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A possible role of airway epithelium in modulating hyperresponsiveness

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1 In order to examine the role of airway epithelium in the responsiveness of smooth muscle in man, we measured the contractile responses to acetylcholine (ACh), histamine, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and the relaxation response to isoprenaline (Isop), in 48 bronchi obtained from 10 patients who received surgery. Responses were measured in the presence and absence of the epithelium.

2 Removal of epithelium (by rubbing the mucosa gently with forceps) significantly increased the contractile responses evoked by ACh, histamine and $PGF_{2\alpha}$.

3 In contrast, removal of epithelium did not alter the relaxation response to Isop.

4 To clarify the mechanism underlying this epithelial inhibitory effect on smooth muscle contraction, we measured the contractile responses of dog trachea with the epithelium removed to increasing concentrations of ACh. After measuring the control response, we added about 0.1 g of the chopped epithelium in the organ chamber, and measured the response again.

5 After adding airway epithelium and incubating with tracheal strips, the contractile response of tracheal strips decreased significantly as compared to the control response.

6 These results show that airway epithelium possesses the ability to decrease the smooth muscle contraction to ACh, histamine and $PGF_{2\alpha}$ in man and dogs.

7 The mechanism of this inhibitory effect of the airway epithelium is not explained by a change in mechanical property of the airway nor the change in diffusion of these drugs to the smooth muscle across the epithelium. Thus, these results suggest that airway epithelium may have an important role in modulating smooth muscle tone, possibly by inactivation of these mediators, or by releasing an epithelium-derived relaxing factor.

Introduction

Airway hyperresponsiveness to a wide variety of stimuli is a characteristic feature of asthma. However, the precise underlying mechanisms of hyperresponsiveness remains uncertain (Boushey *et al.*, 1980; Ramsdale & Hargreave, 1986). It has been reported that airway hyperresponsiveness can be induced following epithelial damage in animals (Fabbri *et al.*, 1984) and in man (Seltzer *et al.*, 1986). One of the possible mechanisms of airway hyperresponsiveness after epithelial damage may be closely linked to airway inflammation (O'Byrne, 1986; Chung, 1986). Recently, it was demonstrated that the removal of airway epithelium increases the responsiveness of smooth muscle in canine and bovine airway, suggesting that airway epithelium may be involved in the inhibitory effect on smooth muscle responses (Flavahan *et al.*, 1985; Barnes *et al.*, 1985). It is possible that airway hyperresponsiveness may be induced when this inhibitory effect is lost. In the vascular system, it is well established that the relaxation of smooth muscle evoked by acetylcholine (ACh) is dependent on the vascular endothelium, and this inhibitory effect is due to an endothelium-derived relaxing factor (EDRF) (Furchgott, 1983; Furchgott *et al.*, 1984). However, little is known about the inhibitory effect of airway epithelium. The first aim of the present study was to determine whether human airway epithelium possesses the ability to decrease the responsiveness of smooth muscle. The second aim was to clarify how airway epithelium may decrease the smooth muscle response in canine trachea.

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Methods

Experiments with human bronchial rings

Forty eight lobar or segmental bronchial rings were removed from 10 patients with lung cancer at the time of surgical operation (4 men and 6 women, mean age 60 years old). We used 2 bronchial rings resected from the same bronchus as a pair in this series of experiments. The epithelium of one of them was removed by gentle rubbing with forceps. Each set of bronchial rings was suspended vertically in the organ chamber filled with 20 ml of Krebs-Henseleit salt solution, bubbled with 95%O_2:5%CO_2 constantly and maintained at 37°C. Krebs-Henseleit salt solution contained (mM):NaCl 118, KCl 5.9, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.6. Isometric tension was measured with a force-displacement transducer (Nihon Kohden, TB-611T) attached to the one end of the bronchial ring, and recorded continuously on a pen recorder (Nihon Kohden, WT-687G). The strips were allowed to equilibrate for 1 h under a resting tension of 2 g, which was found to be optimum for induced contractions in such tissues (Kohrogi *et al.*, 1985). Krebs-Henseleit solution was changed every 20 min. The dose-response curves to ACh, histamine, $PGF_{2\alpha}$, and Isop were cumulative: the drugs were added in molar increments at 3 min intervals, from 10^{-8} to 10^{-2}M for ACh, 10^{-9} to 10^{-3}M for histamine, 1.6×10^{-9} to 1.6×10^{-5}M for $PGF_{2\alpha}$. Contractile responses were measured as the difference between developed peak tension and resting tension. For each dose-response curve, the concentration that would produce 50% of the maximum contraction (EC_{50}) was determined by extrapolation from the linear regression line through the points on the central straight portion. Relaxation responses to Isop (10^{-9} to 10^{-6}M) were measured in the preparations precontracted by 10^{-3}M ACh or histamine. The dose-response curves to these drugs of the bronchi without airway epithelium were compared to those with epithelium intact.

We confirmed histologically whether the bronchial epithelium had been removed after each experiment.

Experiments with tracheal strips of dog

Mongrel dogs were anaesthetized with intravenous sodium pentobarbitone (30 mg kg^{-1}). The trachea was removed rapidly and immersed in oxygenated Krebs-Henseleit solution. The tracheal segment was opened longitudinally through the anterior aspect and dissected free of its epithelium and loose connective tissue. Epithelium was stored in Hanks' balanced salt solution bubbled with 100% O_2 at 37°C. Transverse incisions were made about 2 mm apart through the muscularis portion of the posterior membrane. The strips of trachea so created were mounted in the organ chamber and the tension was measured in the same manner as the experiment 1. After we had measured the control dose-response curve to ACh, we added about 0.1 g of the chopped epithelium (5 mm × 5 mm) in the organ chamber and incubated this with the tracheal strip for 15 min. Then, the dose-response curve was measured again, to examine whether the epithelium added to the chamber might have an inhibitory effect on the smooth muscle response.

Drugs

The following drugs were used; acetylcholine chloride, histamine diphosphate (Wako Chemicals, Japan), prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co, Japan), isoprenaline (isoproterenol) hydrochloride (Sigma).

Statistical analysis

Values of EC_{50} were expressed as geometric means and geometric standard errors (g.s.e.mean). The other values were expressed as the arithmetic means and standard deviations (s.d.). We used paired Student's t test for statistical analysis of pairs, and considered differences to be statistically different when the P value was less than 0.05.

Results

Experiments with human bronchial rings

The histology of a human bronchial cross section showed that the gentle rubbing with the forceps could remove the airway epithelium. The contractile responses of bronchial rings to ACh with and without epithelium are shown in Figure 1. In the preparation with epithelium intact, the bronchial ring started to contract at 10^{-5}M ACh, whereas the bronchial ring without epithelium started to contract at a concentration of 10^{-6}M. For the groups, the dose-response curves of the bronchial rings without epithelium were shifted to the left as compared to those with epithelium intact (Figures 2,3,4). Thus, removal of bronchial epithelium increased the responsiveness of human bronchial rings to ACh, histamine, and $PGF_{2\alpha}$. The EC_{50} values were 3.53×10^{-5}M (g.s.e.mean 1.30) with epithelium and 1.33×10^{-5}M (g.s.e.mean 1.19) without epithelium to ACh, 4.68×10^{-6}M (g.s.e.mean 1.26) with epithelium and 2.82×10^{-6}M (g.s.e.mean 1.40) without epithelium to histamine, 5.29×10^{-6}M (g.s.e.mean 1.25) with epithelium and 2.42×10^{-6}M (g.s.e.mean 1.31) without epithelium to $PGF_{2\alpha}$ respectively (Figure 5). Each of the changes for the values of EC_{50} comparing with and without epithelium was statistically significant ($P < 0.01$ for ACh and $PGF_{2\alpha}$,

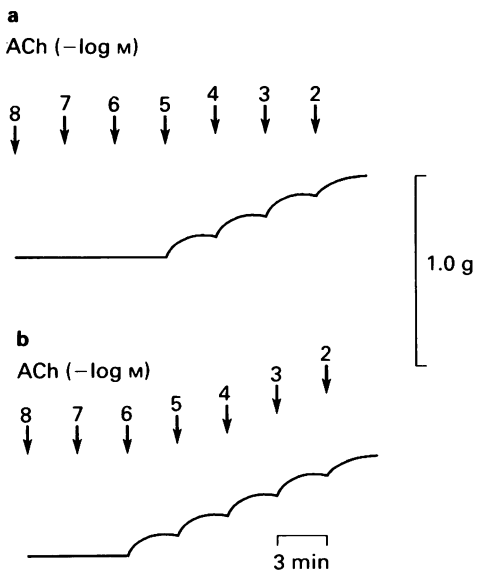

Figure 1 An example of an original tracing from an experiment with human bronchial rings. (a) Contraction induced by increasing concentrations of acetylcholine (ACh) in the bronchial ring with epithelium intact. (b) Enhanced contraction in the bronchus with epithelium removed.

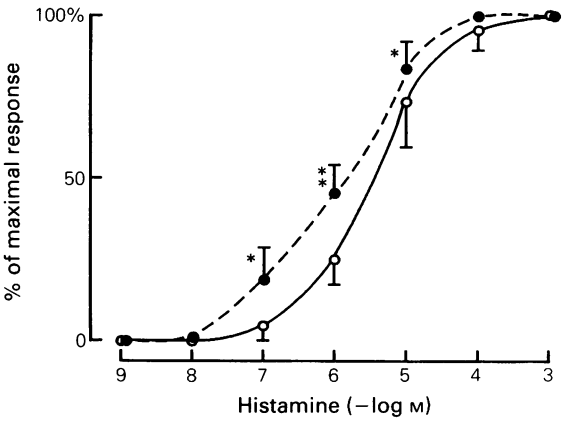

Figure 3 Dose-response curve to histamine, of bronchi with epithelium removed (●), showing a shift to the left as compared to that of bronchi with epithelium intact (○) ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$.

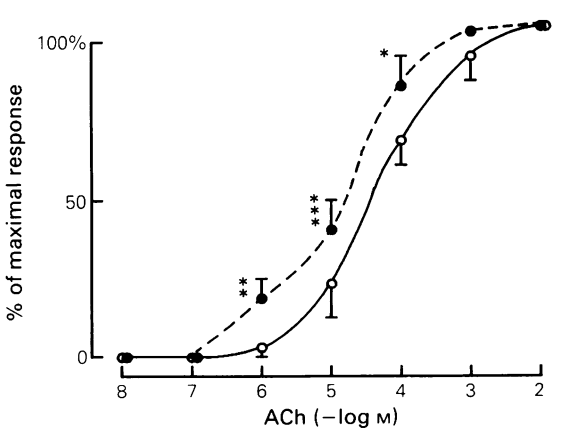

Figure 2 Dose-response curve to acetylcholine (ACh) of bronchi with epithelium removed (●), showing a shift to the left as compared to that of bronchi with epithelium intact (○). Vertical axis shows the percentage of the maximal contraction, and horizontal axis the concentration of ACh ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$.

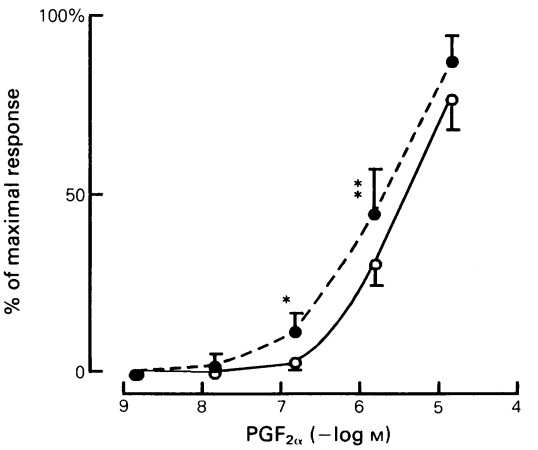

Figure 4 Dose-response curve to prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) of bronchi with epithelium removed (●), showing a shift to the left as compared to that of bronchi with epithelium intact (○). Values are expressed as the percentage to the maximal contraction evoked by acetylcholine 10^{-2}M ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$.

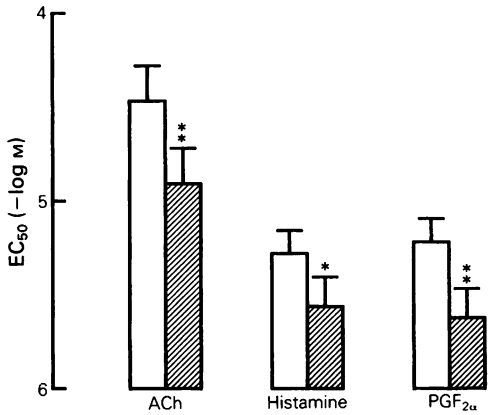

Figure 5 EC_{50} values for acetylcholine (left), histamine (middle), and prostaglandin $F_{2\alpha}$ (right). Each open column represents EC_{50} of bronchi with epithelium intact, and hatched column that of bronchi with epithelium removed. $*P<0.05$; $**P<0.01$.

$P<0.05$ for histamine, respectively). The maximal contractions were not different when tissues were compared with and without epithelium, i.e. 1.4 ± 0.4 g (mean ± s.d.) with epithelium and 1.3 ± 0.7 g without epithelium to ACh, 1.3 ± 0.3 g with and 1.0 ± 0.6 g without epithelium to histamine, respectively.

Although the reversal of the contractile response by Isop was not significantly different between tissues with and without epithelium, the dose-response curve showed a tendency to decrease in the bronchus without epithelium (Figure 6).

Experiments with tracheal strips of dog

In the dog tracheal strips with epithelium removed, the dose-response curve to ACh shifted to the right when the chopped epithelium was added and incubated together in the organ chamber (Figure 7). The EC_{50} values were 2.15×10^{-5}M (g.s.e mean 1.51) without epithelium and 4.77×10^{-5}M (g.s.e.mean 1.56) when incubated with epithelium. This change was statistically significant ($P<0.01$) (Figure 8). To confirm that this change was not time-dependent, we measured the control dose-response curve first and then added the chopped epithelium in 3 strips; in the others, we performed the controls afterward. The results did not differ in the two groups.

Discussion

This study demonstrates that airway epithelium possesses the ability to decrease the contractile responses of smooth muscle in man as it did in dogs and calves

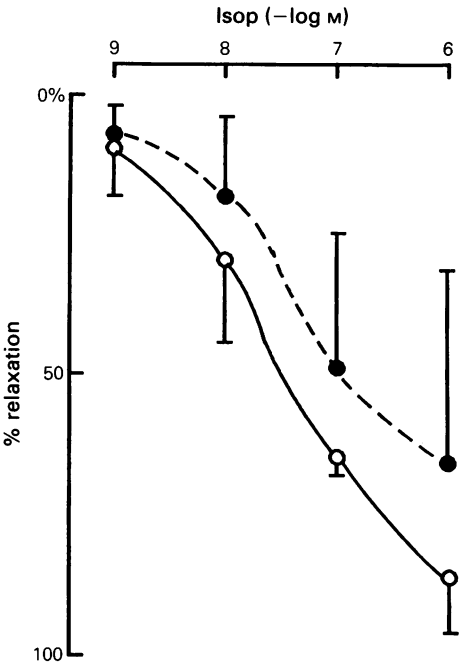

Figure 6 Dose-response curve to isoprenaline (Isop), of bronchi with epithelium intact (○), and of bronchi with epithelium removed (●) ($n = 6$).

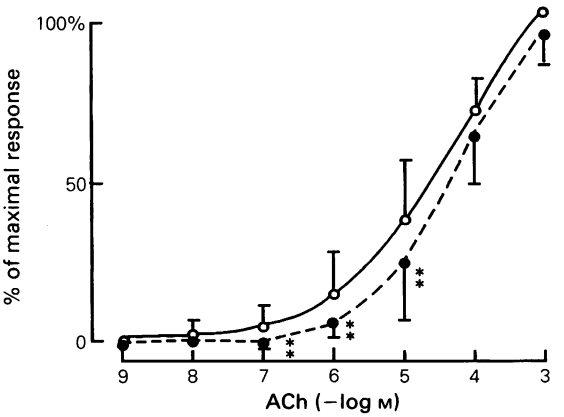

Figure 7 Dose-response curve to acetylcholine (ACh) of dog tracheal strips incubated together with epithelium (●), showing shift to right as compared to the control (○) ($n = 6$). $**P<0.01$.

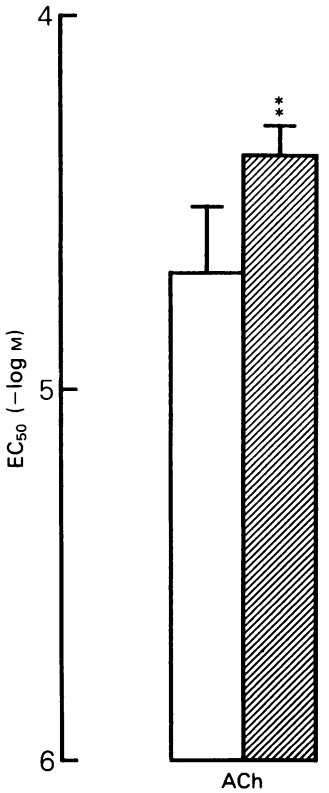

Figure 8 EC_{50} value for acetylcholine (ACh) of tracheal strips incubated together with epithelium (hatched column) increases significantly as compared to the control value (white column). $^{**}P < 0.01$.

(Flavahan *et al.*, 1985; Barnes *et al.*, 1985).

The possible mechanisms underlying this inhibitory effect were considered as follows: First, the change of mechanical property by removal of the epithelium may alter the response of the airway itself. Second, the epithelium may behave as the diffusion barrier for the chemical mediators that move to the smooth muscle across the epithelium. The third possibility may be that the epithelial cells have a metabolic function and inactivate the chemical mediators. Finally, the epithelial cells may be able to synthesize and release some epithelium-derived relaxing factor and modulate the smooth muscle response through its action.

Concerning the first possibility, because the maximal contraction to ACh, histamine and $PGF_{2\alpha}$, and the length-tension relationships did not differ between the bronchi with and without epithelium, a change in the mechanical property of airways appears to be unlikely. Our present results are supported by the data of Flavahan and colleagues (1985). Barnes *et al.* (1985) also demonstrated that the contractile responses to potassium and electrical field stimulation were similar in bovine trachea, with and without epithelium.

The removal of airway epithelium increased the contractile responses to ACh, histamine and $PGF_{2\alpha}$. By contrast, the relaxation response to Isop had a tendency to decrease. This discrepancy in the change of responses between contraction and relaxation cannot be explained by lack of a diffusion mechanism alone. Thus, when the epithelial barrier is lost, the mediators for both contraction and relaxation can easily reach the smooth muscle receptor and can enhance these responses. While contraction responses increased, relaxation responses decreased on removal of the epithelial barrier in these and previous experiments (Flavahan *et al.*, 1985; Barnes *et al.*, 1985). These results suggest that a change in the diffusion path is not likely.

However, we cannot entirely rule out the possibility of involvement of a diffusion factor because of this observation. The changes in responsiveness to various mediators, induced by the removal of the epithelial barrier seem to be dependent on the numbers and localization of each receptor. Thus, the above explanation will only be true, if the receptors for contraction and relaxation are localized on the mucosal side of smooth muscle. We found that the airway epithelium could decrease the contractile response of smooth muscle to ACh, only when incubated with the smooth muscle. In this experiment, the epithelium was not *in situ* and so could not protect the smooth muscle from exposure to chemical mediators, so we can conclude that the diffusion factor did not play an important role.

Results of the experiment with anticholinesterase make the third possibility less likely (Flavahan *et al.*, 1985). It is clear that the epithelial cells play an important role other than that of a simple physical barrier; possibly their metabolic functions are important in decreasing the response of smooth muscle e.g. by inactivation of mediators or releasing a relaxing factor.

In the vascular system, Furchgott and his colleagues demonstrated that the smooth muscle relaxation induced by ACh was dependent on the endothelium and this phenomenon was due to the endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980a,b). Further investigations revealed that the vascular dilatations induced by the Ca ionophore A23187, substance P, bradykinin, ADP, and ATP are also endothelium-dependent (Furchgott *et al.*, 1984). It is well known that EDRF relaxes smooth muscle by increasing cyclic GMP in the smooth muscle cell (Rapoport & Murad, 1983; Furchgott & Jothianandan, 1983). Although the structure of EDRF is not known, it was suggested that EDRF might be the lipoxygenase metabolite of arachidonic acid (Furch-

gott & Zawadzki, 1980a; Chand & Altura, 1981; De Mey *et al.*, 1982), and might require free radicals in this metabolism (Furchgott, 1983; Furchgott *et al.*, 1984; Sata *et al.*, 1986). In the airway system, it has been demonstrated that some relaxations of smooth muscle are accompanied by increase in cyclic GMP (Ito *et al.*, 1985), and that airway epithelial cells have an ability to metabolize arachidonic acid (Holtzman *et al.*, 1983; Hunter *et al.*, 1985). In addition, it was recently reported that airway epithelial cells of rabbit bronchi modulate the responsiveness via a cyclo-oxygenase metabolite of arachidonic acid (Butler *et al.*, 1987). We speculate that the airway epithelial cells have a function similar to the vascular endothelial cells.

The pathophysiological role of the inhibitory effect of epithelial cells is not known in asthma. The possible mechanisms of airway hyperresponsiveness following the epithelial damage may be as follows; first, the sensory nerve endings of the vagal components which terminate in the epithelial layer, are exposed directly to the stimuli by removal of the epithelium, followed by an exaggerated vagal reflex (Widdicomb *et al.*, 1962; Simonsson *et al.*, 1967). Second, airway hyperresponsiveness may occur as a result of airway inflammation (O'Byrne, 1986; Chung, 1986). Thus, when the epithelial cells are stimulated, they release chemotactic factors, followed by an influx of neutrophils into the airways. The chemical mediators, which the neutrophils release in the inflamed airway, make the airway hyperresponsive (Aizawa *et al.*, 1985; Chung *et al.*, 1986). Besides these mechanisms, we can speculate that when the inhibitory effect of the epithelium is gone, the airway may become hyperreactive. In the airway of human asthmatics, these mechanisms may develop, interacting with each other. It is uncertain how important this inhibitory effect of airway epithelium is in this reaction. Some investigators suggest that this inhibitory effect acts as a 'braking mechanism' to decrease or stop these interactions following the damage of airway epithelium (Barnes *et al.*, 1985).

In the present study, we demonstrated the inhibitory effect of airway epithelium on the contractile response of the smooth muscle in human bronchi. We also showed that this inhibitory effect was not due to a change in the mechanical properties of the airway nor to a change in the diffusion path for the chemical mediators to reach the smooth muscle. Thus, we suggest that airway epithelial cells may have an important metabolic function (such as the inactivation of mediators or releasing a relaxing factor) in modulating smooth muscle responsiveness. Further investigations are required, including studies of metabolism of airway epithelial cells, to clarify the role of airway epithelial cells in the development of airway hyperresponsiveness.

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