suggesting that non-specific activation of TP receptors was not solely responsible for the observed variability. Alternatively, the contractions observed may reflect an interaction between these prostanoids and contractile EP receptors. In some species, prostaglandin E_2 is thought to specifically interact with at least three different receptor subtypes within the lung (Coleman et al., 1987; Dong et al., 1986). Activation of EP_1 and EP_3 receptors mediates airway smooth muscle contraction, whereas stimulation of EP_2 receptors produces muscle relaxation. Although the distribution of these receptors in human lung is presently unknown, intra-airway heterogeneity in regard to the distribution and a relative preponderance of contractile EP receptors may exist in human airways. In support of this, McKenniff et al. (1988) demonstrated contraction of large human airways in response to prostaglandin E_2 analogs despite blockade of TP receptors. In contrast, in subsequent studies in which smaller airways (1–4 mm i.d.) were used, no evidence for a contractile response to stimulation of EP receptors was demonstrated (Coleman and Sheldrick, 1989; Armour et al., 1989).

In the current investigation, prostaglandin E_2 and prostacyclin produced a variable degree of relaxation of human isolated bronchial smooth muscle contracted by histamine which was independent of the presence or absence of the epithelium. The apparent lack of efficacy contrasts with data obtained in studies using primate trachea, where addition of prostaglandin E_2 at concentrations ranging from 0.1–4 nM produced complete relaxation of histamine-induced contraction (Krzanowski et al., 1980). The reasons for these differing results are not known. However, since the potency of functional antagonists may partly depend on the pre-existing tone of the preparation (Braunstein et al., 1988) it is possible that level of induced tone may have contributed. Indeed, in the primate study (Krzanowski et al., 1980), the histamine concentration used to contract the tissue prior to generating a relaxant cumulative concentration-effect curve to prostaglandin E_2, produced a contraction which was equivalent to 40% of a maximum contractile response. However, in the current investigation the relaxant effect of prostaglandin E_2, was incomplete when either an EC_{50} or a maximal concentration of histamine were used to induce tone, suggesting that the level of induced tone might not be contributing significantly to this effect.

Despite the modest relaxant response observed following histamine-induced contraction, the addition of exogenous prostaglandin E_2, was sufficient to inhibit subsequent bronchial smooth muscle responses to histamine and significantly, the degree of inhibition was comparable to that originally observed for histamine tachyphylaxis per se (Knight et al., 1992, 1994). Similar results have been reported using canine trachea, where prostaglandin E_2, at a concentration equatable to that generated by cultured epithelial cells, was sufficient to inhibit contractile responses of tracheal smooth muscle to histamine (Yu et al., 1992). The lack of effect of prostaglandin E_2 on the contractile potency of histamine demonstrated in the current study, suggests either that other mechanisms may be contributing to the inhibition of histamine-induced contraction, or that a modest population of inhibitory EP receptors co-exist in human airways with populations of contractile EP and TP receptor subtypes.

In contrast to prostaglandin E_2, exogenous prostacyclin did not significantly influence histamine-induced responses, despite the detection of significant amounts of this prostanoid following histamine stimulation (Knight et al., 1994). The reasons as to why both prostaglandin E_2 and prostacyclin are generated in response to histamine, but only prostaglandin E_2 modulates human isolated bronchial smooth muscle responsiveness is not known, but the results are consistent with the observation that in humans, prostacyclin is a considerably more potent mediator for vascular tissues than airway smooth muscle (Hardy et al., 1988; Haye-Legrand et al., 1987).

When both prostanoids were added simultaneously, the magnitude of inhibition of histamine-induced contraction was not significantly different to that observed for prostaglandin E_2 alone. These data further support the hypothesis that prostaglandin E_2 is the predominant inhibitory cyclooxygenase metabolite in human airways whilst the role of prostacyclin either alone or in enhancing the effects of prostaglandin E_2 on human isolated bronchial smooth muscle responses to histamine is of limited importance. Yu et al. (1992) reported that whilst prostacyclin in isolation, had minimal effects on canine tracheal contraction per se, it significantly enhanced the relaxant activity of prostaglandin E_2 on tracheal smooth muscle contracted by histamine and other agonists. The reasons for these disparate results are not known, although species differences seems likely.

Paradoxically, the addition of a supramaximally effective concentration of prostaglandin E_2 (1 μM) appeared to prevent the reduction in histamine responsiveness, suggesting that very high concentrations of prostaglandin E_2 may contract human isolated bronchial smooth muscle. These results are consistent with previous reports which have shown that high concentrations of prostaglandin E_2 may stimulate contractile TP and EP prostanoid receptors (Coleman and Sheldrick, 1989; Armour et al., 1989; McKenniff et al., 1988). In contrast, Braunstein et al. (1988) demonstrated that prostaglandin E_2 concentration dependently depressed the maximum response and sensitivity to histamine in guinea pig isolated trachea, with the greatest inhibitory effect observed at 1 μM. However, it is generally accepted that the actions of prostaglandin E_2 on isolated airway preparations exhibit marked differences between guinea pig and man (Douglas and Brink, 1987).

In summary, the results of the current study indicate that epithelium-derived prostaglandin E_2, is an important modulator of histamine responses, including the development of tachyphylaxis, in human isolated bronchus, whereas prostacyclin appears to be of little significance. In addition, variable concentration-dependent effects exist for prostaglandin E_2 whereby high concentrations appear to enhance airway smooth muscle responsiveness. These observations are consistent with previous data which suggest that receptors for prostanoid-induced contraction and relaxation are distinct and that prostaglandin E_2 may interact at these multiple receptor subtypes within the airways.

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References

Armour, C.L., P.R.A. Johnson, M.L. Alfredson and J.L. Black, 1986, Characterisation of contractile prostaglandin receptors in human airway smooth muscle, Eur. J. Pharmacol. 165, 215.

Braunstein, G., C. Labat, S. Brunelleschi, J. Benveniste, J. Marsac and C. Brink, 1988, Evidence that the histamine sensitivity and responsiveness of guinea-pig isolated trachea are modulated by epithelial prostaglandin E_2, Br. J. Pharmacol. 95, 300.

Coleman, R.A., P.P.A. Humphrey, I. Kennedy and P. Lumley, 1984, Prostanoid receptors – the development of a working classification, Trends Pharmacol. Sci. 4, 303.

Coleman, R.A. and R.L.G. Sheldrick, 1989, Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP receptors, Br. J. Pharmacol. 96, 688.

Coleman, R.A., I. Kennedy and R.L.G. Sheldrick, 1987, Evidence for the existence of three subtypes of PGE_2 sensitive (EP) receptors in smooth muscle, Br. J. Pharmacol. 91, 323P

Dong, Y.J., R.L. Jones and N.H. Wilson, 1986, Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues, Br. J. Pharmacol. 87, 97.

Douglas, J.S. and C. Brink, 1987, Histamine and prostanoids, Am. Rev. Respir. Dis. 136, s21.

Hardy, C.C., P. Bradding, C. Robinson and S.T. Holgate, 1998, Bronchoconstrictor and anti-bronchoconstrictor properties of inhaled prostacyclin in asthma, J. Appl. Physiol. 64, 1567.

Haye-Legrand, I., B. Bourdillat, C. Labat, J. Cerrina, X. Norel, J. Benveniste and C. Brink, 1987, Relaxation of isolated human pulmonary muscle preparations with prostacyclin (PGI_2) and its analogues, Prostaglandins 33, 845.

Knight, D.A., G.A. Stewart and P.J. Thompson, 1992, Histamine tachyphylaxis in human airway smooth muscle: the role of H_2 receptors and the epithelium, Am. Rev. Respir. Dis. 146, 137.

Knight, D.A., G.A. Stewart, M.L. Lai and P.J. Thompson, 1995, Epithelium-derived inhibitory prostaglandins modulate human bronchial smooth muscle responses to histamine, Eur. J. Pharmacol. 272, 1.

Krzanowski, J.J., W.H. Anderson, J.B. Polson and A. Szentivanyi, 1980, Prostaglandin mediated histamine tachyphylaxis in subhuman primate tracheal smooth muscle, Arch. Int. Pharmacodyn. 247, 155.

Manning, P.J., G.L. Jones and P.M. O'Byrne, 1987, Tachyphylaxis to inhaled histamine in asthmatic subjects, J. Appl. Physiol. 63, 1572.

McKenniff, M., I.W. Rodger, P. Norman and P.J. Gardiner, 1988, Characterisation of receptors mediating the contractile effects of prostanoids in guinea-pig and human airways, Eur. J. Pharmacol. 153, 149.

Norel, X., C. Labat, P.J. Gardiner and C. Brink, 1991, Inhibitory effects of BAY u3405 on prostanoid-induced contractions in human isolated bronchial and pulmonary arterial muscle preparations, Br. J. Pharmacol. 104, 591.

Senior, J., R. Sangha, G.S. Baxter, K. Marshall and J.K. Clayton, 1992, In vitro characterisation of prostanoid FP-, DP-, IP- and TP receptors on the non-pregnant human myometrium, Br. J. Pharmacol. 107, 215.

Shore, S.A., W.S. Powell and J.G. Martin, 1985, Endogenous prostaglandins modulate histamine-induced contraction in canine tracheal smooth muscle, J. Appl. Physiol. 58, 859.

Watanabe, T., R. Duncan, R.F. Lockey and J.J. Krzanowski, Jr., 1988, Histamine tachyphylaxis in young dog airways: compared to adult dog, Arch. Int. Pharmacodyn. Ther. 295, 204.

Yu, X.-Y., W. Hubbard and E.W. Spannhake, 1992, Inhibition of canine tracheal smooth muscle by mediators from cultured bronchial epithelial cell, Am. J. Physiol. 262, L229.