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Epithelium-derived inhibitory prostaglandins modulate human bronchial smooth muscle responses to histamine

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Abstract

The role of the bronchial epithelium and inhibitory prostaglandins in the induction of histamine tachyphylaxis in human isolated bronchial smooth muscle was investigated using bronchi obtained from 14 patients who had been treated with non-steroidal anti-inflammatory drugs (NSAID) for > 2 months and from 14 untreated patients. Epithelium-intact bronchial strips from untreated patients demonstrated tachyphylaxis to histamine with the maximum response (E_{max}) reduced by 30 ± 5% ($P < 0.02$) and the EC_{50} increased 1.86-fold ($P < 0.02$). Tachyphylaxis was not observed in epithelium-denuded strips. In epithelium-intact bronchial preparations from NSAID treated patients, the mean initial maximum tension generated in response to histamine was significantly greater than that for bronchial preparations from untreated patients ($P < 0.05$). In NSAID-treated patients, both epithelium-intact and denuded preparations failed to demonstrate tachyphylaxis. The generation of prostaglandin E_2 and prostacyclin was assessed by radio-immunoassay using bronchi from untreated patients ($n = 8$). In epithelium-intact bronchial preparations, the generation of both prostaglandin E_2 and prostacyclin was significantly increased by histamine exposure ($P < 0.05$) and was completely inhibited by indomethacin. However, the selective histamine H_2 receptor antagonist, ranitidine, selectively inhibited the synthesis of prostaglandin E_2 alone. Production of both prostaglandins was not altered by exposure to acetylcholine. These results suggest that prostaglandin E_2 and prostacyclin are released primarily from the epithelium in response to histamine and may be specifically involved in inhibiting human bronchial smooth muscle responsiveness to this mediator. Significantly, the release of prostaglandin E_2 appears to be selectively controlled by histamine H_2 receptors, resident on the epithelium.

Keywords: Histamine; Prostaglandin E_2; Prostacyclin; Airway; (Human)

1. Introduction

Human lung tissue is capable of releasing a variety of prostanoids in response to different stimuli which modulate airway smooth muscle contractility. The major prostanoids generated at rest, or following passive sensitization with IgE, appear to be prostaglandin E_2 and prostacyclin (Schulman et al., 1982). Stimulation of lung tissue with contractile agonists such as histamine may also generate prostanoids, although the type released appears to be species-dependent and related to the receptor subtype which is being stimulated. For example, in guinea pig (Yen et al., 1976) and human lung (Platshon and Kaliner, 1978), stimulation of histamine H_1 receptors results in the preferential release of contractile prostanoids such as $PGF_{2\alpha}$. In contrast, stimulation of histamine H_2 receptors in guinea pig lung results in the release of inhibitory prostanoids such as prostaglandin E_2 (Yen et al., 1976).

The effects of prostanoids on airway smooth muscle function are also species dependent. Whilst it is generally accepted that inhibition of cyclooxygenase does not alter the basal tension of human isolated airway smooth muscle (Haye-Legrand et al., 1986; Adcock and Garland, 1982), the effect of prostaglandins on agonist-induced airway smooth muscle contraction is unclear. Haye-Legrand et al. (1986) showed that whilst human bronchi generate significant amounts of prostaglandin E_2 in response to histamine, subsequent airway smooth muscle responses to this agonist were not modified. In contrast, other investigators have demonstrated that

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inhibition of cyclooxygenase alters human isolated bronchial smooth muscle responses to stimulation by both histamine (Adcock and Garland, 1982) and antigen (Adams and Lichtenstein, 1985).

Tachyphylaxis to repeated histamine exposure has been demonstrated, both in vivo and in vitro, in the airways of a variety of species including man (Watanabe et al., 1988; Manning et al., 1987; Knight et al., 1992). Although the precise mechanism(s) involved are unclear, in vivo results (Jackson et al., 1988) together with recent data from our laboratory using resected human bronchi, suggest a role for both histamine H_2 receptors and the epithelium in this phenomenon (Knight et al., 1992). In some animal models, it also appears that inhibitory prostanoids, in particular prostaglandin E_2, are involved (Anderson et al., 1983). Although indomethacin treatment has been shown to inhibit histamine tachyphylaxis in vivo (Manning et al., 1987), the role of prostanoids in mediating this phenomenon in human isolated lung tissue has not been delineated.

In this study, the role of prostaglandins in the development of histamine tachyphylaxis in human isolated bronchial smooth muscle was investigated. In addition, the effect of chronic inhibition of prostanoid synthesis was assessed by using tissue from patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) prior to surgery.

2. Materials and methods

2.1. Tissue preparation

Lung tissue was obtained at thoracotomy from 20 male and 8 female patients with a combined mean age of 62.7 year (range 46–78 year). None of the patients had a history of asthma, although four were receiving inhaled β-adrenoceptor agonists and inhaled corticosteroids for chronic obstructive airways disease. All but two patients had a history of long term smoking and all but four had ceased smoking for at least 4 months prior to surgery. Anaesthetic agents were the same for all patients. Fourteen patients (nine male, five female) had been receiving regular non-steroidal anti-inflammatory therapy for a variety of musculoskeletal disorders including osteoarthritis, chronic back pain and sciatica (Table 1) for at least 2 months prior to surgery.

Bronchial tissue from the fourteen patients who had not received histamine receptor antagonists nor non-steroidal anti-inflammatory drugs for at least 14 days prior to surgery were used to assess the production of prostaglandin E_2 and prostacyclin following histamine exposure and to assess the inhibitory effect of acute indomethacin exposure on the induction of histamine tachyphylaxis.

Table 1
Summary of donor patients receiving NSAID therapy prior to surgery

Patient	Age	Sex	NSAID medication
1	61	M	Indomethacin
2	76	M	Sulindac
3	74	M	Ibuprofen
4	67	F	Naproxen
5	64	M	Peroxicam, aspirin
6	60	M	Diclofenac
7	67	F	Diclofenac
8	63	F	Diflusanil
9	62	M	Ketoprofen, indomethacin
10	69	M	Ketoprofen
11	67	F	Indomethacin, aspirin
12	74	M	aspirin
13	54	M	Diclofenac
14	49	F	Ketoprofen

Lung tissue was obtained within 20 min of resection 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) and transported to the laboratory. Macroscopically normal bronchi (4–10 mm i.d.) 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., 1992). In both ex vivo and in vitro experiments, four adjacent bronchial smooth muscle strips (epithelium-intact/denuded, control and test strips) were prepared from the same airway and examined in parallel. 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) 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, submaximal doses of acetylcholine (10^{-5} M) were 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 passively to basal tension.

To assess the development of histamine tachyphylaxis, three cumulative concentration-effect curves were constructed for histamine each separated by a 90 min recovery period. During this period, all preparations were washed (×3) immediately after the maximal response followed by washing (×2) every 10 min for the remaining time.

To measure tissue viability and responsiveness, a cumulative concentration-effect curve was constructed for acetylcholine at the beginning and completion of each experiment, using a bath concentration range of 10^{-7}–10^{-3} M.

2.3. Epithelium-denuded experiments

In experiments designed to assess the modulatory role of the epithelium, adjacent sections from the same airway were used, with one having the epithelium mechanically removed with a moist cotton swab. Care was also taken to ensure the other strip remained intact. Applied force-response relationships were assessed on random samples to ensure that epithelium removal had not altered the contractile apparatus of the underlying smooth muscle. Randomly selected bronchial preparations ($n = 6$) were also assessed in a blinded, semi-quantitative fashion by an independent observer using light microscopy to verify successful removal of the epithelium.

2.4. Inhibition of cyclooxygenase ex vivo

Histamine tachyphylaxis was assessed as described above for both epithelium-intact ($n = 14$) and epithelium-denuded ($n = 7$) preparations obtained from patients who had received one or more of a variety of non-steroidal anti-inflammatory drugs for at least 2 weeks prior to thoracotomy.

2.5. Inhibition of cyclooxygenase in vitro

The cyclooxygenase inhibitor indomethacin (bath concentration, 5 μM) was used to further assess whether prostaglandins were involved in the induction of histamine tachyphylaxis in vitro. Two separate experiments were performed using bronchial preparations from eight patients in each experiment. In one experiment, three histamine cumulative concentration-effect curves were performed after the addition of a single bolus dose of indomethacin to the organ bath 30 min prior to the generation of the first curve but removed prior to subsequent cumulative concentration-effect curves. In the second set of experiments, indomethacin was added to the bath 30 min prior to the generation of the second cumulative concentration-effect curve. This protocol was chosen so as to assess the ability of the tissue to generate prostaglandins following cyclooxygenase inhibition, either before or after histamine stimulation.

2.6. Measurement of prostaglandin E_2 and prostacyclin

2.6.1. Collection of organ bath effluent

Organ bath effluents were collected from experiments involving NSAID naive bronchial preparations, in order to measure prostaglandin E_2 and prostacyclin. For each prostanoid, 1.5 ml of effluent were obtained at baseline prior to any pharmacological challenge, immediately following the maximum response to acetylcholine, 3 min prior to, and immediately following, the maximum response to each of three histamine cumulative concentration-effect curves. All effluents were stored at −85°C until required. Aliquots were also obtained from previously reported experiments in which bronchial preparations were pretreated with the histamine H_2 receptor antagonist ranitidine (Knight et al., 1992).

2.6.2. Sample preparation

Prostaglandin E_2 was measured as bicyclic prostaglandin E_2 (Granstrom et al., 1980), after first adjusting the effluent pH to 11 with 1 M Na_2CO_3. After 24 h the reaction was terminated by the addition of 1 M KH_2PO_4. Prostacyclin was measured as its primary metabolite, 6-keto-$PGF_{1\alpha}$.

Prostanoids were 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 C_2 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].

2.7. Radioimmunoassay

The radioimmunoassay procedures were performed as described by the manufacturer (Amersham, Australia). All assays were performed in duplicate and samples were counted in a β-scintillation counter (Packard, USA). Analysis of results was performed using the Securia 1 (Packard) computer programme and finally expressed as pg prostanoid/mg of tissue.

2.8. 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 (EC_{50}). Tensions developed during the second and third histamine cumulative concentration-effect curves were expressed

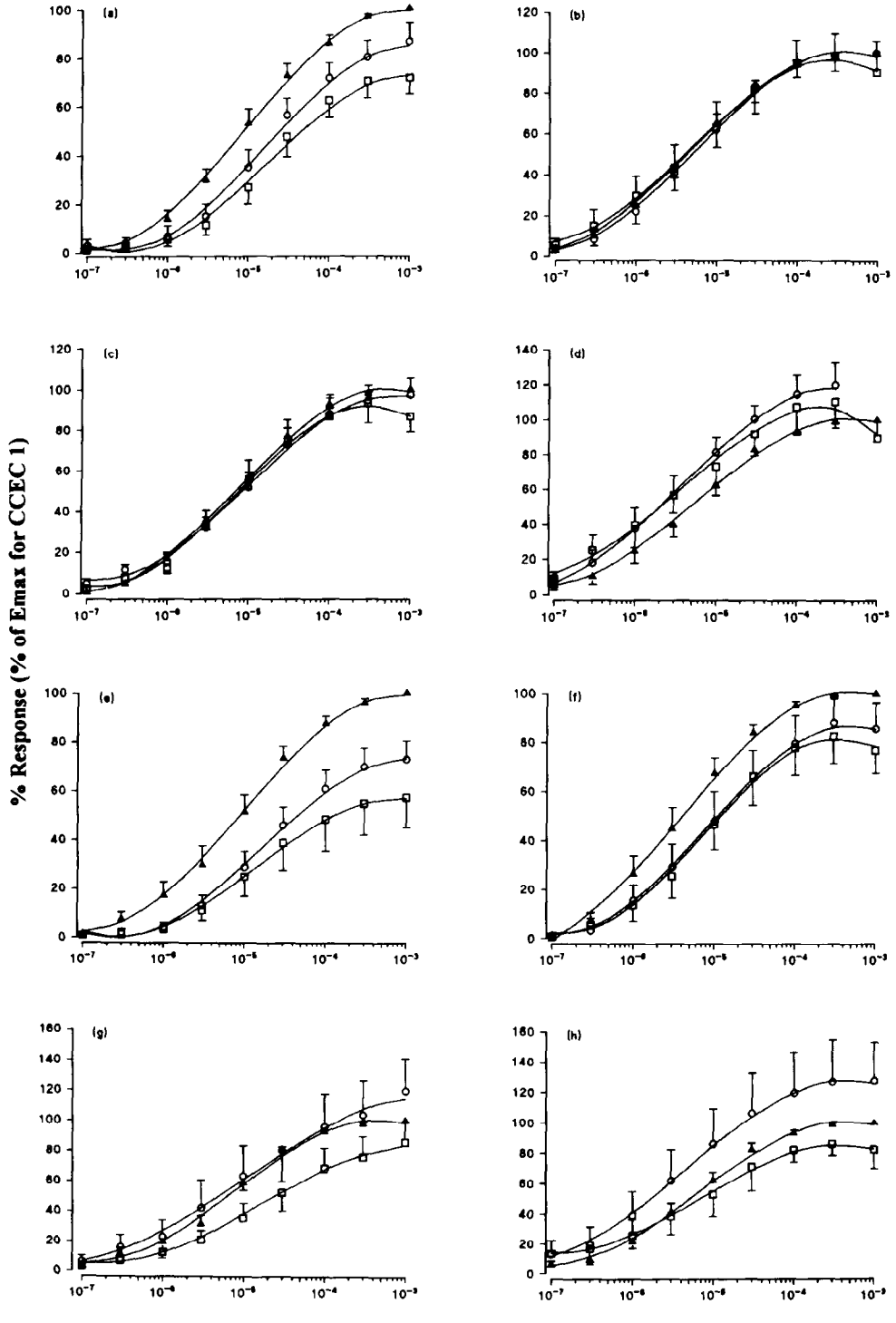

Fig. 1. The effect of three successive histamine cumulative concentration-effect curves in (a) epithelium-intact and (b) epithelium-denuded control human isolated bronchus; (c) epithelium-intact and (d) epithelium-denuded human isolated bronchus treated with non-steroidal anti-inflammatory drugs in vivo; (e) epithelium-intact and (f) epithelium-denuded human isolated bronchus pre-treated with indomethacin in vitro; (g) epithelium-intact and (h) epithelium-denuded human isolated bronchus post-treated with indomethacin in vitro (▲) curve 1, (○) curve 2, (□) curve 3. Each point represents mean ± S.E.M responses from bronchi obtained from at least seven different donors.

Table 2
The effect of epithelium removal and indomethacin treatment on the contractile potency (pD_2) and maximum contractile response (E_{max}) to repeated histamine exposure in human isolated bronchial smooth muscle

Treatment	n	Curve 1		Curve 2		Curve 3	
		pD_2 [a]	E_{max} [b]	pD_2	E_{max}	pD_2	E_{max}
Epithelium intact	12	4.99 ± 0.11	13.80 ± 2.51	4.85 ± 0.20	11.23 ± 2.31 [d]	4.72 ± 0.11 [e]	9.90 ± 2.52 [e]
Epithelium denuded	12	5.37 ± 0.09 [c]	17.41 ± 3.26	5.33 ± 0.11	16.9 ± 3.82	5.28 ± 0.15	15.1 ± 3.31
Epithelium intact/ indomethacin pretreat	8	5.05 ± 0.16	17.58 ± 5.52	4.73 ± 0.14	14.1 ± 5.74	4.63 ± 0.22 [e]	12.1 ± 5.51 [e]
Epithelium denuded/ indomethacin pretreat	8	5.37 ± 0.15	18.68 ± 6.32	5.06 ± 0.18	13.9 ± 4.12	5.06 ± 0.15	12.1 ± 3.32 [c]
Epithelium intact/ indomethacin posttreat	8	5.22 ± 0.10	13.38 ± 3.41	5.08 ± 0.21	15.5 ± 6.62	5.03 ± 0.23	10.4 ± 3.94
Epithelium denuded/ indomethacin posttreat	8	5.36 ± 0.15 [c]	18.61 ± 3.40	5.29 ± 0.21	17.0 ± 2.01	5.26 ± 0.17	13.7 ± 4.22

Results are expressed as means ± S.E.M. n = number of patients from whom bronchi were obtained. [a] pD_2 values calculated as $-\log_{10}(EC_{50})$. [b] E_{max} is expressed in g tension generated/g wet weight of tissue. [c] Value for epithelium-denuded preparations significantly different from corresponding epithelium-intact preparations $P < 0.01$, unpaired t-test. [d] Value sigificantly different to corresponding value for curve 1, $P < 0.05$, ANOVA and Tukey's test. [e] Value significantly different from corresponding value in curve 1, $P < 0.02$, ANOVA and Tukey's test.

as a mean ± S.E.M. percentage of the maximal response achieved during the first histamine cumulative concentration-effect curve. Differences in the results for the three histamine cumulative concentration-effect curves were assessed for statistical significance by one-way ANOVA followed by Tukey's test for multiple comparisons or Student's t-test using the SAS computer programme.

2.9. Drugs and chemicals

The following drugs were used: acetylcholine chloride, indomethacin (Sigma); flurbiprofen (Cayman Chemical); histamine diphosphate (British Drug House); ranitidine hydrochloride (Glaxo). Solutions of acetylcholine and histamine were prepared fresh in 0.9% w/v NaCl. Solutions of indomethacin were prepared by dissolving the powder in 5% Na_2CO_3 with further dilutions in Krebs-Henseleit solution. Flurbiprofen was dissolved in 100% ethanol, with subsequent dilutions in Krebs-Henseleit solution. Ranitidine hydrochloride was obtained in ampoule form. The vehicle itself was shown not to affect the responses of bronchial preparations.

3. Results

Histological examination and assessment of applied force-response relationships from randomly chosen epithelium-denuded preparations revealed no obvious damage to the underlying tissues as a result of epithelium removal.

3.1. Histamine tachyphylaxis in NSAID naive preparations

Repeated histamine challenge significantly reduced bronchial smooth muscle responses to histamine in epithelium-intact preparations. After three histamine cumulative concentration-effect curves, the mean E_{max} was reduced by 30% compared to the E_{max} for the initial curve ($P < 0.02$; Fig. 1a, Table 2). The mean EC_{50} value was increased 1.86-fold following three successive histamine challenges resulting in a significant reduction in pD_2 ($P < 0.02$; Fig. 1a, Table 2).

Following epithelium removal, although there was a tendency for the mean E_{max} value to be significantly enhanced, the difference was not statistically signifi-

Table 3
Effect of in vivo non-steroidal anti-inflammatory drugs on the contractile potency (pD_2) and maximum contractile response (E_{max}) to repeated histamine exposure in human isolated bronchial smooth muscle

Treatment	n	Curve 1		Curve 2		Curve 3	
		pD_2 [a]	E_{max} [b]	pD_2	E_{max}	pD_2	E_{max}
Epithelium intact	14	5.18 ± 0.10	23.02 ± 4.91 [c]	5.17 ± 0.10	20.4 ± 3.21	5.09 ± 0.10	21.7 ± 4.42
Epithelium denuded	7	5.36 ± 0.21	15.91 ± 5.02	5.48 ± 0.20	18.3 ± 4.93	5.51 ± 0.20	16.1 ± 3.42

Results are expressed as means ± S.E.M. n = number of patients from whom bronchi were obtained. [a] pD_2 is calculated as $-\log_{10}(EC_{50})$. [b] E_{max} is expressed in g tension generated/g wet weight of tissue. [c] Value for epithelium-denuded preparations significantly different from corresponding epithelium-intact preparation $P < 0.05$, unpaired t-test.

cant. However, the mean pD_2 value for histamine in these denuded preparations was 2.4-fold greater than observed for the initial histamine-induced contraction in epithelium-intact bronchi ($P < 0.01$; Fig. 1b, Table 2). Tachyphylaxis to repeated histamine exposure was not observed in epithelium-denuded preparations (Fig. 1b, Table 2).

3.2. The effect of chronic exposure to NSAID in vivo, on histamine responses in vitro

Exposure to NSAID in vivo resulted in a significantly augmented mean E_{max} in vitro for the initial cumulative concentration-effect curve in epithelium-intact bronchial strips when compared to control strips from untreated patients being 23.02 ± 4.91 vs. 13.80 ± 2.51 ($P < 0.05$; Fig. 1c, Table 3). However, responses of epithelium-denuded preparations, to the initial histamine challenge were not significantly different from those obtained for epithelium-denuded control strips. Similarly, significant differences between the E_{max} to the initial histamine challenge in epithelium-intact preparations vs. the E_{max} for epithelium-denuded muscle strips were not observed (Table 3). In addition, histamine tachyphylaxis was not observed in epithelium-intact preparations taken from these patients (Table 3). In epithelium-denuded preparations obtained from the same patients, sequential histamine challenges tended to generate greater tension although these values were not significantly different (Fig. 1d, Table 3).

3.3. The effect of acute exposure to NSAIDs in vitro on histamine responses

3.3.1. Pretreatment with indomethacin

Indomethacin did not significantly alter resting tension in either epithelium-intact or denuded preparations. Pretreatment of epithelium-intact bronchial preparations with indomethacin prior to the generation of the first cumulative concentration-effect curve did not significantly modify the tachyphylactic response and the result was similar to that obtained with control epithelium-intact preparations. The mean E_{max} for the third histamine cumulative concentration-effect curve was reduced by 31% compared to the mean E_{max} for

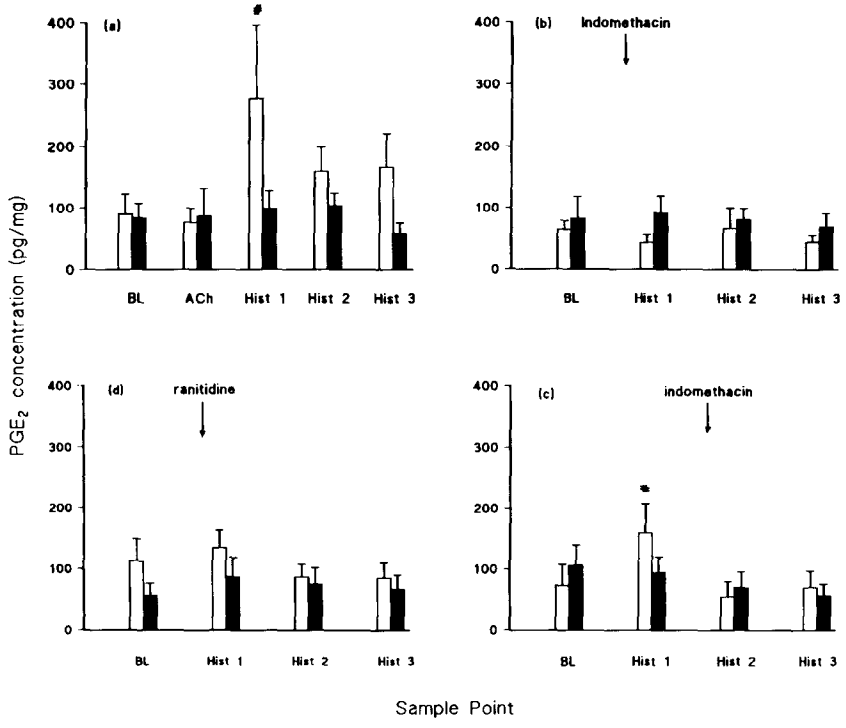

Fig. 2. Relationship between the generation of prostaglandin E_2 and histamine stimulation of epithelium-intact (open columns) and epithelium-denuded (black columns) human isolated bronchial smooth muscle in response to (a) acetylcholine and each of three histamine cumulative concentration-effect curves. (b) The histamine-induced generation of prostaglandin E_2 following indomethacin (5 μM) pretreatment. Indomethacin was added 30 min prior to the first histamine curve (↓). (c) The histamine-induced generation of prostaglandin E_2 following indomethacin (5 μM) post-treatment. Indomethacin was added 30 min prior to the second histamine curve (↓). (d) The histamine-induced generation of prostaglandin E_2 following ranitidine (60 μM) pretreatment. Ranitidine was added 30 min prior to the first histamine curve. #Generation of prostaglandin E_2 significantly greater than basal release, $P < 0.05$. BL = basal generation of prostaglandin E_2; ACh = prostaglandin E_2 measurement obtained at maximum response to acetylcholine; Hist 1, 2, 3 = prostaglandin E_2 measurement obtained at maximum response for each of three histamine cumulative concentration-effect curves. Results are expressed as mean ± S.E.M responses from bronchi obtained from eight donors.

cumulative concentration-effect curve 1 ($P < 0.02$; Fig. 1e) and the mean EC_{50} was increased 2.6-fold ($P < 0.02$; Table 2). This compared to the 1.86-fold increase observed for control preparations.

In contrast to the results obtained with epithelium-denuded control strips, addition of indomethacin to denuded strips prior to histamine exposure resulted in a significant reduction in maximum response to histamine. The mean E_{max} for the third histamine cumulative concentration-effect curve was reduced by 35% compared to that obtained for the first curve ($P < 0.02$; Fig. 1f). The mean pD_2 values also decreased after histamine exposure but the differences failed to reach statistical significance (Fig. 1f, Table 2).

3.3.2. Post-treatment with indomethacin

The addition of indomethacin to epithelium-intact preparations 60 min after the first curve (30 min prior to the second histamine cumulative concentration-effect curve), significantly inhibited the development of tachyphylaxis. The mean E_{max} and pD_2 values obtained for the second and third histamine curves were similar to the mean value obtained for the initial curve ($P > 0.375$; Fig. 1g, Table 2). Tachyphylaxis to repeated histamine exposure was not observed in similarly treated epithelium-denuded bronchial preparations ($P > 0.375$ for both E_{max} and pD_2; Fig. 1h, Table 2). Some of the experiments were repeated using flurbiprofen (5 μM) ($n = 3$) instead of indomethacin with similar results (data not shown).

3.4. Responses to acetylcholine

The mean maximum contractile responses to acetylcholine obtained at the completion of each experiment were 95.4 ± 8.3% of those obtained at the beginning. Similarly, the mean pD_2 values at the beginning and completion of each experiment were not significantly different suggesting that the responses obtained were not due to fatigue or to damage of the contractile elements of the muscle per se.

3.5. Prostaglandin release during tachyphylaxis

3.5.1. Prostaglandin E_2

Basal generation of prostaglandin E_2 was not significantly different between epithelium-intact and denuded preparations (89.31 ± 33.1 pg/mg and 83.76 ±

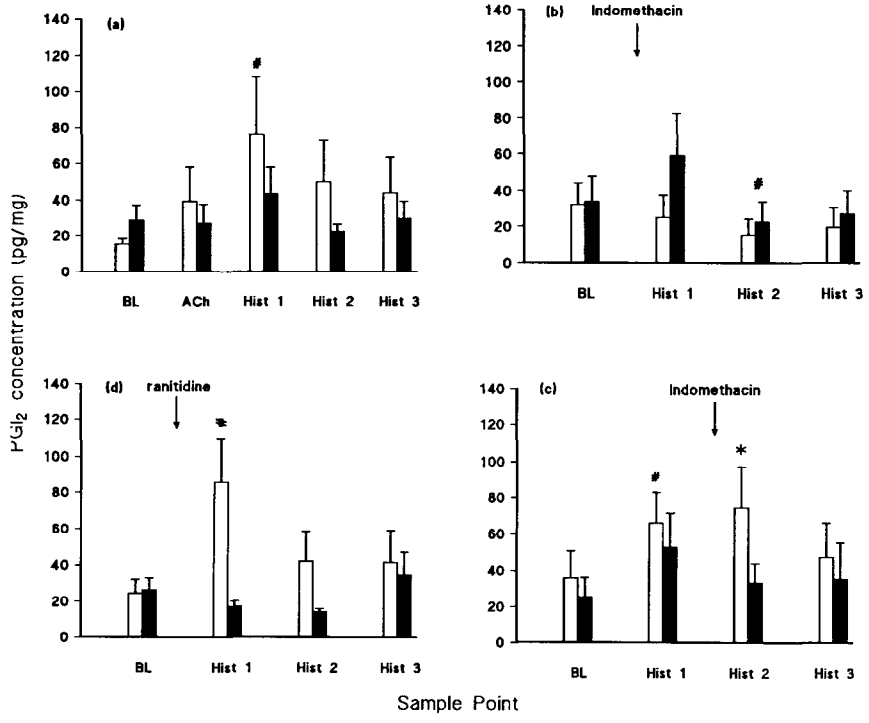

Fig. 3. Relationship between the generation of 6-keto-$PGF_{1\alpha}$ and histamine stimulation of epithelium-intact (open columns) and epithelium-denuded (black columns) human isolated bronchial smooth muscle in response to (a) acetylcholine and each of three histamine cumulative concentration-effect curves. (b) The histamine-induced generation of 6-keto-$PGF_{1\alpha}$ following indomethacin (5 μM) pretreatment. Indomethacin was added 30 min prior to the first histamine curve (↓). (c) The histamine-induced generation of 6-keto-$PGF_{1\alpha}$ following indomethacin (5 μM) post-treatment. Indomethacin was added 30 min prior to the second histamine curve (↓). (d) The histamine-induced generation of 6-keto-$PGF_{1\alpha}$ following ranitidine (60 μM) pretreatment. Ranitidine was added 30 min prior to the first histamine curve. #Generation of 6-keto-$PGF_{1\alpha}$ significantly greater than basal release, $P < 0.05$, * $P < 0.02$, ** $P < 0.01$. BL = basal generation of 6-keto-$PGF_{1\alpha}$; ACh = 6-keto-$PGF_{1\alpha}$ measurement obtained at maximum response to acetylcholine; Hist 1, 2, 3 = 6-keto-$PGF_{1\alpha}$ measurement obtained at maximum response for each of three histamine cumulative concentration-effect curves. Results are expressed as mean ± S.E.M. responses from bronchi obtained from eight donors.

22.35 pg/mg, respectively) (Fig. 2a). In epithelium-intact preparations, the initial histamine exposure resulted in a significantly enhanced mean production of prostaglandin E_2 ($P < 0.05$; Fig. 2a), which returned to within 10% of the initial basal generation following washout. Subsequent histamine challenges resulted in increased synthesis of prostaglandin E_2, but the concentrations measured were lower than those observed for the initial histamine challenge and did not differ significantly from baseline results. In epithelium-denuded muscle strips, repeated exposure to histamine failed to increase prostaglandin E_2 production at any stage (Fig. 2a).

The amount of prostaglandin E_2 generated by bronchial preparations at rest did not statistically correlate with their capacity to generate prostaglandin E_2 in response to histamine ($r = 0.68$, $P = 0.08$). Similarly, basal production of prostaglandin E_2 did not correlate with the magnitude of the reduction in E_{max} observed ($r = -0.29$, $P = 0.5$).

3.5.2. Prostacyclin

Mean basal production of the stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$ was approximately 6-fold less than prostaglandin E_2 production in the same epithelium-intact preparations ($P < 0.04$; Fig. 3a). The basal production of 6-keto-$PGF_{1\alpha}$ in epithelium-denuded strips was greater than that observed for the corresponding epithelium-intact preparations, although this difference did not reach statistical significance. As observed for prostaglandin E_2, the initial histamine exposure induced a significant augmentation in the generation of 6-keto-$PGF_{1\alpha}$ in epithelium-intact preparations ($P < 0.05$; Fig. 3a), but this was not observed with subsequent histamine challenges. In epithelium-denuded preparations, an increase in 6-keto-$PGF_{1\alpha}$ concentrations was detected following histamine exposure, but this failed to reach statistical significance (Fig. 3a).

Significant correlations between concentrations of 6-keto-$PGF_{1\alpha}$ generated at baseline and the production of this prostanoid from the same preparations in response to histamine ($r = 0.42$, $P = 0.34$) or between 6-keto-$PGF_{1\alpha}$ and the magnitude of reduction in E_{max} ($r = -0.07$ $P = 0.8$) were not observed.

3.6. The effect of indomethacin on prostaglandin production

3.6.1. Pretreatment with indomethacin

Basal generation of prostaglandin E_2 from epithelium-intact preparations prior to treatment with indomethacin did not differ from control epithelium-intact preparations ($P > 0.2$; Fig. 2b). Addition of indomethacin prior to the first histamine cumulative concentration-effect curve, abolished the histamine-stimulated generation of prostaglandin E_2 for each of the three histamine curves (Fig. 2b). In epithelium-denuded muscle strips, the basal generation of prostaglandin E_2 was unaffected by the addition of indomethacin, and did not increase following histamine stimulation (Fig. 2b).

Mean baseline production of 6-keto-$PGF_{1\alpha}$ in epithelium-intact strips was not statistically different from control preparations ($P > 0.1$; Fig. 3b). In these same preparations, indomethacin inhibited the histamine-induced release of 6-keto-$PGF_{1\alpha}$ (Fig. 3b), but in epithelium-denuded preparations, the degree of inhibition appeared less effective since the generation of 6-keto-$PGF_{1\alpha}$ tended to be only partially reduced.

3.6.2. Post-treatment with indomethacin

In epithelium-intact preparations, the histamine-induced generation of prostaglandin E_2 was significantly augmented at E_{max} for the initial cumulative concentration-effect curve ($P < 0.05$). However, following the addition of indomethacin, the concentration of prostaglandin E_2 at the E_{max} for subsequent histamine challenges fell dramatically such that significant differences from baseline values were not observed (Fig. 2c). In muscle strips denuded of the epithelium, the mean concentration of prostaglandin E_2 at baseline and following histamine stimulation were comparable to control values (Fig. 2c).

As observed for prostaglandin E_2, the concentration of 6-keto-$PGF_{1\alpha}$ was substantially increased following the initial histamine cumulative concentration-effect curve in both epithelium-intact and denuded preparations, although statistical significance was only observed for epithelium-intact preparations ($P < 0.02$; Fig. 3c). However, despite the presence of indomethacin, subsequent histamine exposure still significantly enhanced the production of 6-keto-$PGF_{1\alpha}$ from epithelium-intact preparations ($P < 0.05$; Fig. 3c).

3.7. The influence of histamine H_2 receptors on prostaglandin production

In epithelium-intact preparations, blockade of histamine H_2 receptors with ranitidine, prior to histamine exposure, significantly reduced the histamine-induced generation of prostaglandin E_2 (Fig. 2d) such that the concentration measured after the E_{max} for the first cumulative concentration-effect curve was not significantly different from baseline production ($P > 0.2$). In agreement with data obtained with control epithelium-denuded strips, the drug did not influence the generation of prostaglandin E_2 from epithelium-denuded preparations (Fig. 2d).

In contrast to the effects on prostaglandin E_2, ranitidine did not affect the histamine-induced generation of 6-keto-$PGF_{1\alpha}$ by epithelium-intact preparations (Fig.

3d). Thus, in the presence of the drug, the initial histamine stimulation induced a significant augmentation in the production of 6-keto-$PGF_{1\alpha}$ ($P < 0.05$).

3.8. The generation of prostaglandins in response to acetylcholine

Indomethacin did not alter responses to acetylcholine (10^{-3} M) and the mean concentration of prostaglandin E_2 and 6-keto-$PGF_{1\alpha}$ generated by both epithelium-intact and epithelium-denuded preparations following exposure to acetylcholine (10^{-3} M) were not significantly different from baseline concentrations (Fig. 1a, 2a).

4. Discussion

Tachyphylaxis to repeated histamine challenge in human isolated bronchial smooth muscle has been previously demonstrated in our laboratory and shown to be both epithelium and histamine H_2 receptor-dependent (Knight et al., 1992). In the current study, these earlier observations were confirmed and, more importantly, that tachyphylaxis was associated with the epithelial generation of prostaglandin E_2 and prostacyclin. As such, it seems likely that the generation of these prostanoids is involved in the modulation of histamine responses and the subsequent development of tachyphylaxis to histamine in human airways.

The profile of prostaglandins generated by airway tissue is dependent on the species and experimental conditions used. In passively sensitised human lung and bronchi (Schulman et al., 1982) as well as in histamine-stimulated canine trachea (Shore et al., 1985), the stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$ has been reported to be the predominant cyclooxygenase metabolite released, with significantly smaller amounts of prostaglandin E_2 being detected. In contrast, in the present study, prostaglandin E_2 was demonstrated to be the primary cyclooxygenase metabolite released by human airways in vitro, although the production of smaller amounts of 6-keto-$PGF_{1\alpha}$ was also observed.

The reduction in contractile responses to histamine, in concert with the augmented generation of prostaglandin E_2 and prostacyclin, strongly suggests that these mediators may influence airway muscle responses to histamine. In contrast to these observations, Haye-Legrand et al. (1986) and Cerrina et al. (1989) although not examining histamine tachyphylaxis per se, also described significant increases in prostaglandin E_2 generation by bronchial preparations following histamine exposure, but concluded that this prostaglandin did not alter muscle responses to a subsequent histamine challenge.

The reasons for the dissimilarities between our data and those reported earlier are not clear. The maximum histamine-induced generation of prostaglandin E_2 detected in the study of Haye-Legrand et al. (1986) was similar to the basal generation of prostaglandin E_2 measured in the present investigation. In the present study and in those of Haye-Legrand et al. (1986) and of Cerrina et al. (1989), the quantity of generated prostanoid was normalised for the amount of tissue and as such, differences in tissue mass are unlikely to have influenced the results. However, this does not preclude the existence of regional differences in the generation of prostanoids in relation to airway size (Stuart-Smith and Vanhoutte, 1988a). The bronchial preparations used in the current investigation were all less than 6 mm internal diameter whereas in previous investigations (Haye-Legrand et al., 1986; Cerrina et al., 1989), data from both large and small airways were pooled. In addition, it is possible that pre-operative medications may have influenced previously reported results. The current study clearly demonstrates that certain medications such as NSAIDs may have profound effects on airway muscle responsiveness to histamine.

Another possible and important factor contributing to the lower amounts of prostaglandin E_2 detected in previous studies may relate to the radioimmunoassay methodology. Prostaglandin E_2 is highly labile, and in blood at least is rapidly degraded (Granstrom et al., 1980). The radioimmunoassay protocol used in the present study utilised a procedure which unified the metabolites of prostaglandin E_2 into one measurable compound (11-deoxy-13,14-dihydro-15-keto-11β,16ϵ cyclo-prostaglandin E_2) whereas previously (Haye-Legrand et al., 1986; Cerrina et al., 1989), antibodies to native prostaglandin E_2 were used.

Indomethacin has been shown to prevent tachyphylaxis to repeated histamine challenge in patients with mild asthma (Manning et al., 1987), supporting a role for inhibitory prostanoids. In the present study, similar findings were demonstrated using bronchial preparations obtained from patients receiving chronic NSAID therapy in vivo. Both epithelium-intact and epithelium-denuded bronchial preparations from these patients failed to demonstrate tachyphylaxis to repeated histamine challenge. Similarly, in muscle strips from untreated patients, indomethacin added in vitro also prevented histamine tachyphylaxis in epithelium-intact bronchial preparations. However this was only observed when the drug was added following an initial histamine challenge (post-treatment). The reasons for this observation are presently unknown, although similar findings have also been reported in canine airways in vitro and in vivo (Antol et al., 1988). That this effect was not modified by repeated washing, initially suggested that under resting conditions, cyclooxygenase

activity is not inhibited by indomethacin. However, indomethacin pretreatment (i.e. added prior to histamine exposure), suppressed the histamine-induced generation of prostaglandin E_2 and prostacyclin from epithelium-intact preparations. Since indomethacin added after the initial histamine challenge blocked the development of histamine tachyphylaxis and also inhibited prostanoid synthesis, the data suggest that in addition to the production of prostaglandin E_2 and prostacyclin either muscle contraction or alternatively, histamine exposure, is necessary for this phenomenon to occur.

Antol et al. (1988) speculated that receptor uncoupling or down-regulation maybe responsible for the prostanoid-independent inhibition of histamine responses. In support of this suggestion, recent studies by Leurs et al. (1991) using the guinea-pig ileum have demonstrated that histamine H_1 receptors may uncouple from the intracellular second messenger. Whether prostanoids are also involved in the uncoupling or other desensitising mechanisms of histamine H_1 receptors remains unknown.

Alternatively, it is conceivable that other mediators derived from the epithelium or muscle may be involved. It is now recognised that two isoforms of the prostaglandin H endoperoxide synthetase (PGHS) enzyme exist: a constitutive form (PGHS-1) and an inducible form (PGHS-2) (Holtzman et al., 1992; Masferrer et al., 1992; Meade et al., 1993). In some systems PGHS-2, when exposed to NSAIDs, may metabolise arachidonic acid to 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) (Holtzman, 1992; Meade et al., 1993). This mediator is the major arachidonic acid metabolite produced by human respiratory epithelium (Holtzman et al., 1991), however its effects in the airways remain unknown. In addition, indomethacin appears to only inhibit PGHS-1, not that which is induced by inflammatory mediators (Meade et al., 1993). Hence, it is possible that the unregulated expression and activity of PGHS-2, following exposure to histamine may produce mediators which regulate airway muscle tone.

The histamine-induced generation of prostacyclin from epithelium-intact preparations appeared to be relatively unaffected by indomethacin, particularly when the drug was added after the initial histamine exposure (post-treatment). This observation is supported by data from other in vitro and in vivo studies (Shephard et al., 1985; Shaik et al., 1980). However, since functional tachyphylaxis to histamine was not observed following post-treatment with indomethacin, the data suggest that prostacyclin plays a limited role in mediating human bronchial smooth muscle responses to histamine.

Long term exposure to NSAIDs caused a significant augmentation in the initial responsiveness to histamine in epithelium-intact bronchial preparations, suggesting that the generation of inhibitory prostanoids had been reduced. Similar results have been reported in canine airways (Shore et al., 1985). However, both in the current investigation and in canine trachea (Antol et al., 1988), pretreatment with indomethacin resulted in an apparent tachyphylaxis to histamine in epithelium-denuded airway preparations. Together these data raise the possibility that following histamine stimulation, the epithelium-denuded airway is capable of generating predominantly contractile prostaglandins (in contrast to the epithelium which generates inhibitory prostanoids) and that these are inhibited by indomethacin.

The cellular mechanisms by which histamine induces prostanoid release are unknown. Whilst the design of the current study did not specifically address this question, it has been demonstrated that the release of prostaglandins was not enhanced by acetylcholine. This suggests that increased production of prostaglandin E_2 and prostacyclin in human airways, may be specifically related to stimulation of histamine receptors. Similar results have been obtained in canine (Stuart-Smith and Vanhoutte, 1988b) and human airways (Dunlop and Smith, 1976). However, it has also been reported (Schulman et al., 1982; Steel et al., 1979) that prostaglandin generation occurs non-specifically in response to muscle contraction. However, in these latter studies, the amounts of prostanoids generated by agonists such as methacholine accounts for only a relatively small fraction of that which the tissue is capable of producing (Adkinson et al., 1983). Furthermore, the current data and the results of other studies (Walters et al., 1984) suggest that prostaglandins do not appear to affect airway smooth muscle responses to exogenous acetylcholine. More importantly, since tachyphylaxis to acetylcholine was not observed, it appears that the modulatory role of prostaglandins may relate to a cellular event specific to a limited number of mediators including histamine.

Significantly, the specific histamine H_2 receptor antagonist, ranitidine, selectively blocked the histamine-induced release of prostaglandin E_2, but not prostacyclin. Thus, prostaglandin E_2 generation appears to be coupled to the stimulation of histamine H_2 receptors. This finding is in general agreement with results previously reported for guinea-pig trachea (Yen et al., 1976) and it now seems likely that prostaglandin release may be selectively controlled by different histamine receptor subtypes. In further support of this, a recent study using human umbilical vein endothelial cells has recently demonstrated that prostacyclin release is selectively controlled by histamine H_1 receptors (Bull et al., 1992).

These data highlight that the long term administration of preoperative medications and in particular NSAIDs significantly impact on airway smooth muscle

responses and thus should be noted when using resected human tissue in experimental procedures and furthermore may be relevant to physiological responses observed both in vivo and in vitro. More importantly, although the regulation of airway smooth muscle contraction by the epithelium and the role of prostanoids remains complex, it seems likely that prostaglandin E_2 and prostacyclin are generated by human bronchial epithelium as a consequence of histamine exposure and play a role in the development of histamine tachyphylaxis. The generation of prostaglandin E_2 in response to stimulation of histamine H_2 receptors serves to highlight the different roles that histamine receptor subtypes may play in human airways.

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