Role of Respiratory Epithelium in the Development of Hyperreactivity of Bronchial Smooth Muscles

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In guinea pigs sensitized with ovalbumin the respiratory epithelium lost its ability to modulate the responses of airway smooth muscles to histaminergic stimuli. Incubation of bronchial segments with IL-5 potenntiated the contractile responses of bronchial smooth muscles to histamine in both intact and sensitized animals. Incubation of bronchial segments with IL-5 receptors moderated contractile activity of segments from sensitized pigs, but not in the segments from intact controls.

Key Words: *respiratory epithelium; smooth muscle; hyperreactivity*

Modern physiology and medicine focus on the mechanisms regulating electrical and contractile properties of smooth muscles (SM) in the viscera. The study of these properties clarifies the intimate mechanisms of pathogenesis and outlines novel approaches at correction of pathological states. These studies are extremely important for the respiratory system, because many respiratory diseases are associated with disturbances in the regulatory mechanisms in airway respiratory epithelium and SM cells [1-4].

Our aim was to examine the role of respiratory epithelium in the regulation of contractile activity of airway SM in guinea pigs during the development of bronchial hyperreactivity.

MATERIALS AND METHODS

The study was carried out on isolated bronchial SM rings from mature male guinea pigs. Animals of the experimental group were sensitized with 0.25% ovalbumin in physiological saline (three subcutaneous injections, 0.1 ml/100 g body weight). The period between injections was 3-4 days. On postinjection day 21, the pigs were subjected to inhalation of aerosol prepared from the same solution. The control pigs were injected with physiological saline.

Mechanical tension of SM rings was recorded in a quasi-isometric mode in aerated Krebs solution (37°C) using a 6MKh1B mechanotron. The experiments were carried out on segments with intact epithelium and on denuded ones. In the later case, the epithelium was removed by mechanical rotation of a wood spatula in the lumen for 1 min.

Before measurements, the segments were tested in a high-potassium Krebs solution (40 mM) and this SM response was taken as 100%.

The composition of Krebs solution was (in mM): 120.4 NaCl; 5.9 KCl; 2.5 $CaCl_2$; 1.2 $MgCl_2$; 11.5 $C_2H_{12}O_6$; 15.5 Tris(hydroxymethyl)aminomethane; pH 7.36±0.01. The reagents were histamine and ovalbumin (ICN Biomedicals), human recombinant IL-5 (Biosource International), and IL-5 receptor (R&D Systems).

RESULTS

Histamine applied in concentrations of 0.1 nM-100 μM induced dose-dependent contractions of all bronchial segments from control guinea pigs (Fig. 1, *a*). The maximum contraction amplitude (CA) of bronchi with intact epithelium (n=15) observed during applica-

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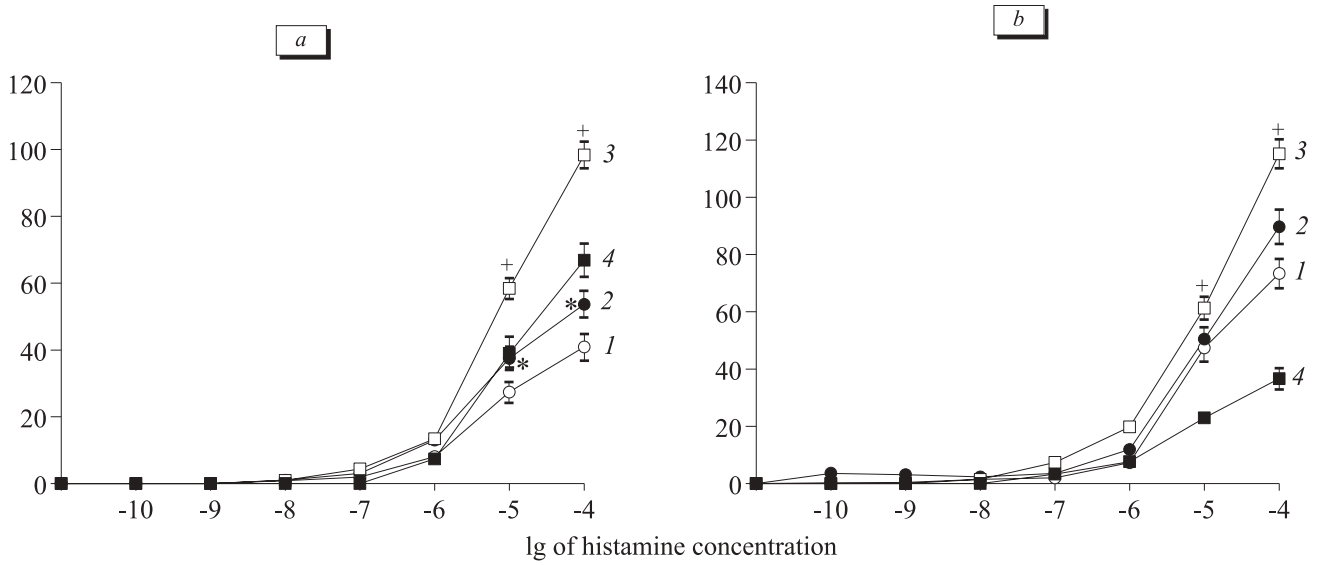

Fig. 1. Dose-dependent histamine-induced contractions of smooth muscle in bronchial segments of intact (*a*) and sensitized (*b*) guinea pigs. Ordinate: contraction amplitude in percent to control response induced by high-potassium Krebs solution. *1*) segments with intact epithelium; *2*) denuded segments; *3*) denuded segments incubated with IL-5; *4*) denuded segments incubated with IL-5 receptor (1 μg/ml). **p*<0.05 in comparison with *1*; +*p*<0.05 in comparison with *2*.

tion of histamine at 100 μM was 40.96±2.44% of control CA observed in high-potassium Krebs solution.

Denuded segments also demonstrated dose-response contractions induced by 0.01-100.00 μM histamine (Fig. 1, *a*). The maximum CA at 100 μM histamine surpassed that observed for intact segments: 53.65±1.82% ($p<0.05$, $n=9$). The differences in the contractile responses of denuded and intact segments can be explained by the fact that histamine induces production of relaxing factors (*e.g.* NO) in epithelium thereby moderating the contractile responses [2,6]. Therefore, removal of epithelium enhanced CA in SM.

Histamine (0.1-100 μM) induced contraction of segments with intact epithelium from sensitized guinea pigs (Fig. 1, *b*), the maximum CA being 73.36± 6.32% ($n=13$). At concentrations of 10 and 100 μM, CA of experimental segments from sensitized animals significantly surpassed that of control segments ($p<0.05$).

Denuded segments from sensitized guinea pigs also responded to 0.1-100.0 μM histamine by dose-dependent contractions. The maximum CA was 89.74± 7.49 at 100 μM histamine ($n=18$, Fig. 1, *b*). Thus, the histaminergic contractile reactions of intact and denuded segments of sensitized animals did not differ by their amplitude. It can be hypothesized that ovalbumin-induced sensitization damages the epithelium and decreases production of relaxing factors.

In control and sensitized guinea pigs incubation with IL-5 significantly potentiated histamine-induced contraction of denuded bronchial segments. At histamine concentrations of 10 and 100 μM, this increase was observed in control ($p<0.05$, $n=13$) and experimental group ($p<0.05$, $n=9$, Fig. 1).

Since incubation with IL-5 significantly changed responsiveness of segments to histamine, it can be hypothesized that IL-5 affects functional state of SM. The effect of this cytokine can be mediated via the receptor complex [7,8]. The IL-5 receptors were found in airway SM cells [5,9]. Probably, IL-5 can modulate the contractile properties of SM via receptors of SM membranes. This hypothesis can explain the phenomenon of IL-5 mediated bronchial hyperreactivity in tissues without eosinophil-produced damage [5].

In order to prove receptor-specific effect of IL-5, the segments were incubated in a medium with IL-5 and α-subunit of its receptor in the concentrations of 0.1, 0.5, and 1.0 μg/ml. In both groups, addition of IL-5 receptor to the medium containing IL-5 significantly decreased CA of histamine-evoked responses in contrast to the experiments where only IL-5 was added to the medium. The decrease of CA was dose-dependent.

Incubation of SM-segment with α-subunit of IL-5 receptor in the absence of the agonist moderated histaminergic contractile reactions only in segments from sensitized animals, but produced no effect in control segments. These findings suggest that tissue level of endogenous IL-5 is increased in sensitized animals, but its receptors added to the incubation medium "trap" the agonist molecules.

Therefore, sensitization disturbs the integrity of the epithelial layer and provokes irreversible changes in airways, which lead to deficiency of NO-ergic bronchial relaxation and loss of epithelial endocrine func-

tions [3]. The epithelium loses the ability to modulate the response of SM to histaminergic stimulation, which explains the absence of differences between contractile responses of bronchial segments of sensitized guinea pigs with and without epithelium.

Potentiation of contractile responses of bronchial SM to histamine in the animals of both groups by IL-5 results from its interaction with the corresponding receptors on SM cells. Probably, the observed decrease in contractile responses of bronchial SM after incubation with IL-5 receptor attests to ability of dissolved receptor to bind both exogenous IL added to the medium during incubation and endogenous IL produced by cells in airway bronchial walls in sensitized animals.

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