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Airway epithelium modulates the reactivity of guinea-pig respiratory smooth muscle [1]

Douglas W.P. Hay [2], Stephen G. Farmer, David Raeburn, Victor A. Robinson, William W. Fleming * and Jeffrey S. Fedan [3]

*Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, 944 Chestnut Ridge Road, and * Department of Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV 26505, U.S.A.*

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The influence of the epithelium on the reactivity of guinea-pig tracheal smooth muscle in vitro was investigated. Mechanical removal of the epithelium produced contrasting effects on methacholine-, histamine- and K^+-induced contractions of tracheal strips. Epithelium removal resulted in 2.1-fold shifts to the left of histamine and methacholine concentration-response curves, and an increase in the maximum response to histamine; the maximum response to methacholine was unaffected. Epithelium removal had little effect on the sensitivity, but decreased the maximum response to K^+. The results suggest that inhibitory and excitatory factor(s) are released from epithelial cells, and that these factors modulate the reactivity of the smooth muscle. Indomethacin (1 μM) produced in epithelium-containing preparations qualitatively identical effects on the sensitivity to methacholine and on the maximum response to histamine as removal of the epithelium. However, indomethacin was without effect on the sensitivity to histamine in the presence or absence of the epithelium. Alterations in the production or release of epithelial cell-derived factors may contribute to the airway hyperreactivity observed in respiratory disorders.

Airway smooth muscle; Reactivity; Airway epithelium; Indomethacin; Trachea; (Guinea-pig)

1. Introduction

It has been reported recently that mechanical removal of the airway epithelial layer augments histamine-, methacholine-, acetylcholine-induced and neurogenically induced contractile responses of the smooth muscle of isolated airway preparations from the dog (Flavahan et al., 1985), bovine (Barnes et al., 1985) and rabbit (Raeburn et al., 1986). The relaxant effect of isoproterenol (Flavahan et al., 1985) and the antagonistic action of verapamil (Raeburn et al., 1986) also have been demonstrated to be reduced in dog and rabbit airways devoid of epithelium. These findings suggest that an inhibitory factor, released from airway epithelial cells, may interact with the underlying smooth muscle to modulate its reactivity.

Recent evidence indicates that the epithelial cells lining the respiratory tract may be involved in the pathophysiology of asthma, insofar as epithelial cell loss or damage accompanies airway hyperreactivity in asthmatics (Laitinen et al., 1985).

The main purpose of this study was to examine the influence of the epithelium on the reactivity of

[1] Mention of brand name does not constitute product endorsement.

[2] Present address: Smith Kline and French Laboratories, Department of Pharmacology, P.O. Box 7929, Spring Garden Street, Philadelphia, PA 19101, U.S.A.

[3] To whom all correspondence should be addressed: Physiology Section, NIOSH, 944 Chestnut Ridge Road, Morgantown, WV 26505, U.S.A.

the smooth muscle of the guinea-pig isolated trachea. The guinea-pig was chosen because of its wide use in models of airway hyperreactivity, and to compare the role of the epithelium in guinea-pig airways with that reported in canine, bovine and rabbit airways. In addition, the effects of the cyclooxygenase inhibitor, indomethacin (Vane, 1971), on responses of preparations with and without epithelium also were examined for the following reasons: (1) cultured airway epithelial cells are capable to releasing prostanoids (Leikauf et al., 1985); (2) prostaglandins cause bronchoconstriction, bronchodilation, are involved in the maintenance of basal tone, and modulate the release of neurotransmitters (Fanburg, 1973; Orehek et al., 1975; Coleman and Kennedy, 1980; Brink et al., 1981; Hadhazy et al., 1982; Walters et al., 1984); and (3) aspirin, another cyclooxygenase inhibitor, can induce an attack in a small proportion of asthmatics (Szczeklik, 1982).

A preliminary account of this work has appeared (Hay et al., 1985).

2. Materials and methods

2.1. Preparation of tissues

Male English short-hair guinea-pigs (340-780 g; Camm Research Institute, Inc., Wayne, NJ) were killed by cervical dislocation and bled. The trachea was removed, placed in a modified Krebs-Henseleit solution and cleaned. The trachea was slit through the cartilage along its longitudinal axis, and strips consisting of two adjacent cartilage rings were prepared. The strips were placed in 10 ml water-jacketed organ baths (37°C) containing Krebs-Henseleit solution, and tied to force-displacement transducers for the measurement of isometric tension responses. The tissues were equilibrated for 1 h under an optimum resting load of 1 g, and washed every 15 min before the start of each experiment. The modified Krebs-Henseleit solution contained (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.7. The solution was gassed with 95% O_2-5% CO_2 to give a pH of 7.4 at 37°C.

The epithelium was removed by gently rubbing the luminal surface with a cotton-tipped applicator. To determine the effectiveness of this procedure, at the end of each experiment sample control and rubbed tissues were examined using light microscopy (hematoxylin and eosin staining). To avoid possible differences in reactivity due to regional variations, adjacent strips from the same area of trachea were used, i.e. one intact and one rubbed. The area of trachea used was randomized with respect to the agonist studied. This method permits paired analysis between epithelium-containing and epithelium-free preparations from the same animal.

2.2. Concentration-response curves

Concentration-response curves for KCl, histamine and methacholine were constructed from responses to cumulative additions of these agents. Only one concentration-response curve was obtained from each preparation.

Where appropriate, indomethacin was added 40-45 min before, and remained present during, concentration-response determinations. It was observed that indomethacin relaxed the preparations under resting tension (vide infra). Therefore, when calculating the effects of indomethacin on the maximum response to an agent, the amount of this relaxation was subtracted from the total developed tension to obtain the corrected value for the maximum contraction developed from the original baseline.

2.3. Statistical analysis

The results were expressed as % maximum contraction and g developed tension and are given as means ± S.E.; n is the number of separate experiments. Geometric mean EC_{50} values (the concentration producing 50% of the maximum response) were determined from linear regression analysis of probit-transformed data. The data were evaluated for differences using Student's t-test for paired samples; the 0.05 level of probability was considered significant. In the figures the S.E. is shown by the vertical bars, unless enclosed within the symbol.

2.4. Drugs and solutions

The drugs used were histamine diphosphate, methacholine chloride (Sigma Chemical Co., St. Louis, MO, USA) and indomethacin (Merck, Sharp and Dohme, West Point, PA, USA).

Ethanol, the solvent for indomethacin, in a final bath concentration of ca. 0.1%, was without effect on baseline tension or methacholine, histamine and KCl concentration-response curves in both intact or rubbed preparations (n = 3-13).

3. Results

3.1. Histological examination of tissues

Histological sections showed that the epithelium was removed by rubbing the luminal surface with a cotton-tipped applicator (fig. 1). There was no noticeable damage to the mucosal and muscle layers.

3.2. Lack of effect of epithelium removal on tissue mechanical properties

To determine whether removal of the epithelial cell layer affected the mechanical properties of the tissue, responses to nearly equieffective concentrations (vide infra) of methacholine (2 μM in intact tissues, 1 μM in rubbed tissues, EC_{60} values) were evaluated in preparations placed under progressively increasing resting loads (0.25-4 g, n = 5-6). There was no qualitative or quantitative difference in the applied load vs. developed tension relationship between intact and rubbed tissues (data not shown). The applied 1 g initial load used in these experiments was optimal for the development of contractile responses of tissues both containing or lacking the epithelium.

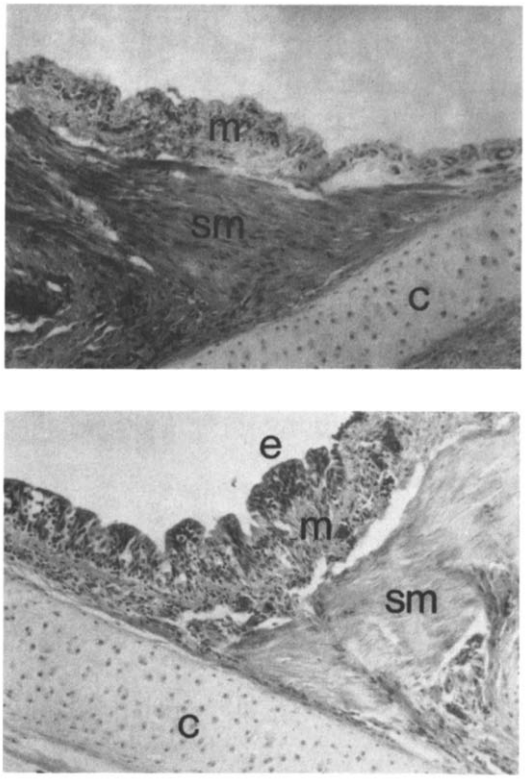

Fig. 1. Histological sections (5 μm; hematoxylin and eosin staining; ×240 magnification) of guinea-pig tracheal strips demonstrating that the epithelial cell layer was removed by rubbing the luminal surface with a cotton-tipped applicator (top panel). An unrubbed, adjacent tracheal strip is shown in the bottom panel. E: epithelium, M: mucosa, SM: smooth muscle, C: cartilage.

TABLE 1

EC_{50} values for KCl, histamine and methacholine in intact and rubbed guinea-pig tracheal strips.

Agent	n	$-\log EC_{50}$ (M)		Maximum contraction (g)	
		+ Epithelium	− Epithelium	+ Epithelium	− Epithelium
KCl	8	1.85 ± 0.02	1.94 ± 0.05	0.80 ± 0.15	0.55 ± 0.08
Histamine	14	5.37 ± 0.06	5.70 ± 0.05 [a]	0.81 ± 0.06	1.00 ± 0.09 [a]
Methacholine	19	5.94 ± 0.04	6.25 ± 0.05 [a]	1.10 ± 0.08	0.96 ± 0.12

[a] Significantly different from + Epithelium.

3.3. Effects of epithelium removal on concentration-response relationships

The removal of the epithelial layer produced several, agent-specific effects on contractile responses of tracheal strips. Although epithelium removal increased responses of the preparations to low concentrations of KCl, there was no effect on the EC_{50} value (fig. 2A, table 1). In contrast, significant increases in sensitivity to methacholine and histamine (ca. 2- to 2.1-fold) occurred in rubbed strips (fig. 2C,E, table 1).

After epithelium removal, the maximum contractile response to histamine was increased (fig. 2D), whereas the maximum response to methacholine was unaffected (fig. 2F). In rubbed preparations there was a marked decrease (20-67%) in the maximum response to KCl in 5 of 8 animals, and an increase (8-38%, n = 3) in the remaining tissues; consequently, the differences in the mean

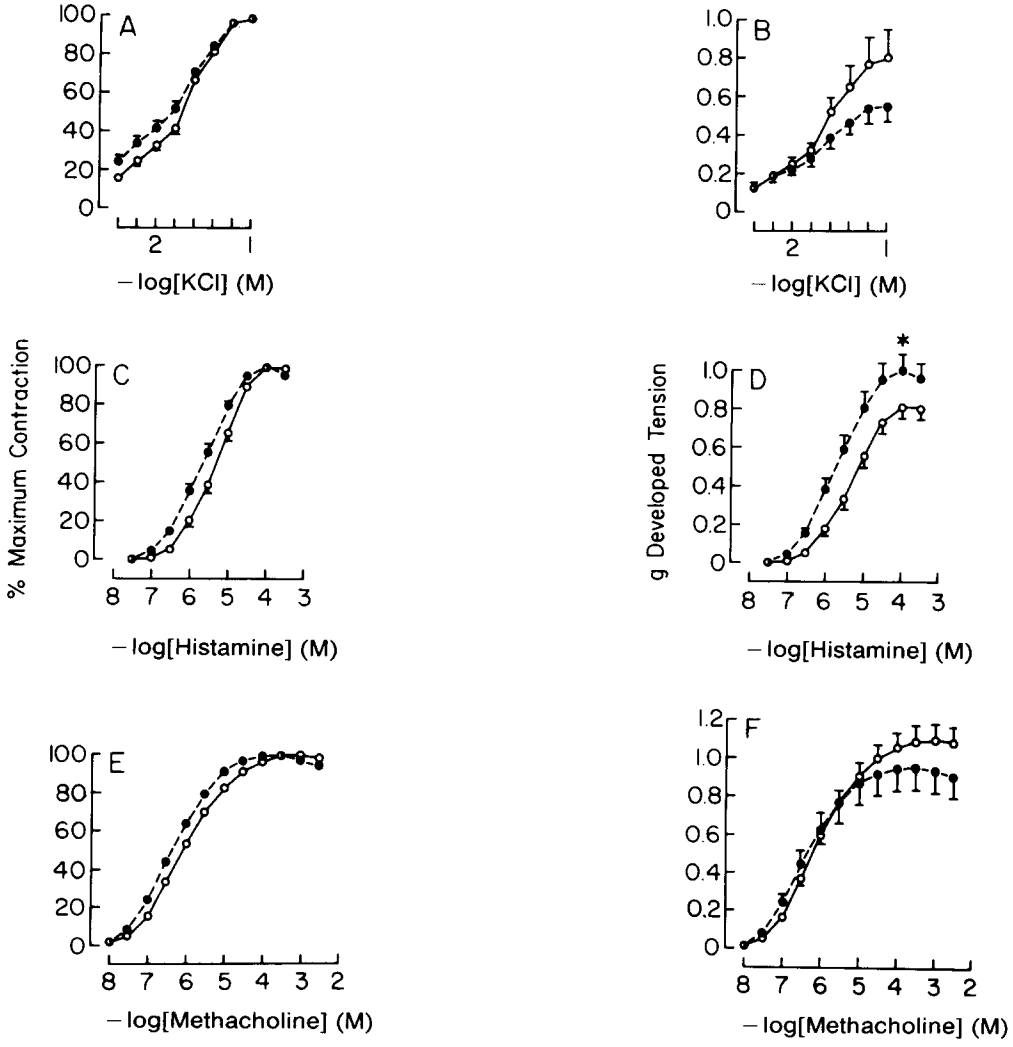

Fig. 2. Concentration-response curves for KCl (A,B), histamine (C,D) and methacholine (E,F) in the presence (open symbols, solid line) and absence (filled symbols, dashed line) of epithelium. Results are expressed as % maximum response (A,C and E) and g developed tension (B,D and F). n = 8 (A,B), n = 14 (C,D), n = 19 (E,F).

maximum responses between unrubbed and rubbed tissues (fig. 2B), while quite large, failed to achieve statistical significance.

3.4. Effects of indomethacin on concentration-response relationships

In the presence and absence of the epithelium, indomethacin (1 μM) produced a slowly developing and substantial decrease in baseline tension. The relaxation of rubbed preparations was significantly smaller than that of unrubbed strips (0.41 $\pm$ 0.04 vs. 0.28 $\pm$ 0.04 g for intact and rubbed preparations, respectively; n = 18) and developed significantly faster (time to 50% relaxation: 17.0 $\pm$ 2.0 vs. 7.9 $\pm$ 1.1 min for intact and rubbed preparations, respectively; n = 18).

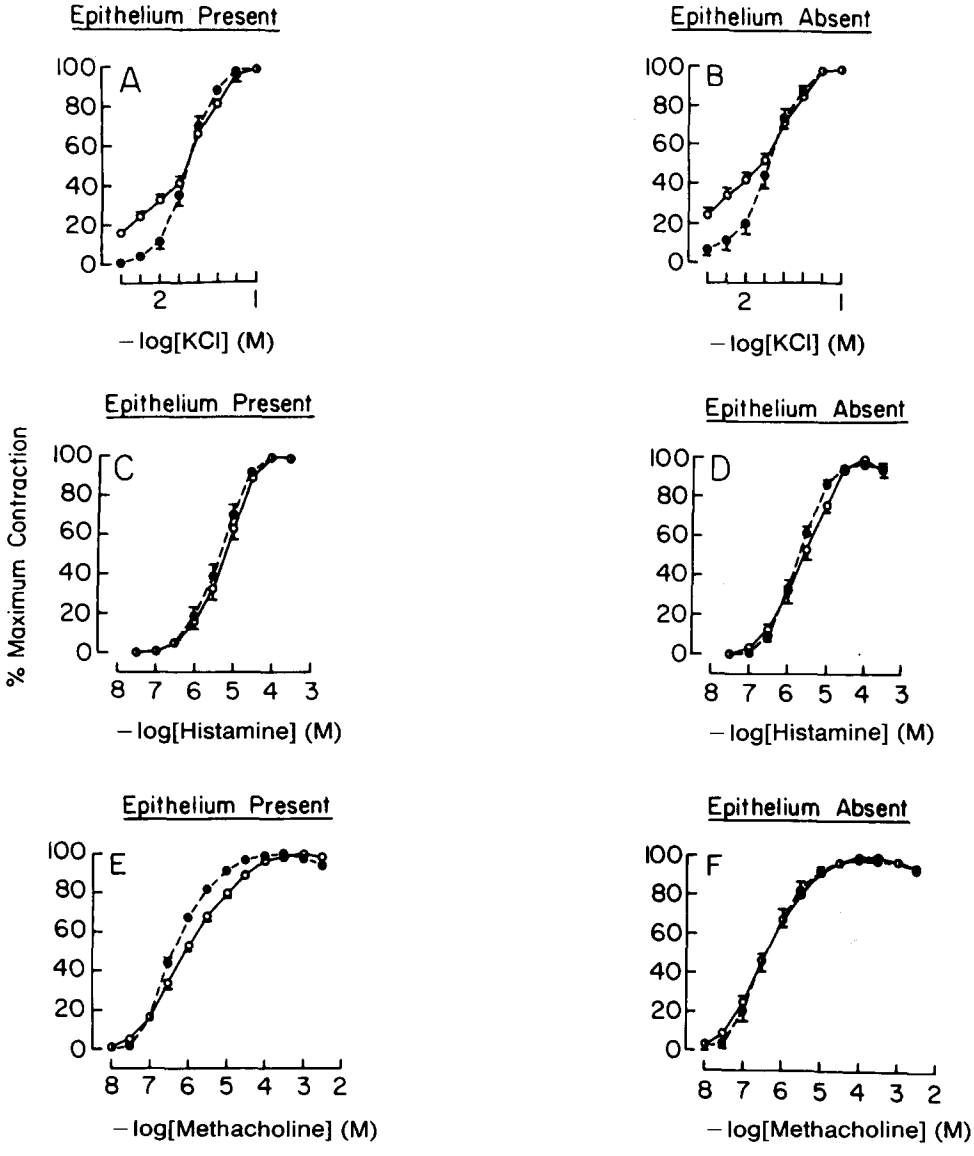

Fig. 3. Effects of indomethacin (1 μM) on KCl, histamine and methacholine concentration-response curves in the presence (A,C,E) and absence (B,D,F) of the epithelium. Results are expressed as % maximum response. n = 7 (A,B), n = 6-7 (C,D), n = 6-8 (E,F). Open symbols: control; closed symbols: + indomethacin.

In the presence and absence of the epithelium responses to low concentrations of KCl were reduced by indomethacin (fig. 3A,B). The KCl EC_{50} value of unrubbed preparations was decreased significantly by indomethacin, but the value for rubbed strips was not affected (table 2). Indomethacin increased significantly the maximum response to KCl in the absence of the epithelium, but not in the presence of the epithelium (table 2). These effects of indomethacin, which are different from the effects of epithelium removal, suggest that a prostanoid may be involved in the response to KCl via mechanisms that are epithelium-dependent and epithelium-independent.

The sensitivities of both intact and rubbed preparations to histamine were unaffected by indomethacin (fig. 3C,D, table 2). However, in the presence of indomethacin, the maximum contractile response of epithelium-containing tissues to histamine was increased, an effect which was not evident in tissues lacking the epithelium (table 2).

Indomethacin increased the sensitivity of intact strips to methacholine but was without effect on the sensitivity of rubbed preparations (fig. 3E,F, table 2), and it did not modify the maximum response of intact or rubbed preparations to methacholine (table 2). Thus, with respect to the sensitivity of the preparations to methacholine and to the maximum response to histamine, the effects of epithelium removal, and of indomethacin in intact preparations, were similar.

4. Discussion

The results indicate that the epithelial cell layer modulates the reactivity of guinea-pig isolated airway smooth muscle. This finding is in accord with other studies on canine, bovine and rabbit airways (see Introduction). The qualitatively different effects of epithelium removal on responses to histamine, methacholine and KCl suggest that the alterations in reactivity are not due to removal of a diffusion barrier, or to damage of the smooth muscle. Contractile responses to the agents were quantitated when the plateau was reached, i.e. at a time when equilibrium had been established between a given concentration of agent and the response of the muscle. Moreover, the applied load vs. developed tension data indicate that the effect of epithelium removal is not attributable to alterations in the mechanical properties of the tissue.

Our findings suggest that an inhibitory factor(s) and, perhaps in the case of KCl, an excitatory factor(s), is released from epithelial cells to modulate the reactivity of the underlying smooth muscle. It cannot be determined from the present experiments whether the factor(s) is produced and released continually, or only in response to the stimulant agents.

The results of this study and similar studies in other species (vide supra) suggest that the involvement of the epithelium in the modulation of air-

TABLE 2

Effects of indomethacin (1 μM) on EC_{50} values and maximum responses to KCl, histamine and methacholine in intact and rubbed guinea-pig tracheal strips.

Agonist	n	$-\log EC_{50}$ (M)		Maximum contraction (g)	
		Control	+ Indomethacin	Control	+ Indomethacin
+ Epithelium					
KCl	7	1.83 ± 0.03	1.73 ± 0.04 [a]	0.80 ± 0.15	0.93 ± 0.21
Histamine	6	5.27 ± 0.06	5.38 ± 0.10	0.80 ± 0.10	1.14 ± 0.14 [a]
Methacholine	8	5.92 ± 0.07	6.12 ± 0.03 [a]	1.10 ± 0.15	1.00 ± 0.14
− Epithelium					
KCl	7	1.93 ± 0.06	1.81 ± 0.05	0.52 ± 0.09	0.84 ± 0.13 [a]
Histamine	7	5.64 ± 0.09	5.68 ± 0.06	1.04 ± 0.15	0.93 ± 0.14
Methacholine	6	6.33 ± 0.04	6.24 ± 0.15	0.84 ± 0.12	0.73 ± 0.13

[a] Significantly different from controls.

way smooth muscle reactivity may be a general feature of the mammalian respiratory tract.

There are both qualitative and quantitative differences in the results obtained in the present study compared with those in dog (Flavahan et al., 1985) and bovine airways (Barnes et al., 1985). For example, epithelium removal from dog bronchus resulted in an approximately 4-fold increase in sensitivity to acetylcholine and histamine (Flavahan et al., 1985) compared with the approximately 2-fold increase observed with methacholine and histamine in this study. Furthermore, there was no increase in the maximum response to histamine in dog bronchus stripped of the epithelium (Flavahan et al., 1985). Although the contrasting results obtained may be explained by species differences, another possibility is that it reflects an anatomical difference, as the two studies examined different airway generations. A previous study from our laboratory using rabbit airways indicated that the effects of removal of the epithelium on smooth muscle reactivity exhibit marked regional variation (Raeburn et al., 1986). In bovine trachea there was an increase in the maximum response produced by acetylcholine in epithelium-free preparations (Barnes et al., 1985), an effect not observed using guinea-pig trachea.

Since submission of the present paper, there have been two other publications on the influence of the epithelium on the reactivity of guinea-pig trachea (Goldie et al., 1986; Holroyde, 1986), the results of which differ in a number of respects from our findings. For example, in the study by Goldie et al. (1986), epithelium removal had no influence on responses produced by cholinergic agonists, or on the maximum response to histamine. Goldie et al. (1986) did, however, observe a greater increase in sensitivity to histamine than that obtained in our investigation. These differences are surprising, but could reflect the fact that different strains of guinea-pig from different suppliers have been used. Holroyde (1986) reported that there was an increase in sensitivity to histamine, acetylcholine and 5-hydroxytryptamine, and to the relaxant agents adenosine and isoproterenol, in preparations lacking the epithelium; there was no effect of epithelium removal on the maximum response produced by the contractile agents. Holroyde (1986) concluded that the effects of epithelium removal are due to the loss of a diffusion barrier, an epithelial cell-derived factor(s) not being involved. However, we have shown recently that loss of a diffusion barrier cannot be invoked to explain several effects seen after epithelium removal. The reactivity of intact and rubbed strips of rabbit trachealis to contractile agents (i.e. methacholine, KCl) was identical (Raeburn et al., 1986). The relaxation of guinea-pig tracheal preparations produced by papaverine, salbutamol or isoproterenol (in the presence of corticosterone to inhibit epithelial extraneuronal catecholamine uptake; Hay et al., 1986) is not affected by epithelium removal (Hay et al., 1986).

Comparisons of the effects of epithelium removal vis à vis the effect of indomethacin in intact airway preparations have led to conflicting results. In contrast to the reported lack of effect of indomethacin on acetylcholine-induced responses of bovine airway smooth muscle (Barnes et al., 1985), in the present study indomethacin increased both the sensitivity to methacholine and also the maximum response to histamine only in those preparations containing the epithelium. On the other hand, indomethacin did not affect the sensitivity of the guinea-pig preparations to histamine in the presence or absence of the epithelium. Thus, indomethacin to some degree mimics the effects of epithelium removal. This perhaps indicates that more than one factor may be released from the epithelium, and/or that its modulatory influence will depend on the stimulus being applied. Holroyde (1986) reported that there was no difference in the effects of indomethacin on both the sensitivity and the maximum contraction to histamine in epithelium-containing and epithelium-free preparations of guinea-pig trachea. In contrast, indomethacin increased the sensitivity of dog bronchus to methacholine in the presence but not the absence of the epithelium (Flavahan et al., 1985); these investigators did not comment on the effect of indomethacin on the maximum response, and did not examine the effect of indomethacin on histamine-induced responses.

The marked reduction in baseline produced by indomethacin confirms previous findings in the

guinea-pig trachea (Orehek et al., 1975; Brink et al., 1981), and contrasts with the lack of effect of indomethacin on baseline tension in dog trachea (Hadhazy et al., 1982) or in human bronchi (Brink et al., 1980; Raeburn, 1984). The significantly smaller indomethacin-induced relaxation in epithelium-free compared with epithelium-containing preparations perhaps indicates that some of the cyclooxygenase products involved in the maintenance of intrinsic tone are synthesized by, and released from epithelial cells. Furthermore, the much faster development of relaxation produced by indomethacin in rubbed preparations may be due to the loss of a diffusion barrier to sub-epithelial sites of prostanoid synthesis.

Our findings suggest, at least in guinea-pig airways, that the factor(s) involved in modulating the sensitivity to methacholine and the maximum response to histamine may be a prostanoid, or, alternatively, that the synthesis or release of this factor is regulated by a prostanoid. Some evidence to support this suggestion comes from the findings that epithelial cells from dog and human airways cultured in vitro release prostanoids under basal conditions, and that this release is increased by bradykinin (Leikauf et al., 1985). The interpretation of this finding must be tempered by the acknowledged differences in the actions of prostaglandins in different animal species.

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