

EFFECT OF RESPIRATORY EPITHELIUM ON SMOOTH MUSCLES OF THE GUINEA PIG TRACHEA

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Just as endothelial cells of vessels can modulate the tone of the vascular smooth muscle (SM) [3, 6], injury to or dysfunction of cells of the respiratory epithelium may play a role in the development of hyperreactivity of the bronchial SM in bronchial asthma [5].

The aim of this investigation was to study the effect of epithelium of the air passages on reactivity of SM, on a model of contraction of the tracheal SM.

METHODS

Experiments were carried out on male and female guinea pigs weighing from 335 to 598 g, sacrificed by exsanguination under general anesthesia (thiopental sodium, 80 mg/kg, intraperitoneally). Pairs of tracheal rings (1-1.5 mm wide) were divided in the region of the cartilage. The layer of epithelium was removed from one strip of each pair by gentle rubbing with a cotton-covered probe. Removal of the epithelium was confirmed histologically (Fig. 1a, b). The tracheal strips were placed in a 2 ml receptacle containing Krebs—Henseleit solution, aerated with a mixture of 95% O₂ + 0.5% CO₂ (pH 7.4), and fixed with one end securely to the receptacle, and with the other end to the sensor of a mechanical-to-electrical transducer [1] for measurement under isometric conditions. The tracheal preparation was stretched for 1 h by a force of 2 g when assessing contractions to histamine, and of 1 g when assessing relaxation by isoproterenol. During stretching the rate of rinsing with Krebs—Henseleit solution was 70 ml/h. Contractile activity of SM was then estimated by a cumulative technique, in the form of a dose—response to histamine curve. When relaxation with isoproterenol was studied the preparation was first contracted by histamine solution in a concentration inducing 50% of maximal contraction. Results obtained on strips of trachea with mechanically removed epithelium were compared with those obtained on strips with intact epithelium. A solution of histamine hydrochloride (Merck) was made up in Krebs—Henseleit solution. A solution of isoproterenol (Sigma) was made up in physiological saline containing 14 mM ascorbic acid as antioxidant. The maximal ascorbic acid concentration in the receptacle did not exceed 141 mM. pD₂, the negative logarithm to base 10 of the molar concentration of the preparation causing contraction with a force of 50% of maximal on addition of histamine or 50% of maximal relaxation for isoproterenol (ED₅₀) — was determined. The value of ED₅₀ was obtained by the linear regression method, by analyzing the linear part of the dose—response curve (20-80% of the maximal response). The results were assessed as mean values ± standard error, and significance was determined by Student's test.

RESULTS

Removal of the epithelium increased the reactivity of SM of the tracheal rings to histamine, as shown by a shift of the dose—response curve to the left compared with the control curve with intact epithelium (Fig. 2). To

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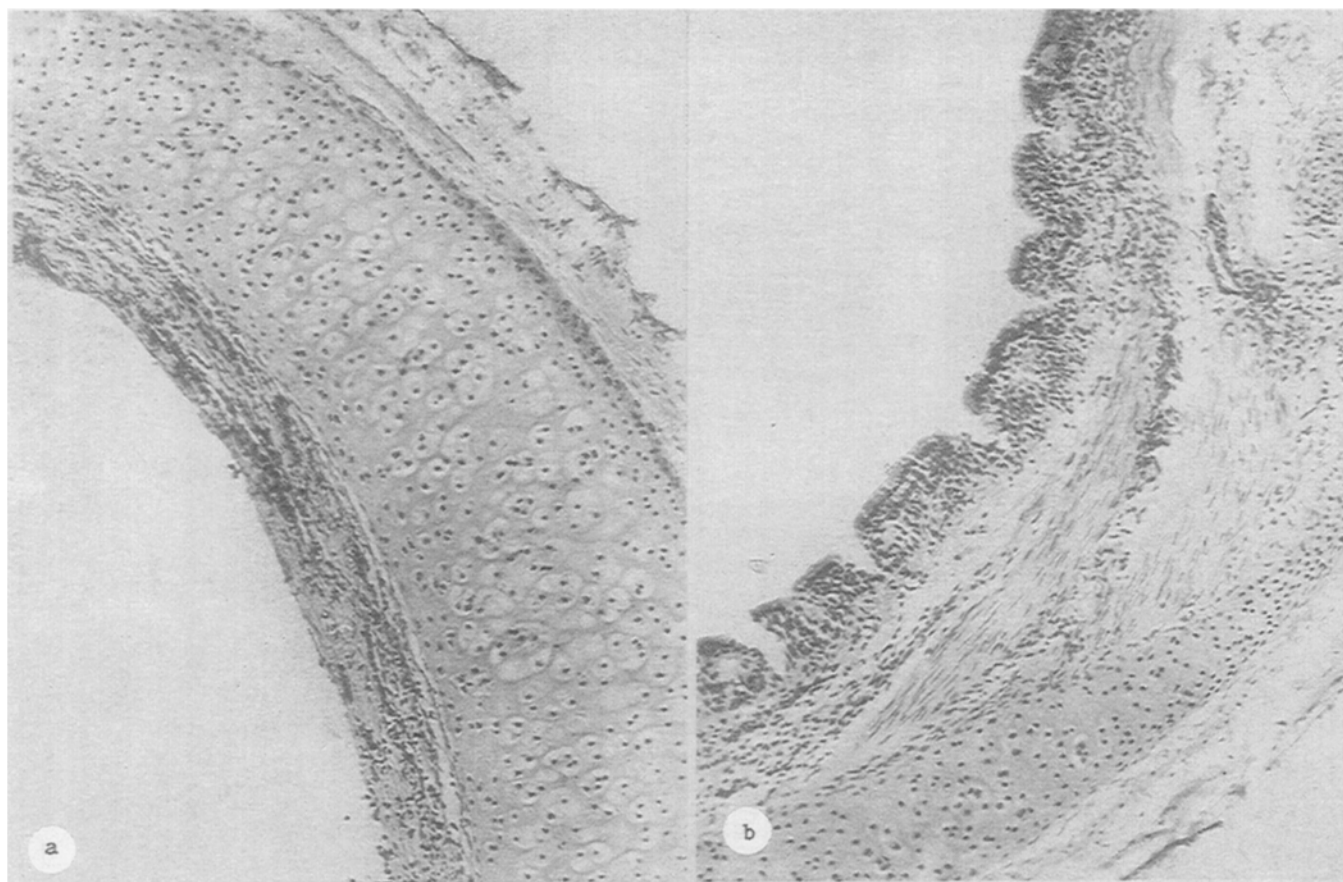


Fig. 1

Fig. 1. Section through trachea: a) with epithelium; b) after mechanical removal of epithelium. Hematoxylin—eosin. 168 \times .

construct the dose—response curve histamine was used in concentrations of 10^{-7} to 10^{-4} M. pD_2 for histamine on the preparations without epithelium was 5.85 ± 0.09 , and on preparations with epithelium it was 5.19 ± 0.06 ($p < 0.05$).

Isoproterenol in concentrations of $5 \cdot 10^{-10}$ M to $8.25 \cdot 10^{-9}$ M were added to tracheal strips, previously contracted by histamine ($5 \cdot 10^{-6}$ M), as contraction flattened out on a plateau. In deepithelized preparations of tracheal SM the dose—response to isoproterenol curve was largely shifted to the right compared with the control (Fig. 3). This indicates a decrease in sensitivity of the preparations without epithelium to isoproterenol. pD_2 for isoproterenol on preparations with epithelium was 9.11 ± 0.038 , whereas for the deepithelized preparations it was 8.88 ± 0.04 ($p < 0.01$).

The increase in reactivity of the preparation of tracheal SM obtained in these experiments was not the result of mechanical injury, for the isometric characteristics and the force of maximal contraction were virtually indistinguishable in the deepithelized and control preparations.

One possible cause of the increased reactivity of the deepithelized preparations to histamine is absence of the relaxing factor whose production by the epithelium is modulated by the H_1 -receptor-mediated contractile response of SM [4].

The stimuli-inducing production of this factor may be β -adrenomimetics. It has been shown that the density of β -adrenoreceptors on the tracheal epithelium is almost twice as high as their density on smooth muscles [4]. This may probably account for the weakening of the response of the preparations to isoproterenol after removal of the

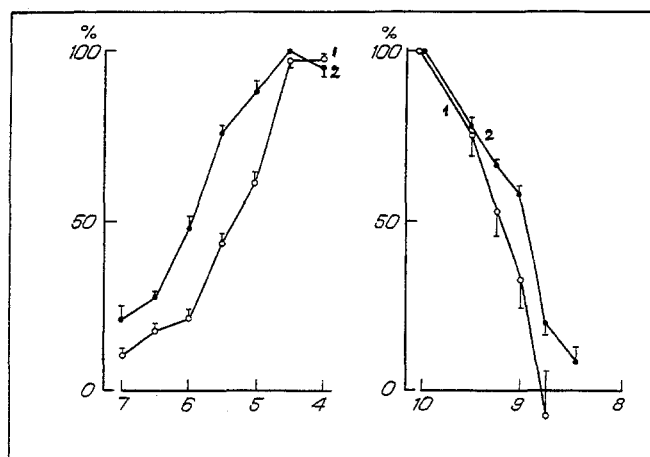


Fig. 2

Fig. 3

Fig. 2. Contractile response of tracheal SM to histamine. 1) Intact preparations of SM; 2) deepithelized preparations of trachea. Abscissa) histamine concentration ($-\log M$); ordinate) force of contraction, in % of maximal.

Fig. 3. Effect of isoproterenol on tone of tracheal SM, contracted beforehand by histamine ($5 \cdot 10^{-6}$). Abscissa) concentration of isoproterenol ($-\log M$); ordinate) relaxation, in % of maximal.

epithelium, observed in the present investigations. This may be manifested in vivo by an increase in reactivity to bronchoconstrictor stimuli [2] and it is probably one of the mechanisms leading to the formation of hyperreactivity.

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