Age-Related Characteristics of Epithelial-Muscular Interactions in the Rat Trachea

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The interactions between epithelial and muscular cells of tracheal wall were studied by a mechanographic technique in rats of different ages. The state of the epithelium was assessed using hematoxylin-eosin stained sections. The epithelium-dependent relaxation was found to be most pronounced in 10-12-week-old rats. It was not observed in younger rats probably due to the low sensitivity of guanylate cyclase to activating stimuli. Destructive changes in the respiratory epithelium after 24 weeks of life reduced the amplitude of epithelium-dependent relaxation in old rats.

Key Words: *epithelium; trachea; smooth muscles; age*

It has been recently shown that the respiratory epithelium plays an active role in the regulation of contractile activity of airways' smooth muscles [4,5,12]. Through the production of relaxing factors it affects both the basal tone of bronchi and their responsiveness to biologically active substances.

Sensitive targets of epithelial factors are the ion-transporting systems of the sarcolemma and the contractile proteins of muscle cells.

Acetylcholine, histamine, and catecholamines stimulate the production of a relaxing factor by bronchial and tracheal epithelium. The effects of epinephrine on the carbachol-precontracted tracheal segments of rats are largely epithelium-dependent [1].

In the available literature we did not find any data on age-specific characteristics of relaxing epithelial effects on the muscular walls of the airways. At the same time, numerous data indicate that significant morphological and functional changes occur in the tracheobronchial system during ontogenesis [8,10,11].

In the present study we investigated the interactions between the tracheal epithelial and muscular cells in rats of different ages.

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MATERIAL AND METHODS

Experiments were carried out on outbred male albino rats divided into three age groups: group 1, immature rats, 2-4 weeks old; group 2, mature rats, 10-12 weeks old; group 3, old rats, aged over 22-24 weeks. These ages were selected in accordance with the data on age-related characteristics of the respiratory system in rats [10].

The portion of the trachea from the larynx to the bifurcation was separated from adipose and connective tissues and sectioned into 2-3-mm ring segments. If necessary, the epithelium was mechanically removed by a wooden spatula rotated in tracheal lumen for 1 min.

Four ring segments were fixed in a constant-temperature bath filled with a constantly aerated Krebs solution containing (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 segments was recorded by a 6MC-2B mechanotrons under close to isometric conditions.

Morphological changes in the tracheal wall were studied on preparations stained with hematoxylin and eosin by standard methods.

RESULTS

The epithelium-dependent relaxation of tracheal muscular cells induced by epinephrine (1 μM) was mea-

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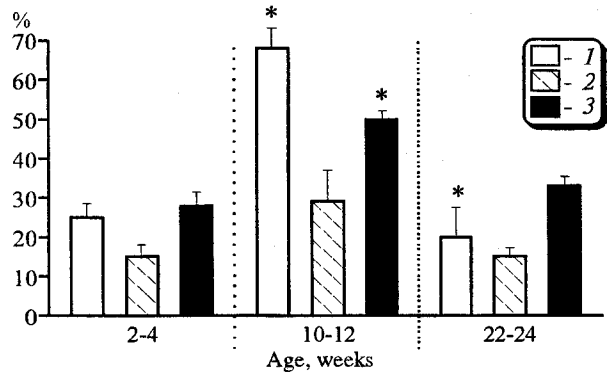

Fig. 1. The amplitude of relaxation of tracheal segments in rats of different age groups (in percent of the amplitude of precontraction). *1*) epinephrine (1 μM) effect in the presence of epithelium; *2*) epinephrine (1 μM) effect in the absence of epithelium; *3*) sodium nitroprusside (1 μM) effect on epithelium-free segments, $^*p<0.05$ in comparison with the preceding age group.

sured as a percentage of the amplitude of carbachol (5 μM)-induced precontraction. Under these conditions the epithelium contributes to the catecholaminergic responses [2] (Fig. 1).

The tracheal segments without the epithelium responded to epinephrine by only minor relaxation in all age groups. Slight differences in the responsiveness were probably due to age-related variations in the activity of muscular adenylate cyclase [8].

By contrast, the responses of tracheal segments with intact epithelium revealed significant age-related differences. Thus, the epithelium-dependent relaxation was most pronounced in the intermediate age group, while these reactions were suppressed in preparations from both the youngest and the oldest rats. Therefore, the interactions between the epithelial and muscular cells undergo considerable ontogenetic alterations.

The age-related differences can be attributed to changes in either epithelial or muscular cells. To analyze this question, we studied the effects of sodium nitroprusside on the mechanical tension of epithelium-free tracheal segments obtained from rats of different age groups.

As seen from Fig. 1, the lowest amplitude of the nitroprusside-induced relaxation was recorded in the youngest rats.

Nitroprusside activates guanylate cyclase [3,7,9], which is a crucial enzyme in the epithelium-dependent muscular relaxation [1,6]. These data indicate that the

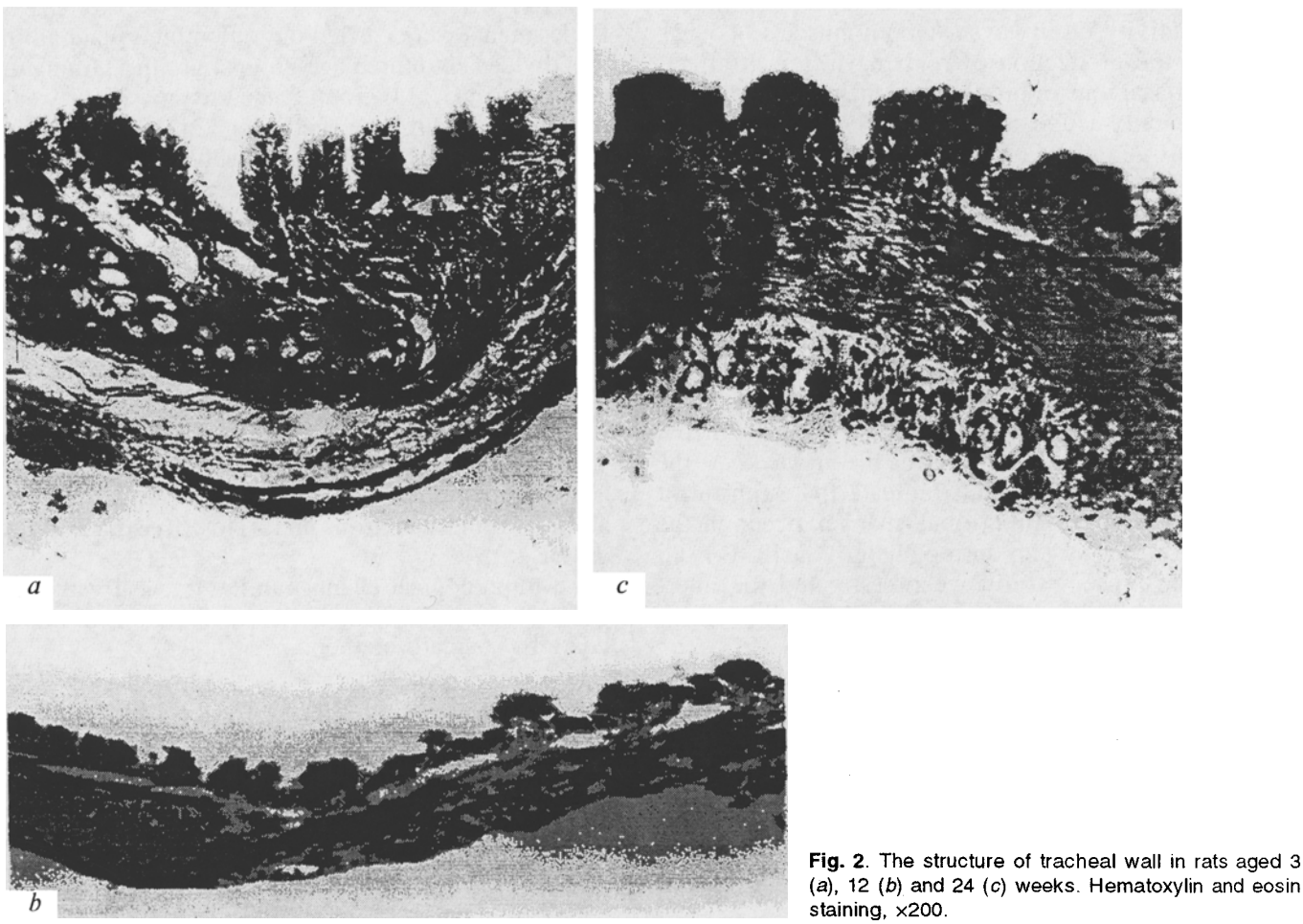

Fig. 2. The structure of tracheal wall in rats aged 3 (*a*), 12 (*b*) and 24 (*c*) weeks. Hematoxylin and eosin staining, ×200.

activation is not pronounced in young animals and develops only after 10-12 weeks of life.

Sections prepared from the trachea of 2-4-week-old rats (Fig. 2, *a*) showed no signs of impaired epithelial layer morphology. Epithelium was arranged as a monolayer at the level of the cartilage plate and had normal appearance.

Visual inspection of tracheal sections from 12-14-week-old rats (Fig. 2, *b*) revealed thin epithelial layer with nearly cubiform epitheliocytes. A hyperchromic cytoplasm and moderate edema of the subepithelial membrane were noted. Thus, although changes in epithelial morphology were present at this age, they could not be considered as destructive and did not affect the ability of epitheliocytes to produce a relaxing factor.

Epithelial exfoliation was observed in the sections prepared from the trachea of rats older than 24 weeks (Fig. 2, *c*). The thickness of the epithelium tended to decrease, the number of goblet cells increased. The terminal parts of glands in the subepithelial membrane were hyperplasic, the membrane showed signs of hardening due to the appearance of numerous cellular structures. These manifestations can be considered as destructive being inevitably accompanied by the reduction of functional activity.

It is likely that a low amplitude of epithelium-dependent relaxation of the tracheal segments in young rats is explained by low sensitivity of the smooth muscle cell guanylate cyclase to activating factors. This sensitivity appears on the 10th-12th week of life and the epithelium-dependent reactions become most pronounced.

After 24 weeks, the respiratory epithelium shows destructive transformations which are accompanied by reduction in its functional activity. This results in suppression of the epithelium-dependent relaxation induced by different substances.

The data obtained point to one of the possible causes of an increased risk of bronchiospastic reactions in young and old age.

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