PHYSIOLOGY

Role of Guanylate Cyclase and Arachidonic Acid Metabolites in the Regulation of Epithelium-Dependent Relaxation of Tracheal Smooth Muscles in Rats

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The effects of guanylate cyclase modulators and inhibitors of the arachidonic acid metabolism on epithelium-dependent smooth muscle reactions of rat tracheal segments were studied by a mechanographic technique. Application of phenylephrine to the tracheal segments precontracted by high-potassium Krebs' solution reduced their mechanical tension. This reaction was considerably less pronounced in the tracheal segments from which the epithelium had been removed. Phenylephrine-induced relaxation was suppressed by preincubation with methylene blue, but was not affected by preincubation with either indomethacin (a cyclooxygenase inhibitor) or nordihydroguaiaretic acid (a lipoxygenase inhibitor). These data suggest that the epithelium-dependent relaxation of the tracheal smooth muscle is mediated by guanylate cyclase activation, and arachidonic acid metabolites do not significantly contribute to this reaction.

Key Words: *epithelium; trachea; guanylate cyclase; arachidonic acid*

Disturbances of the regulatory mechanisms of the airway smooth muscles contribute to the pathogenesis of respiratory diseases [3]. Therefore, considerable attention has been focused on the mechanisms of regulation of smooth muscle contractile and electrical activity by various biologically active substances [8]. Recently, it has been shown that epithelial cells affect smooth muscle cells modulating the effects of endogenous regulators, i.e., transmitters and hormones [4,5,12].

The mechanisms of this activity can be attributed to the ability of the respiratory epithelium to produce relaxing factors, whose nature and mechanisms of action remain unclear [6,7,9]. There is similarity between endothelial and epithelial relaxing factors and their possible cross-reactivity [1]. From these data it can be suggested that similar to vascular endothelium, respiratory epithelium produces nitric oxide and/or arachidonic acid (AA) metabolites that exert the relaxing effects.

In this study we investigated the epithelium-dependent reactions of rat tracheal segments in response to substances modulating guanylate cyclase activity and AA metabolism.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats weighing 150-200 g. Part of the trachea from the larynx to the bifurcation was separated from adipose and connective tissues and sectioned into 2-3-mm ring

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segments. If necessary, the epithelium was mechanically removed by a wooden spatula rotated in the tracheal lumen for 1 min.

Four ring segments were fixed in a constant-temperature bath filled with aerated Krebs solution containing in mM: NaCl 120.4, KCl 5.9, $CaCl_2$ 2.8, $MgCl_2$ 1.2, HEPES 10, glucose 11.5, (pH 7.35). The contractile activity of the segments was recorded with a 6MC-2B mechanotrons under close to isometric conditions.

RESULTS

Rat tracheal segments precontracted by high-potassium solution Krebs' (40 μM KCl) were tested by phenylephrine (Phe, 1 μM) under conditions of β-adrenoreceptor blockade with propranolol (1 mM). Phenylephrine reduced the mechanical tension of intact segments by 27.2±8.0% (Fig. 1, *1*). Phenyl-ephrine-induced relaxation was much lower in the segments with removed epithelium, where mechanical tension decreased only by 8.7±2.0%.

α-Adrenoreceptor agonists were shown to relax rat tracheal segments in an epithelium-dependent manner [1]. It has been suggested that α-adrenoreceptors are located on the membrane of epitheliocytes and stimulate the production of relaxing factor through the cytosolic calcium mobilization [9,10]. It was shown that smooth muscle guanylate cyclase is involved in the regulation of epithelium-dependent relaxation revealed and is the target of NO and NO-producing compounds [2,8]. After preincubation with methylene blue (50 μM, 20 min), the segments with intact epithelium exhibited significantly smaller relaxation in response to Phe application (Fig. 1, *2*], which was similar to that in the epithelium-free segments.

This finding suggests an important role of guanylate cyclase in the mechanisms of Phe-induced epithelium-dependent relaxation of rat tracheal smooth muscles. On the other hand, many researchers have noted a high concentration of eicosanoids in the airway wall epithelium and attributed epithelial relaxing effects to AA metabolites (prostaglandin E_2, prostacyclins) [9,11].

We have examined the effects of some inhibitors of AA metabolism on the epithelium-dependent relaxation. The segments were preincubated with the cyclooxygenase inhibitor indomethacin (5 μM) or nordihydroguaiaretic acid (5 μM), a lipoxygenase inhibitor. The indices of Phe-induced relaxation in these preparations, both intact and epithelium-free, did not differ from the corresponding control values (Fig. 1, *3* and *4*). It can be concluded, therefore, that AA metabolites are not involved in the epithelium-dependent relaxation of rat tracheal smooth muscles.

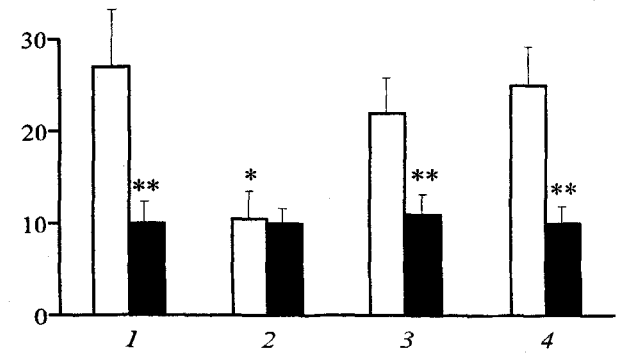

Fig. 1. Amplitude of phenylephrine-induced relaxation of rat tracheal segments (1 μM; percent of the amplitude of KCl-induced precontraction, 40 mM). *1*) control; *2*) pretreatment with methylene blue (50 μM); *3*) pretreatment with indomethacin (5 μM); *4*) pretreatment with nordihydroguaiaretic acid (5 mM). Light bars: segments with epithelium. Dark bars: segments without epithelium. $p<0.05$, *in comparison with the control; **in comparison with intact segments.

Our results indicate that the relaxing factor produced by respiratory epithelium in response to Phe stimulation arises primarily from NO metabolism, and the products of epithelial AA metabolism do not contribute significantly to the mechanisms of epithelium-dependent relaxation of the respiratory tract smooth muscles.

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