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Histamine-induced contraction of human isolated bronchus is enhanced by endogenous prostaglandin $F_{2\alpha}$ and activation of TP receptors

Darryl A. Knight [a,*], Geoffrey A. Stewart [b], Philip J. Thompson [a]

[a] *Asthma and Allergy Research Unit, Department of Medicine, University of Western Australia, Nedlands, Western Australia 6009, Australia*
[b] *Department of Microbiology, University of Western Australia, Nedlands, Western Australia 6009, Australia*

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Abstract

The effect of histamine on the production of prostaglandin $F_{2\alpha}$ and the actions of prostaglandin $F_{2\alpha}$ on the responsiveness of human isolated bronchial smooth muscle were examined by organ bath techniques using bronchi from lung tissue resected from 18 patients. Following exposure to histamine, epithelium-intact bronchi generated 34.26 ± 16.3 pg of prostaglandin $F_{2\alpha}$/mg of tissue and epithelium-denuded preparations produced 32.62 ± 11.83 pg/mg, suggesting that histamine-induced release of prostaglandin $F_{2\alpha}$ was from non-epithelial sources, presumably smooth muscle. The histamine H_2 receptor antagonist ranitidine did not affect the release of prostaglandin $F_{2\alpha}$, suggesting that its generation may have resulted from histamine H_1 receptor activation. Carbachol did not influence prostaglandin $F_{2\alpha}$ generation. Contractile responses to histamine, prostaglandin $F_{2\alpha}$ and carbachol were measured in the presence and absence of the prostaglandin TP receptor antagonist SQ 29,548 ([1*S*-[1α,2β(5*Z*),3β,4α]]-7-[3[[2-[9-phenylamino)carbonyl]hydrazino]methyl-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (0.4 μM). SQ 29,548 abolished responses to prostaglandin $F_{2\alpha}$ suggesting that contractions were mediated via TP receptors. Exposure to SQ 29,548 also produced a 3-fold rightward shift in the concentration-effect curve for histamine ($P = 0.01$) without influencing the maximum response. SQ 29,548 did not affect responses to carbachol. These results suggest that histamine selectively stimulates the generation of prostaglandin $F_{2\alpha}$ from epithelium-denuded human airway tissue (presumably from the smooth muscle), which in turn, amplifies the contractile responses of human airway smooth muscle to histamine.

Keywords: Airway, human; Histamine; Prostaglandin $F_{2\alpha}$

1. Introduction

Prostaglandins are generated by a variety of tissues and cells within the lung and may act as local hormones (Smith, 1989). Although arachidonic acid, the precursor to prostanoids, is probably ubiquitous within this environment, biologically active prostanoids such as prostaglandin E_2 and prostaglandin $F_{2\alpha}$ appear to be synthesised in a cell-specific manner. For example, endothelial cells from large blood vessels synthesise primarily prostacyclin (prostaglandin I_2) (Baenziger et al., 1979), whereas prostaglandin E_2 is the predominant cyclooxygenase metabolite generated by bronchial epithelial cells (Mattoli et al., 1990). With respect to contractile prostanoids, prostaglandin D_2 has been identified as the major cyclooxygenase product generated by mast cells, while prostaglandin $F_{2\alpha}$ is produced by macrophages and to a lesser degree monocytes (Godard et al., 1982; Holgate et al., 1984, Niiro et al., 1995) but whether other cell types such as the smooth muscle have the capacity to generate significant quantities of this mediator is unknown. However, prostaglandin $F_{2\alpha}$ is a potent contractile agonist of non-asthmatic (Black et al., 1986; Featherstone et al., 1990), and to a greater extent, asthmatic airways (Beasley et al., 1987) and also potentiates contractions mediated by vagal stimulation and histamine (Leff et al., 1985), suggesting a significant influence of prostaglandin $F_{2\alpha}$ on airway smooth muscle responses.

Current opinion suggests that prostaglandin receptors be classified by the prostaglandin which has the greatest activity (Coleman et al., 1984). Recent evidence obtained from experiments on human bronchial smooth muscle suggests that all prostaglandin-induced contraction of this

* Corresponding author. Present address: U.B.C. Pulmonary Research Laboratory, St. Paul's Hospital, 1081 Burrard Street, Vancouver, B.C. V6Z 1Y6, Canada. Tel.: (1-604) 682-2344 (ext. 2702); Fax: (1-604) 631-5351; e-mail: dknight@prl.pulmonary.ubc.ca

tissue is mediated via the TP receptor subtype for which several selective antagonists are available (Coleman and Sheldrick, 1989; Armour et al., 1989).

Previous data from our laboratory demonstrated that histamine, acting through the histamine H_2 receptor subtype, selectively induces the epithelial generation of prostaglandin E_2, which in turn, is capable of inhibiting the histamine-induced contraction of human bronchial smooth muscle (Knight et al., 1995a,b). In contrast, in the guinea pig at least, activation of histamine H_1 receptors leads to the generation of prostaglandin $F_{2\alpha}$ (Yen et al., 1976). These results suggest that the histamine-induced release of prostaglandins may be controlled by the stimulation of a particular histamine receptor subtype and this, in turn, may determine whether an inhibitory or excitatory feedback response is observed.

The aim of the current investigation was to determine whether histamine-induced release of prostaglandin $F_{2\alpha}$ plays a role in modulating human isolated bronchial smooth muscle responses. To achieve this, we used the selective prostaglandin TP receptor antagonist SQ 29,548 and compared the responses to histamine, prostaglandin $F_{2\alpha}$ and the cholinomimetic carbachol.

2. Materials and methods

2.1. Collection of lung specimens

Macroscopically normal bronchi were obtained from 18 patients undergoing thoracotomy and placed in ice-cold Krebs-Henseleit solution (composition in mM: NaCl 121, KCl 5.4, $MgSO_4$ 1.2, Na_2HPO_4 1.2, $NaHCO_3$ 15, $CaCl_2$ 2.5, glucose 11.5, previously gassed with 95% O_2/5% CO_2), within 20 min of resection and transported to the laboratory. Macroscopically normal bronchi (4–7 mm internal diameter) were dissected free of all visible blood vessels and parenchyma and used either on the day of resection or stored for 24 h at 4°C.

2.2. Organ bath protocol

Human bronchial smooth muscle preparations were examined as previously described (Knight et al., 1995a). In all experiments, adjacent bronchial smooth muscle strips were prepared from the same airway and examined in parallel. Muscle strips designated as epithelium-denuded were mechanically denuded of the epithelial cell layer by gentle rubbing with a moist cotton swab. Applied force-response relationships were measured to ensure that the denuding process did not significantly influence the contractile apparatus of the muscle (Knight et al., 1992). Muscle strips were mounted in 20 ml organ baths containing Krebs-Henseleit solution maintained at 37°C and gassed with 95% O_2/5% CO_2. Bronchial preparations were allowed to equilibrate (60–90 min) under a previously determined optimum tension of 1 g. Any changes in this basal tension were compensated for by readjustment of the apparatus to maintain the muscle tension at 1 g. Changes in isometric tension were measured with Grass FTO3C transducers (Grass Instruments, Quincy, MA, USA) coupled to a Rikadenki L50 multipen chart recorder (Rikadenki, Tokyo, Japan). During the equilibration period, the bathing fluid was exchanged every 15 min. At the beginning of each experiment, a submaximal dose of carbachol (10^{-6} M) was added to the organ bath to assess tissue viability and response reproducibility. Once the tissues had generated their maximum response, all preparations received multiple washes in fresh carbogenated, prewarmed Krebs-Henseleit solution and allowed to return to basal tension.

A cumulative concentration-effect curve was constructed for carbachol using a bath concentration range of 10^{-8} to 10^{-5} M. Following washout and a suitable recovery period, a cumulative concentration-effect curve was constructed for prostaglandin $F_{2\alpha}$, in the concentration range of 10^{-9} M to 3×10^{-4} M. Preparations were allowed to return passively to basal tension and then treated with either the TP receptor antagonist SQ 29,548 (0.4 μM) or the appropriate vehicle solution for 30 min and a second cumulative concentration-effect curve was then constructed for prostaglandin $F_{2\alpha}$. An identical protocol for adjacent epithelium-intact and epithelium-denuded muscle strips was followed for histamine with the concentration range being 10^{-7} to 10^{-3} M.

For experiments examining the effects of SQ 29,548 on human isolated bronchial smooth muscle responses to prostaglandin $F_{2\alpha}$, muscle preparations were pre-treated with the non-steroidal anti-inflammatory drug flurbiprofen for 30 min prior to exposure to the prostaglandin. For comparison, the responses to carbachol in the presence of SQ 29,548 were recorded also. For histamine experiments, and where appropriate, the effect of the H_2 receptor antagonist ranitidine (60 μM) was assessed. This concentration was selected on the basis of previous investigations (Knight et al., 1992).

2.3. Measurement of prostaglandin $F_{2\alpha}$

2.3.1. Collection of organ bath effluent

Organ bath effluents were collected for prostaglandin $F_{2\alpha}$ measurement from experiments involving 6 bronchial preparations. For each experiment, 1.5 ml of effluent were obtained at baseline prior to any pharmacological challenge, immediately following the maximum response to carbachol and 3 min prior to, and immediately following, the maximum response to histamine. All effluents were stored at −85°C until assayed.

2.3.2. Sample preparation

Prostaglandin $F_{2\alpha}$ was extracted from organ bath effluents after adjusting the pH to 3 by the addition of 2 M

HCl. The sample was then applied to Amprep C2 reverse phase extraction columns (Amersham, Australia) previously conditioned with methanol followed by water. Columns were washed sequentially with 5 ml of distilled water, 10% (v/v) ethanol and hexane and the bound prostaglandin was then eluted with 5 ml of methyl formate. The eluate was evaporated to dryness at 37°C under nitrogen and resuspended in 1 ml of 0.1 M phosphate buffer (pH 7.6, containing 0.9% (w/v) NaCl, 0.1% (w/v) gelatin; thiomersol).

2.4. Enzymeimmunoassay (EIA)

The EIA procedures were performed as described by the manufacturer (Cayman Chemical, Ann Arbor, MI, USA). All assays were performed in duplicate; results were finally expressed as pg prostanoid produced/mg of tissue.

2.5. Analysis of results

The maximum tension (E_{max}) and potency (pD_2) were determined from the raw data. Agonist potency, pD_2, was calculated as the $-\log$ of the concentration producing 50% of the maximum response (E_{max}). Differences in the results between each cumulative concentration-effect curve were assessed for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons or by Student's *t*-test using the SAS computer program.

2.6. Drugs and chemicals

Carbamylchloride (carbachol) and histamine diphosphate were purchased from Sigma (Sydney, Australia). SQ 29,548, flurbiprofen and prostaglandin $F_{2\alpha}$ tromethamine were purchased from Cayman (Ann Arbor, MI, USA). All dilutions of carbachol, histamine and prostaglandin $F_{2\alpha}$ were made in 0.9% (w/v) saline. Stock solutions were stored at −20°C. Stock solutions of flurbiprofen and SQ 29,548 were made by dissolving the powder in 100% ethanol. Further dilutions were made in combinations of ethanol and 0.9% saline. At the final working concentrations, the vehicle alone did not significantly affect contractility of human isolated bronchial smooth muscle preparations.

3. Results

3.1. Generation of prostaglandin $F_{2\alpha}$ from human isolated bronchial smooth muscle

The generation of prostaglandin $F_{2\alpha}$ by both epithelium-intact and epithelium-denuded bronchial preparations at resting tension and following agonist stimulation is shown in Fig. 1a. Prior to stimulation, the mean levels of prostaglandin $F_{2\alpha}$ generated by intact ($n = 6$) or denuded

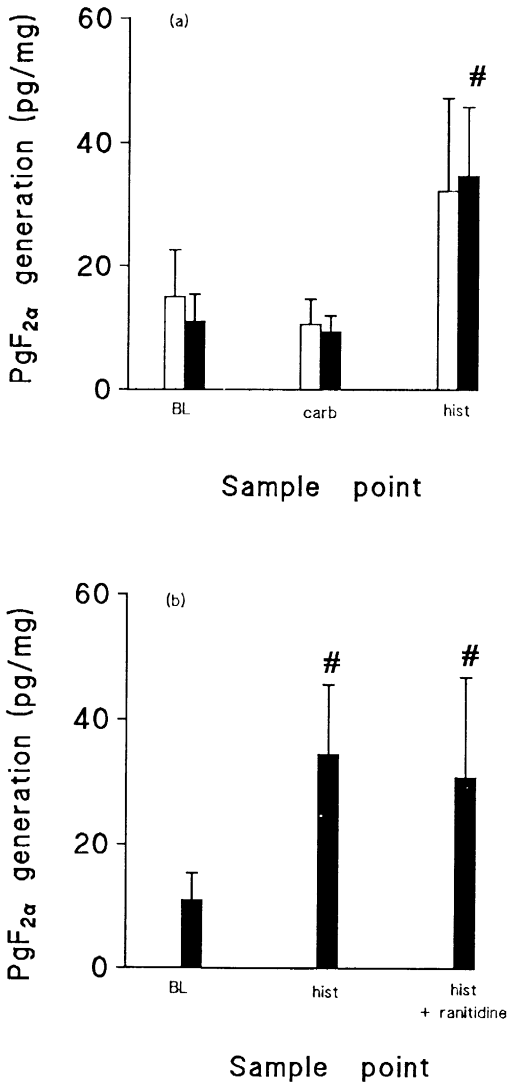

Fig. 1. (a) Relationship between the generation of prostaglandin $F_{2\alpha}$ and stimulation of epithelium-intact (□) and epithelium-denuded (■) human isolated bronchial smooth muscle in response to carbachol and histamine and (b) the effect of the histamine H_2 receptor antagonist ranitidine (60 μM) on prostaglandin $F_{2\alpha}$ generation from epithelium-denuded preparations. # Generation of prostaglandin $F_{2\alpha}$ significantly greater than basal release, $P < 0.05$. BL = basal release of prostaglandin $F_{2\alpha}$. carb = the concentration of prostaglandin $F_{2\alpha}$ obtained at maximum response to carbachol. hist = the concentration of prostaglandin $F_{2\alpha}$ obtained at maximum response to histamine. Results are expressed as mean ± S.E.M. response from bronchi obtained from 6 donors.

($n = 6$) bronchi were not significantly different, being 14.96 ± 7.79 pg/mg and 10.97 ± 3.05 pg/mg for intact and denuded preparations respectively. Exposure to the muscarinic agonist carbachol did not influence the generation of prostaglandin $F_{2\alpha}$ from either epithelium-intact or denuded bronchi (Fig. 1a). In contrast, histamine exposure significantly enhanced the amount of prostaglandin $F_{2\alpha}$ generated by both epithelium-intact and denuded bronchial preparations. Epithelium-intact preparations generated 34.26 ± 16.3 pg/mg of prostaglandin $F_{2\alpha}$ whilst epithelium-denuded bronchi produced 32.62 ± 11.83 pg/mg of

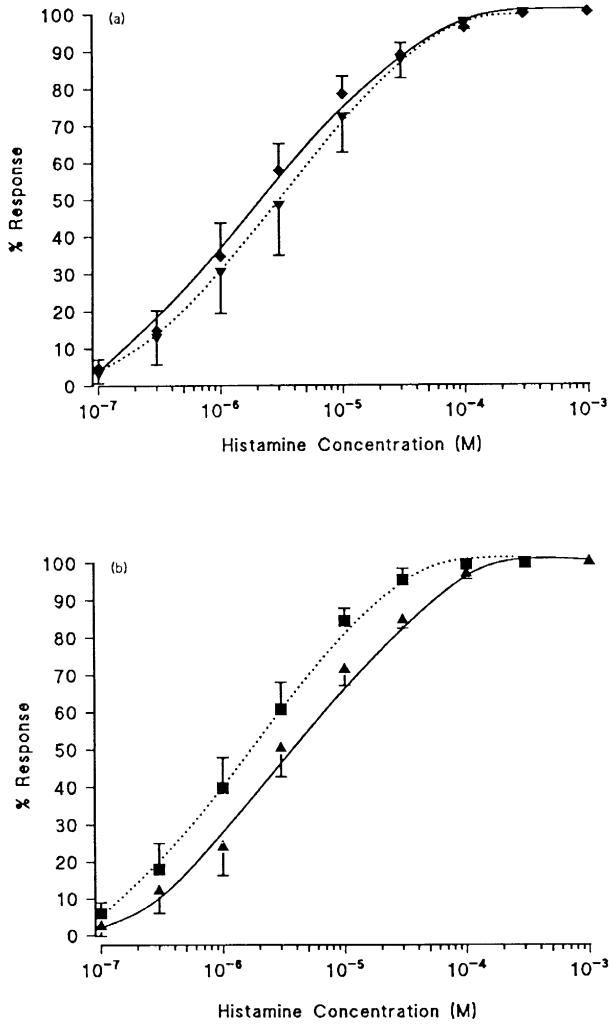

Fig. 2. Mean ± S.E.M. responses of epithelium-denuded human isolated bronchial smooth muscle to histamine in the (a) absence and (b) presence of the TP receptor antagonist SQ 29,548 (0.4 μM). (a) Control preparations were exposed to vehicle 30 min prior to the construction of the second cumulative concentration-effect curve to histamine (solid line). (b) Bronchial preparations were treated with SQ 29,548 for 30 min prior to the construction of the second cumulative concentration-effect curve to histamine (solid line). Responses are given as a percentage of individual E_{max} values.

prostaglandin $F_{2\alpha}$ (Fig. 1a). These results suggest that prostaglandin $F_{2\alpha}$ is generated primarily from non-epithelial sources, presumably the airway smooth muscle itself. Pre-incubation of epithelium-denuded preparations with the selective histamine H_2 receptor antagonist ranitidine (60 μM) did not alter the histamine-induced generation of prostaglandin $F_{2\alpha}$ (Fig. 1b), suggesting that the histamine H_2 receptor is not involved in prostaglandin $F_{2\alpha}$ release.

3.2. The effect of SQ 29,548 on human isolated bronchial smooth muscle responses to histamine

Since prostaglandin $F_{2\alpha}$ appeared to be generated primarily from non-epithelial sources, experiments examining the role of endogenous prostaglandin $F_{2\alpha}$ were performed only on epithelium-denuded bronchi.

Table 1
The effect of the TP receptor antagonist SQ 28 549 on contractile responses of human isolated bronchial smooth muscle to histamine, carbachol and prostaglandin $F_{2\alpha}$

Agonist	Control		SQ 29,548	
	Curve 1	Curve 2	Curve 1	Curve 2
Histamine				
E_{max} [a]	26.11 ± 5.26	29.04 ± 5.40	29.13 ± 4.04	25.99 ± 5.14
pD_2 [b]	5.56 ± 0.22	5.55 ± 0.15	5.72 ± 0.09	5.47 ± 0.11 [c]
Carbachol				
E_{max}	19.71 ± 6.67	19.47 ± 6.96	22.78 ± 6.27	22.63 ± 6.72
pD_2	6.26 ± 0.08	6.23 ± 0.15	6.30 ± 0.06	6.27 ± 0.04
Prostaglandin $F_{2\alpha}$				
E_{max}	23.63 ± 10.22	24.22 ± 8.08	20.06 ± 6.97	0.81 ± 0.11 [d]
pD_2	5.14 ± 0.23	5.16 ± 0.20	5.47 ± 0.29	N/A

Results expressed as mean ± S.E.M. [a] E_{max} expressed as g tension generated/wet weight of tissue. [b] pD_2 is derived from the mean EC_{50} values. Control preparations were treated with vehicle only for 30 min prior to cumulative concentration-effect curve 2. Test preparations were exposed to SQ 29,548 for 30 min prior to cumulative concentration-effect curve 2. [c] pD_2 for test preparation significantly different to corresponding value for cumulative concentration-effect curve 1, $P < 0.01$, paired *t*-test. [d] E_{max} value for test preparation significantly different to corresponding value for cumulative concentration-effect curve 1, $P < 0.0001$, paired *t*-test.

Histamine caused a concentration-dependent contraction of all human bronchial preparations tested with a mean pD_2 of 5.72 ± 0.1 (Table 1). Addition of the TP receptor antagonist SQ 29,548 (0.4 μM) produced variable, but insignificant effects on resting tension of bronchial strips. The concentration of SQ 29,548 used in this study equated

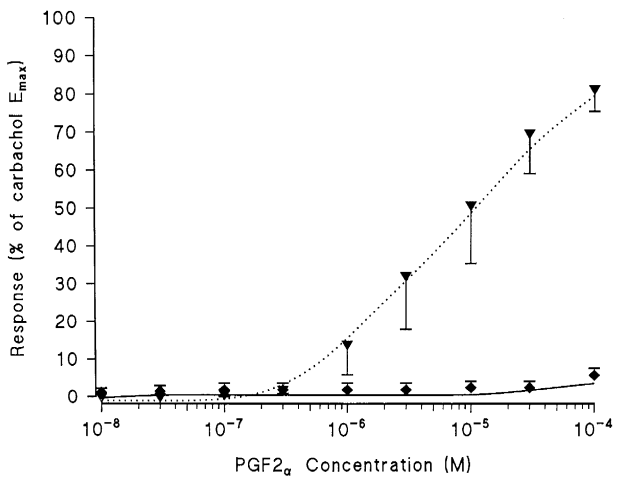

Fig. 3. Mean ± S.E.M. responses of epithelium-denuded human isolated bronchial smooth muscle to prostaglandin $F_{2\alpha}$ in the presence (b) of the TP receptor antagonist SQ 29,548 (0.4 μM). SQ 29,548 was added 30 min prior to the construction of the second cumulative concentration-effect curve to prostaglandin $F_{2\alpha}$. Responses are expressed as a percentage of the carbachol E_{max}.

well with its calculated pK_B value of 6.28 ($K_i = 5.2 \times 10^{-7}$ M) and is based on previously published concentrations of SQ 29,548 used to antagonize TP receptor-mediated platelet aggregation (Eglen and Whiting, 1988) and contraction of guinea pig tracheal spirals (Ogletree et al., 1985). The concentration-response curve to histamine in the presence of this drug was shifted significantly to the right, such that the pD_2 value in the presence of SQ 29,548 was 5.47 ± 0.1 ($P = 0.01$, $n = 6$) (Table 1; Fig. 2b). In contrast, the mean maximum contractile response to histamine was not significantly altered by SQ 29,548 (Table 1). In adjacent control preparations treated with vehicle alone, neither the potency nor E_{max} values for the second histamine cumulative concentration-effect curve were significantly different from values for the first histamine cumulative concentration-effect curve (Fig. 2a; Table 1, $n = 6$).

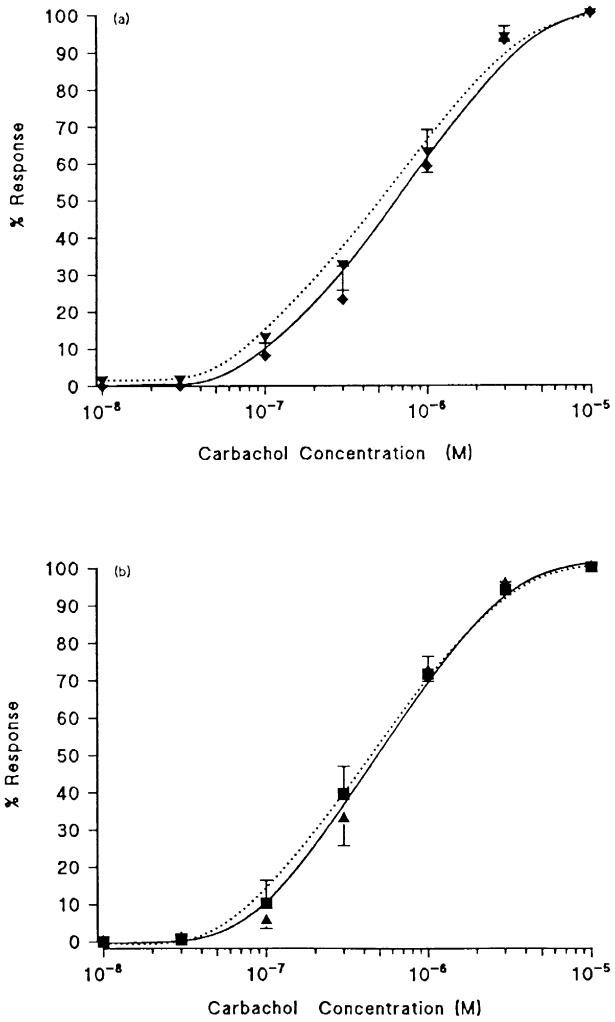

Fig. 4. Mean ± S.E.M. responses of epithelium-denuded human isolated bronchial smooth muscle to carbachol in the (a) absence and (b) presence of the TP receptor antagonist SQ 29,548 (0.4 μM). (a) Control preparations were exposed to vehicle 30 min prior to the construction of the second cumulative concentration-effect curve to carbachol (solid line). (b) Bronchial preparations were treated with SQ 29,548 for 30 min prior to the construction of the second cumulative concentration-effect curve to carbachol (solid line). Responses are given as a percentage of individual E_{max} values.

3.3. The effect of SQ 29,548 on human isolated bronchial smooth muscle responses to prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ also produced concentration-dependent contractions of human isolated bronchial smooth muscle (Fig. 3; Table 1). For all preparations contraction in response to prostaglandin $F_{2\alpha}$ in the presence of SQ 29,548 was either small (< 10% of control contraction at E_{max}) or absent (Fig. 3). Thus in the presence of SQ 29,548 an accurate determination of EC_{50} values (and therefore pD_2) was not possible. In control preparations, the two cumulative concentration-effect curves constructed for prostaglandin $F_{2\alpha}$ were statistically indistinguishable (Table 1).

3.4. The effect of SQ 29,548 on human isolated bronchial smooth muscle responses to carbachol

There was no significant generation of prostaglandin $F_{2\alpha}$ from human isolated bronchial smooth muscle stimulated with carbachol, which was therefore used to examine whether SQ 29,548 produced a non-specific effect on human isolated bronchial smooth muscle contraction. SQ 29,548 did not alter the cumulative concentration-effect curve to carbachol (Fig. 4b; Table 1). The pD_2 values for carbachol in the absence and presence of the drug were 6.31 ± 0.07 and 6.29 ± 0.04 ($n = 6$) respectively and were not statistically different. Similarly, E_{max} values for carbachol before and after treatment were statistically indistinguishable (Table 1).

4. Discussion

Previous data from this laboratory have demonstrated that histamine, acting via the histamine H_2 receptor subtype, evoked the generation of prostaglandin E_2 and, to a lesser extent, prostaglandin I_2 from the epithelium of human isolated airways (Knight et al., 1995a). Prostaglandin E_2 in turn was shown to inhibit bronchial smooth muscle responses to further histamine stimulation (Knight et al., 1995b). In the current investigation, histamine was shown to induce the generation of significant quantities of prostaglandin $F_{2\alpha}$, most likely from non-epithelial sources such as airway smooth muscle and this prostaglandin, acting via TP receptors, appeared to amplify the contractile activity of histamine. Significantly, the generation of prostaglandin $F_{2\alpha}$ appears to be agonist selective, since the cholinergic agonist carbachol did not stimulate the generation of prostaglandin $F_{2\alpha}$ and the contractile responses to this agent were not influenced by blockade of TP receptors.

All bronchial preparations released detectable amounts

of prostaglandin $F_{2\alpha}$ at rest which increased following stimulation with histamine. Following washout of histamine, levels of prostaglandin $F_{2\alpha}$ returned to baseline. Previously reported data obtained with both human and guinea-pig airways have demonstrated that histamine is capable of inducing the generation of both prostaglandin $F_{2\alpha}$ and prostaglandin E_2 (Yen et al., 1976; Schulman et al., 1982), although the site of production has not been reported. In the current study, the levels of prostaglandin $F_{2\alpha}$ released by epithelium-intact and denuded preparations were virtually identical suggesting a non-epithelial source and that the smooth muscle itself may be a major source of prostaglandin $F_{2\alpha}$. Although cultured respiratory epithelial cells have also been reported to generate prostaglandin $F_{2\alpha}$, the relative amounts of these prostanoids measured appear to be dependent on the particular culture conditions used (Holtzman et al., 1988; Churchill et al., 1989). Equally, in these conditions, the amount of prostaglandin $F_{2\alpha}$ released in comparison to inhibitory prostanoids such as prostaglandin E_2, is small (Holtzman et al., 1988). Similarly, small amounts of prostaglandin $F_{2\alpha}$ release have been detected in other cell types such as monocytes and macrophages (Godard et al., 1982; Holgate et al., 1984), but the overall level of prostaglandin $F_{2\alpha}$ contributed by these cells in isolated bronchial smooth muscle preparations is likely to be relatively insignificant. However, in addition to prostaglandin $F_{2\alpha}$, the action of other cyclooxygenase metabolites such as thrombaxane A_2 (generated from vascular tissue) and prostaglandin D_2 (generated from mast cells) which would also presumably interact with TP receptors cannot be discounted.

The production of prostaglandin $F_{2\alpha}$ from the non-epithelial source appears to be the result of histamine H_1 receptor activation since the selective histamine H_2 receptor antagonist ranitidine had no effect on the release of prostaglandin $F_{2\alpha}$ from epithelium-denuded bronchi. Histamine H_2 receptors have been functionally localised to the bronchial epithelium (Knight et al., 1992, 1994) and stimulation of these receptors results in the generation of inhibitory prostanoids such as prostaglandin E_2 (Knight et al., 1995a). In contrast, it seems likely that activation of histamine H_1 receptors on airway smooth muscle cells leads to production of the contractile prostaglandin prostaglandin $F_{2\alpha}$ which in turn amplifies histamine-induced airway smooth muscle contraction. Further confirmation of these findings awaits similar studies using histamine H_1 receptor-specific antagonists. This hypothesis is in agreement with previously reported effects of prostaglandin $F_{2\alpha}$ on human isolated bronchial smooth muscle (Heaton et al., 1984). Thus it appears that histamine has a complex role in regulating airway smooth muscle contraction both to itself and potentially for other contractile mediators.

The relative importance of contractile prostaglandins in modulating airway responses requires an understanding of the specific receptors for these mediators. Receptors for prostaglandins are currently classified into at least 5 different subtypes based on the rank order of agonist potencies in different tissues or cells (Coleman et al., 1984). Thus, TP receptors are the naturally occurring binding sites for thromboxane A_2, whereas prostaglandin E_2 preferentially binds to the EP receptor subtypes and prostaglandin $F_{2\alpha}$ binds selectively to the FP receptor subtype (Coleman et al., 1984). Following the recent development of selective TP receptor antagonists such as SQ 29,548 (Ogletree et al., 1985; Eglen and Whiting, 1988), it has become apparent that prostanoid-induced contraction of human airways is mediated through the TP receptor subtype (Coleman and Sheldrick, 1989; Armour et al., 1989). In large, proximal airways some contractile responses may also occur through stimulation of EP-1 receptors (McKenniff et al., 1988). Although FP receptor subtypes, the natural binding site for prostaglandin $F_{2\alpha}$, have been identified in various tissues such as human non-pregnant myometrium (Senior et al., 1992), significant populations of these receptors have not been observed previously in human airways. The data from the current investigation support this concept since exposure to SQ 29,548 almost completely abolished contractions to exogenous prostaglandin $F_{2\alpha}$ suggesting that this prostanoid too, acts via the TP receptor subtype. In contrast, Ogletree et al. (1985) showed that SQ 29,548 had no effect on histamine-induced contraction and only partially inhibited contractions elicited by prostaglandin $F_{2\alpha}$ in guinea pig trachea. These data suggest that in guinea pig tracheal preparations, other prostaglandin receptor populations exist which also mediate contractile responses to prostaglandin $F_{2\alpha}$.

The observation that SQ 29,548 elicited a variable but minimal effect on basal tone suggests that prostaglandin $F_{2\alpha}$ and other contractile cyclooxygenase metabolites are not significantly involved in the maintenance of resting tone in human airways, but are selectively released in response to, and amplify histamine-induced contraction.

Contractile responses to exogenously added carbachol were not affected by SQ 29,548. These results conflict with those of Jongejan and colleagues (Jongejan et al., 1990) who demonstrated that prostaglandin $F_{2\alpha}$ produced a very small but nonetheless significant increase in the sensitivity of human bronchial smooth muscle to methacholine. The reason for these conflicting results is unknown, although differences in the concentrations of prostaglandin $F_{2\alpha}$ used may be significant. When expressed as a molar concentration, the amount of prostaglandin $F_{2\alpha}$ generated following histamine exposure in the current study was 0.36 nM. The lowest concentration of prostaglandin $F_{2\alpha}$ used in the study of Jongejan et al. (1990) was 1 nM and even at this concentration, the effect of prostaglandin $F_{2\alpha}$ on methacholine responses appeared to be relatively minor. In support of our data, Heaton et al. (1984) demonstrated that prostaglandin $F_{2\alpha}$, in concentrations too small to directly contract airway smooth muscle, selectively augmented histamine-induced bronchoconstriction without affecting methacholine responsiveness in vivo.

In conclusion, human airway smooth muscle preparations at rest produce detectable amounts of prostaglandin $F_{2\alpha}$, the levels of which are selectively enhanced following exposure to histamine. The prostaglandin $F_{2\alpha}$ generated acted via TP receptors presumably present on airway smooth muscle cells and was capable of producing a leftward shift in the dose-response curve to histamine. This finding, coupled with the previous observation that histamine-induced, epithelial production of prostaglandin E_2 (Knight et al., 1995a,b) inhibits histamine-induced airway smooth muscle contraction, provides further evidence that with respect to histamine and prostaglandins, separate but inter-related feedback systems operate at the epithelial and smooth muscle levels. In this setting, histamine appears to play an important role in regulating and coordinating human airway contractile responses.

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