J. Pharm. Pharmacol. 1991, 43: 392–395
Received December 13, 1990

Epithelium Modulates the Reactivity of Sensitized Guinea-pig Trachea: Influence of the Surface of Drug Entry

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Abstract—A technique by which drug access was restricted to either the mucosal or the adventitial surface of tracheal rings isolated from sensitized guinea-pigs was applied to study the role of the epithelium in modulating responses to KCl, acetylcholine, histamine and antigen (bovine serum albumin, BSA). Epithelium removal did not alter the responsiveness or sensitivity of tracheal rings to KCl. In contrast, a leftward shift occurred for concentration-response curves to acetylcholine (concentration ratio (CR) = 4·1), histamine (CR = 2·9) and BSA (CR = 33·9) entering from the mucosal surface of de-epithelialized trachea. This shift was not associated with changes in the maximal effect of the spasmogens. Response to the adventitial entry of these spasmogens was not altered by epithelial stripping. These results confirm that the epithelium modulates tracheal responses to certain spasmogens including antigen challenge. This role was exclusively exerted for mucosal drug entry. The mechanism underlying this protective effect of epithelium remains to be determined.

Non-specific bronchial hyperresponsiveness is a characteristic feature of human asthma but its pathogenic mechanisms still remain incompletely understood (Nadel et al 1987). Morphological studies have demonstrated that damage and loss of airway epithelium accompanies bronchial hyperreactivity in asthmatics (Laitinen et al 1985). Pharmacomechanical and bioassay experiments indicate that the epithelium of the airways modulates the pharmacological reactivity of the underlying smooth muscle through the production of epithelial-derived factors (Vanhoutte 1988; Fedan et al 1988).

The consequences of epithelium removal on airways reactivity should be studied in circumstances where drugs enter the airway wall from either the mucosal or the adventitial surface alone, as previously reported in this (Iriarte et al 1990) and other laboratories (Munakata et al 1988, 1989; Small et al 1990).

The immunization of laboratory animals has become a widely adopted method used to create in-vivo and in-vitro models of allergic asthma that, as closely as possible, resemble the disease state in man. This purpose makes desirable the existence of hyperreactivity in the experimental model. We have previously characterized the presence of a non-specific hyperreactivity to pharmacological stimuli in airway smooth muscle isolated from actively sensitized guinea-pigs (Morcillo et al 1984; Ortiz et al 1989).

The aim of the present study was to analyse the influence of the epithelium in the responses to various spasmogens after selective entrance (mucosal or adventitial) to tracheal rings isolated from actively sensitized guinea-pigs.

Materials and Methods

Guinea-pigs of either sex, 300–450 g, were sensitized as previously described (Ortiz et al 1989). Briefly, on day 0 the animals were injected subcutaneously with 0·25 mL of Freund's complete adjuvant plus 1·25 $\mu g\ g^{-1}$ of bovine serum albumin (BSA) dissolved in 0·25 mL saline and on days 2 and 4 the animals received the same amount of Freund's complete adjuvant and BSA by the intramuscular route. The animals were used for experiments on days 21 to 25. Animals were killed by stunning and bleeding. Tracheae were excised, dissected free of extraneous tissue and cut into rings of about 4 mm width. At least 6 rings were obtained from each trachea. Rings were randomly allocated to provide paired preparations serving as epithelium-intact and epithelium-denuded preparations for either luminal or drug entry. The epithelium was removed by gently rubbing the mucosal surface with a wet filter paper. Rings were suspended under a resting tension of 2 g in tissue baths containing Krebs bicarbonate solution (composition in mM: NaCl 115, KCl 4·6, $CaCl_2$, 1·6 KH_2PO_4 1·2, $MgSO_4 \cdot 7H_2O$ 1·2, $NaHCO_3$ 25, EDTA 0·02 and glucose 11·1) bubbled with 95% O_2-5% CO_2 at 37°C (pH = 7·4). An equilibration period of 60 min was used. Tension was measured with a Grass FTO3 isometric transducer and recorded on a model 7 Grass polygraph.

The method to select the surface of drug entrance to the tracheal wall was as previously described (Iriarte et al 1990). In brief, Vaseline was applied to cover completely either the adventitial or the mucosal surface of the ring, thus restricting drug entry to the mucosal or the adventitial surface, respectively.

Experimental protocol

After the equilibration period, cumulative concentration-response curves to either BSA, KCl, acetylcholine or histamine were constructed. The contact time for each concentration of the spasmogens was such as to allow the development of almost all the tension rise attainable by that concentration. For BSA this was 15 min, for KCl 12 min, for acetylcholine 3 min, and for histamine 6 min. Only one concentration-response curve to an agonist was generated in

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Table 1. Effect of KCl, acetylcholine, histamine and bovine serum albumin, in sensitized guinea-pig trachea under conditions of mucosal or adventitial drug entry. Maximal effects (E_{max}) and negative $\log_{10}$ EC50 values are given for concentration-response curves constructed in intact (E+) or epithelium-denuded (E−) preparations. Shift in curves is expressed as $\log_{10}$ (concentration-ratio) (a negative sign signifies a leftward shift) at the EC50.

	KCl		Acetylcholine		Histamine		Bovine serum albumin	
	Mucosal	Adventitial	Mucosal	Adventitial	Mucosal	Adventitial	Mucosal	Adventitial
E_{max} (g)								
E+	2·42±0·12	2·56±0·11	2·03±0·12	2·19±0·09	1·47±0·09	1·33±0·15	1·07±0·33	1·11±0·41
E−	2·68±0·15	2·48±0·11	2·30±0·21	2·21±0·15	1·52±0·10	1·42±0·11	1·12±0·27	1·23±0·38
$-\text{Log}_{10}$EC50								
E+	1·86±0·09	1·86±0·07	4·16±0·05	5·00±0·06◆	4·60±0·07	5·03±0·05◆	2·95±0·07	2·59±0·10◆
E−	1·80±0·07	1·83±0·04	4·77±0·13*	4·88±0·08	5·06±0·05*	5·07±0·03	4·48±0·10*	2·81±0·09◆

	Log_{10} (concentration-ratio)			
	Mucosal E− vs E+	Adventitial E− vs E+	Presence of epithelium adventitial vs mucosal	Absence of epithelium adventitial vs mucosal
KCl	0·06±0·04	0·02±0·09	0·01±0·10	−0·03±0·03
Acetylcholine	−0·61±0·09	0·12±0·04◆	−0·84±0·04	−0·11±0·09*
Histamine	−0·46±0·06	−0·04±0·04◆	−0·43±0·03	−0·02±0·03*
Bovine serum albumin	−1·53±0·03	−0·22±0·04◆	0·36±0·03	1·67±0·03*

Data are means ± s.e.m. of 5 experiments in each group. * $P < 0.05$ from rings with epithelium. ◆ $P < 0.05$ from values for mucosal drug entry.

each ring. Concentration-response curves were obtained in intact or epithelium-denuded preparations for mucosal or adventitial drug access. Those preparations where BSA was not used as a spasmogen were challenged at the end of the experiment with BSA (1 mg mL^{-1}) to confirm the existence of an antigen-induced contraction.

Efficacy (E_{max}) and potency ($-\log_{10}$ EC50) of the agonists was calculated from individual concentration-response curves. Differences in sensitivity to the agonists under different conditions (with and without epithelium; luminal or adventitial drug entry) were quantified by measurement of concentration ratios (CR) at the EC50 and expressed as $\log_{10}$ CR. The results are given as means ± s.e.m. Statistical analysis of the results was performed by analysis of variance (ANOVA) followed by Duncan's test. Differences were considered significant when $P < 0.05$.

Drugs and solutions

The following chemicals were used: acetylcholine chloride (Sigma), bovine serum albumin (Sigma), Freund's complete adjuvant (Difco) and histamine dihydrochloride (Sigma). KCl was of analytical grade. Drugs were prepared and diluted in distilled water and added to the tissue bath in small volumes. Drug concentrations are presented as final bath concentrations (M or mg mL^{-1}, as appropriate), with the exception of KCl where the stated concentration was that in excess of KCl provided by the Krebs solution.

Results

BSA, KCl, acetylcholine and histamine entering from either the mucosal or adventitial surface of the trachea produced concentration-dependent contractions of intact (epithelium present) rings. The maximal effects of each spasmogen entering by the mucosal route or by the adventitial route, did not differ significantly (Table 1). The concentration-response curves for KCl entering from the mucosal or adventitial surfaces were superimposable (curves not shown). The concentration-response curve for mucosal BSA entry was slightly displaced to the left compared with that for adventitial entry. By contrast, the concentration-response curves for mucosal acetylcholine and histamine entry lay to the right of those obtained after adventitial drug entry (Fig. 1).

The concentration-response curves and the maximal effects of KCl entering by the mucosal or adventitial route were not significantly altered by removal of the epithelium (curves not shown, Table 1). In contrast, a significant leftward displacement occurred for concentration-response curves to BSA, acetylcholine and histamine entering from the mucosal surface of de-epithelialized trachea (Fig. 1). The order of leftward shift was BSA (CR = 33·9) > acetylcholine (CR = 4·1) ≥ histamine (CR = 2·9). This displacement was not accompanied by changes in the maximal effect of the spasmogens (Table 1). The concentration-response curves and the maximal effects for adventitial entry of BSA, acetylcholine and histamine were not altered in epithelium-denuded preparations (Table 1, Fig. 1).

Discussion

A previous study from this laboratory showed the consequences of epithelium removal on the responses to spasmogens entering the guinea-pig isolated trachea from the mucosal or from the adventitial surface alone (Iriarte et al 1990). Selective drug entry was achieved by coating the appropriate surface of tracheal rings with Vaseline. This technique has been applied in the present study to tracheal rings isolated from guinea-pigs actively sensitized to BSA. The influence of epithelium removal on the responses to various spasmogens, including specific antigen challenge, has been previously reported (Frossard & Muller 1986; Hay et al 1986; Undem et al 1988) but, as far as we are aware, it has not yet been examined for selective drug entry.

Contraction of sensitized trachea to KCl was not altered by epithelium removal irrespective of whether KCl entry was from either the mucosal or the adventitial surface. Hay et al

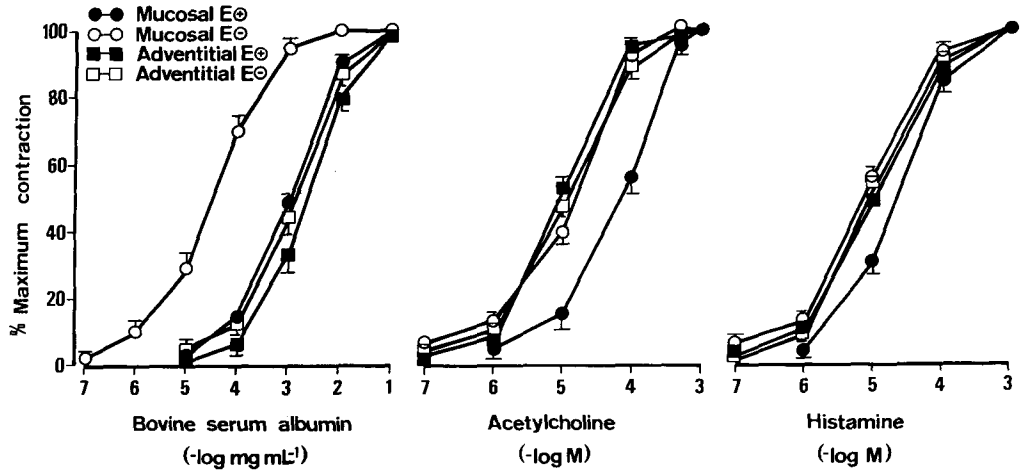

FIG. 1. Log concentration-response curves to bovine serum albumin (left panel), acetylcholine (middle panel) and histamine (right panel) in tracheal rings isolated from sensitized guinea-pigs. Responses were obtained in intact (closed symbols) or epithelium-denuded (open symbols) preparations for mucosal (●, ○) or adventitial (■, □) entry of drugs. Data are expressed as percentage of maximal response to each agonist and are shown as means of 5 experiments with s.e.m. shown by vertical bars.

(1986) found a significant, but very small (CR = 1·2), leftward shift of the concentration-response curve to KCl. The present data suggest that the epithelium is neither a diffusion barrier for KCl nor a site producing a relaxing factor in response to this spasmogen. These results are similar to those found for KCl in non-sensitized guinea-pig trachea with the same technique (Iriarte et al 1990). The finding of equal sensitivity and responsiveness of tracheal rings to KCl irrespective of the route of drug entry and the presence of epithelium indicate that the technical procedure used in this study did not cause overt damage to the smooth muscle layers or artifactual changes in drug-induced responses.

In contrast to the results for KCl, removing the epithelium potentiated the contractions elicited by acetylcholine and histamine entering from the mucosal surface while maximal effects remained unaltered. These results are in agreement with those of Hay et al (1986) and Undem et al (1988) for unrestricted drug entry in sensitized guinea-pig trachea. The mechanism underlying the enhancing effect of epithelial removal on the contraction to certain spasmogens is not precisely known. Thus, the epithelium may be a passive diffusion barrier or a site of metabolic loss or may produce relaxing factors in response to different agonists (Fedan et al 1988; Vanhoutte 1988).

The sensitivity and responsiveness of sensitized tissue to acetylcholine and histamine entering from the adventitial side was similar in intact and epithelium-free preparations, respectively. This may be due to insufficient concentration of agonists reaching the epithelium after crossing the tracheal wall, or incapacity of the epithelium to release an inhibitory factor following stimulation through its adventitial surface.

Methodological differences among laboratories make it difficult to compare the influence of epithelium removal in normal vs sensitized tracheal preparations. Hay et al (1986) found that stripping of the epithelium equally enhanced the contractile responses to histamine and methacholine (unrestricted drug entry) in control and sensitized tissues. The concentration ratio (with and without epithelium) for histamine (unrestricted entry) in sensitized guinea-pig trachea has been reported to be 2·6 (Undem et al 1988) and 1·9 (Hay et al 1986). These values are smaller than those reported in non-sensitized guinea-pig trachea by some authors (8·2 (Holroyde 1986); 6·7 (Tschirhart et al 1987)) but not by others (3·8–2·4 (Goldie et al 1986); 1·9 (Hay et al 1986); 2·0 (Iriarte et al 1990)). In trachea isolated from normal guinea-pigs, epithelial removal had a greater influence on sensitivity to acetylcholine than on sensitivity to histamine when these agonists entered from the mucosal surface (Munakata et al 1989; Iriarte et al 1990). Although a similar trend was observed in sensitized trachea in this study, differences did not reach statistical significance. In addition, only small differences were found between normal (Iriarte et al 1990) and sensitized tissues in this study in the potentiation of the responses to acetylcholine and histamine (mucosal entry) after epithelial removal.

This part of the study confirms the role of the epithelium in controlling responses to certain spasmogens in sensitized trachea. However, this modulatory role appears to be equally exerted in normal and sensitized tissues, i.e. immunization did not result in a patent loss of the protective effect of epithelium against agonist-induced spasm. It follows that the non-specific hyperreactivity existing in this model (Morcillo et al 1984; Ortiz et al 1989) cannot be attributed to epithelial damage. First, rubbing off epithelium in normal trachea did not produce non-specific hyperreactivity but preferentially enhanced the contraction produced by certain agonists (Iriarte et al 1990). Second, non-specific hyperreactivity in this model seems related to alterations of Ca^{2+} movements in trachealis muscle (Perpiñá et al 1990). In fact, the presence or absence of epithelium did not influence basal and stimulated ^{45}Ca uptake by guinea-pig trachea (Raeburn et al 1987).

The stripping of the epithelium augmented contractions produced by BSA entering from the mucosal surface of sensitized guinea-pig isolated trachea. This was not observed for BSA when it was added to the adventitial surface of the preparations. Maximal effects were not altered by epithelium removal. Potentiation of the spasm produced by specific antigen challenge (unrestricted entry) after rubbing the

epithelium from sensitized guinea-pig trachea has been previously reported (Hay et al 1986; Undem et al 1988). Contraction to antigen is attributed to the release of mediators triggered by the antigen-antibody reaction. It is presently not known whether an epithelial-derived factor may effectively inhibit mediator release from the appropriate cells, e.g. mast cells. It is also uncertain whether the antigen challenge constitutes an appropriate stimulus to induce the release of epithelial factors. Experiments using a "sandwich" preparation suggest that the epithelium releases an inhibitory factor in response to antigen (Hay et al 1987) but results from cascade superfusion experiments do not support this conclusion (Undem et al 1988). Finally, the influence of epithelium removal on the direct contractile effects produced by mediators other than histamine released after antigen challenge is unknown. Certainly, the extent of the leftward shift in the BSA concentration-response curve caused by epithelium removal was greater than that for histamine in agreement with the reports of Hay et al (1986) and Undem et al (1988).

Pharmacomechanical data from this study for the selective entry of BSA are coincident with those obtained by Undem et al (1988) when measuring histamine release after antigen exposure to either the luminal or serosal surface of superfused tracheal tubes. Taken together, these results indicate that the primary effect of epithelium on tracheal responses to mucosal antigen entry is to serve as a diffusion barrier opposing the penetration of antigen macromolecules into the airway tissue. Stripping of the epithelium facilitates antigen entrance through the mucosal surface of the trachea and this translates into greater sensitivity to antigen challenge. Therefore, the epithelial cell damage and loss reported in asthmatic patients (Laitinen et al 1985) may directly alter the sensitivity of the airways to inhaled allergens predisposing to episodes of antigen-induced asthma.

Acknowledgements

This investigation was supported by a grant from the Ministerio de Educación y Ciencia from Spain (CAICYT PB-85-0228; PB-86-0428).

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