Experimental Evidence on Metasympathetic Nervous Mechanisms of Asthma

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Electrical activity from tracheal and bronchial smooth muscles, either spontaneous or induced by transmural electrical stimulation of muscles and nerves, was recorded in experiments with local anesthetics, ganglioblockers, and biologically active substances (histamine and vasoactive intestinal peptide). A hypothesis on mechanisms the underlying genesis of asthmatic state was formulated.

Key Words: *asthma, metasympathetic nervous system, trachea and bronchi*

Allergic reaction is often manifested in bronchial asthma with bronchospasm as a compulsory component of its pathogenesis.

Hyperreactivity of the bronchi is associated with hypersensitivity of their receptors [6,10] and autonomic nervous system disorders [4,5,9]. Epithelium and smooth muscle here are the links of the same reflex arc, connected either centrally or locally or having the axon-reflex origin [9].

In addition to extraorganic sympathetic and parasympathetic innervation, the trachea and bronchi are controlled locally by their own metasympathetic nerve system (MNS). MNS nerve cells are arranged in functional modules consisting of oscillatory, sensory, tonic, and effector neurons and interneurones. These nerve modules provide motor, secretory, and excretory functions and regulate regional circulation, endocrine, and immune processes [1,2]. We studied the role of certain elements of the MNS modules in the initiation of bronchial constriction.

MATERIALS AND METHODS

Experiments were performed *in vivo* on random-bred albino rats (n=20) weighing 180-300 g, anesthetized with Urethane (1.2-1.5 g/kg), and *in vitro* on isolated tracheal segments (n=27). The surgery procedure, methods of muscle stimulation, mechanical and electrical activity registration and *in vitro* preparation maintenance were described elsewhere [7,8]. Muscular tension in cervical segments (6 rings below the larynx) and in thoracic segments (6 rings above the bifurcation) were recorded with an electromechanical detector of displacement. Initial preloading was 500 mg. Transmural electrical stimulation (5-20 Hz, 15 V, 0.3-3.0 sec) with an ESL-1 stimulator was applied to tracheal segments for 10 sec. By varying stimulus duration one can selectively excite muscle or nerve fibers [8].

The results were statistically analyzed by Student's t test.

RESULTS

In spontaneously breathing animals, electrical activity in tracheal and in bronchial smooth muscles was recorded as discharge bursts occurring during inspiration. The mean frequency in a burst was 49.0±0.3 pulses/sec. The muscle tone changed in step with respiration. Constriction started in the second half of inspiration or at the beginning of the expiration and was 451.0±102.4 mg. When respiration was of low frequency (30 cycles/min or less) or shallow, the rhythmic activity was weak or absent. By contrast, deep inspiration increased the

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discharge frequency. In artificially ventilated curarized animals, electrical activity of tracheal muscle changed in a similar manner as in spontaneously breathing animals. At low frequency ventilation, the bursts disappeared and appeared again with increased ventilation volume. During a short standstill (10-15 sec) the bursting activity remained; however, the interburst intervals became longer. After bilateral vagotomy, rhythmic discharges continued in 78% experiments, although the discharge frequency decreased to 58.4±18.8%. This coincided with a decrease in tracheal segment constriction (to 35.7±5.4%, $p<0.05$). These findings imply that when the air flow runs along the respiratory pathways it excites the tracheobronchial receptors that activate the MNS regulatory modules with subsequent generation of rhythmic smooth muscle activity in the tracheal and bronchial walls. To verify this supposition, experiments with the local anesthetic procaine, ganglioblocker benzohexonium, and histamine were performed.

Procaine (2%, 0.05 ml) being applied on the tracheal epithelium at the site of the action potential recording increased the discharge frequency to 118.7±2.6%. The burst discharge pattern was replaced by the uniform pattern (Fig. 1) and was restored after 20-25 min. These experiments showed that receptor inhibition in spontaneously breathing rats causes discharge and muscular tone changes resulting in reduced respiratory tract cross-section area.

Intravenous benzohexonium (100 mg/kg) increased the tracheal muscle discharge frequency to 224±10%. The rhythmic bursts disappeared 5 min after injection and the discharges became uniform. The rhythmic segmental constriction practically stopped, and the muscle tone increased 1.53-fold ($p<0.05$). Twenty minutes after the injection, the muscle activity returned to the initial level and became rhythmic. These findings indicate that peripheral nervous mechanisms associated with the MNS modules play the key role in the generation of the muscle rhythmic discharge.

To check up this hypothesis, *in vitro* experiments with isolated tracheal segments were performed, in which electrical stimulation and various concentrations of both substances were applied. Perfusion of isolated tracheal preparation with solution containing procaine (100-1000 μM) induced a dose-dependent constriction of thoracic segments and had no effect on the muscular tone of cervical segments. Procaine also produced different effects on the response of thoracic and cervical segments to transmural electrical stimulation. Regardless the parameters of stimulation, procaine (1-1000 μM) decreased the constriction of the cervical part of the trachea in response to stimulation; the effect varied

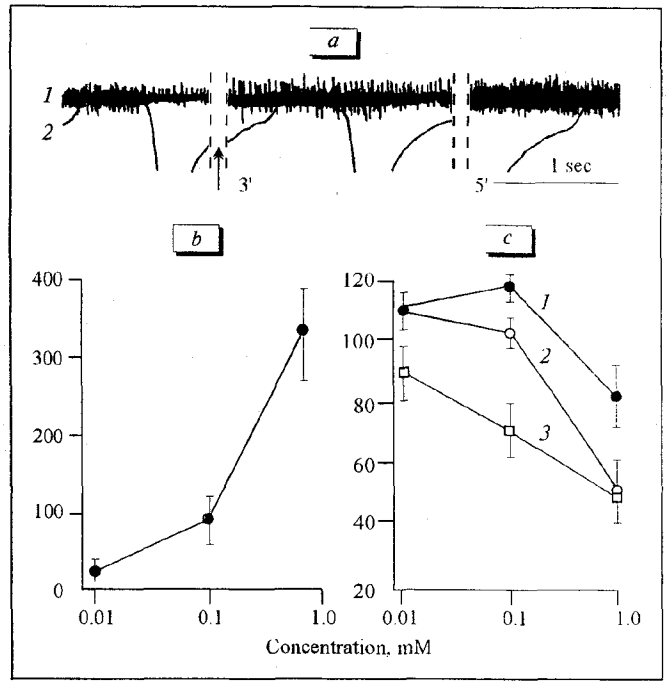

Fig. 1. Effect of procaine on electrical activity (*a*), muscular tension (*b*), and responses to transmural stimulation (*c*) of rat tracheal smooth muscle. *a*) myogram (*1*), airflow (*2*), time after procaine injection (3' and 5'); *b*) ordinate: muscular tension increment, mg; *c*: thoracic (*1,2*) and cervical (*3*) tracheal part responses to stimulation of preganglionic (*1*) and postganglionic (*2,3*) nerve fibers; ordinate: response, %.

linearly with concentration. A different situation arose with the thoracic segments. At 10 μM procaine, constriction in these segments in response to stimulation increased to 130.8±8.7% ($p<0.05$). Only at higher concentrations the anesthetic decreased the response amplitude.

Benzohexonium (100 μM) did not affect the smooth muscle tone in cervical segments and caused marked constriction in thoracic segments: 737±186 mg. This contraction was characterized by depolarization of myocytes; both amplitude and frequency of discharges increased 1.5-fold. The muscarinic receptor blocker atropine (10 μM) abolished these effects. Perfusion of the preparation with a saline containing benzohexonium altered not only spontaneous activity of tracheal muscle but also the response of thoracic segments to nerve fiber stimulation. At 10 μM benzohexonium, the amplitude of the response reached 140% of the initial value. At 100 μM benzohexonium, the amplitude of the response decreased to 50% ($p<0.01$). It should be noted that under these conditions no changes were observed in muscular responses to transmural stimulation of the muscle *per se*.

Histamine (0.01-1.0 mg/ml) had a weak effect on the tracheal muscle tone. There was no difference in its effect on the thoracic and cervical

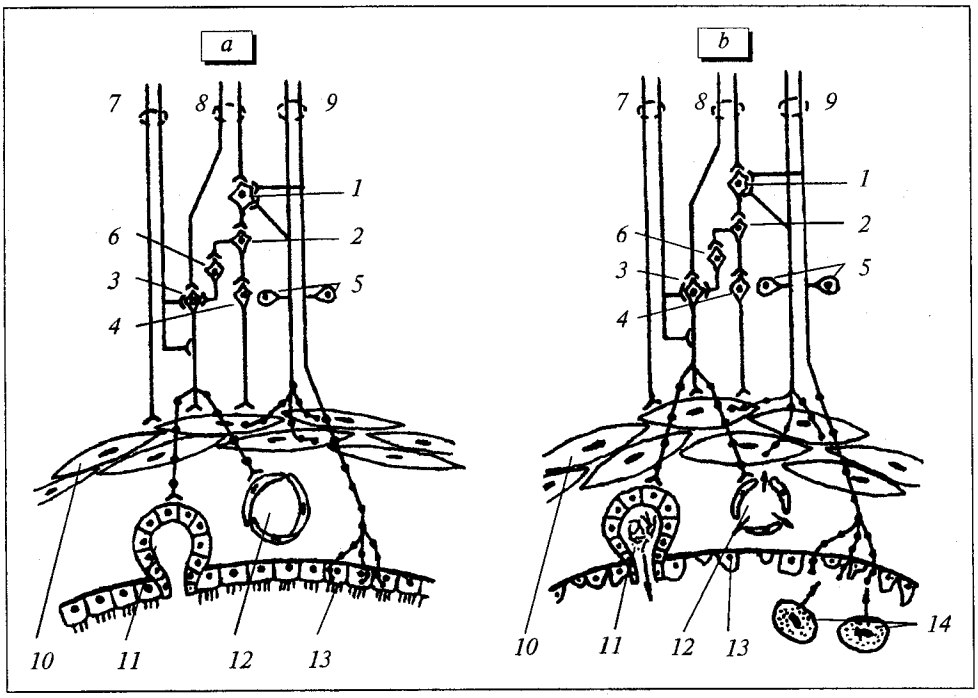

Fig. 2. Scheme of tracheal and bronchial innervation in normal (*a*) and in asthmatic bronchospasm (*b*). Neurons: rhythm generator (*1,2*), excitatory (*3*) and inhibitory (*4*) effector, sensory (*5*), tonic (*6*); sympathetic (*7*) and parasympathetic (*8*) and nerve fibers (*9*); smooth muscle (*10*); gland (*11*); blood vessel (*12*); epithelium (*13*); inflammation cells (*14*).

segments. Selective transmural stimulation of muscles and nerves induced similar reactions in thoracic and cervical trachea. Vasoactive intestinal peptide (0.01-0.5 μM; n=19) did not affect the muscular tone of thoracic and cervical segments. However, it produced a substantial effect on the constriction reactions evoked by transmural stimulation, the maximum effect (168.8±6.5%) being observed at the peptide concentration of 1 nM (p<0.01).

Thus, rhythmic burst activity in tracheal muscles is generated if there is an air flow in the respiratory tract. The activity is preserved after bilateral vagotomy and is modified by local anesthetics. This finding suggests that tracheobronchial receptors are involved in the smooth muscle activity regulation, and, in particular, in the generation of rhythmic bursts [12,13]. Thus, there are reflex influences on the respiratory tract smooth muscles rising from the tracheobronchial receptive fields. These data indicate that MNS modules are responsible for these viscero-visceral interactions. Strictly speaking, MNS modules close the local reflex arc. They represent independent low-level neural centers (like microprocessors incorporated into the tracheobronchial wall) that function locally without influencing the upper bulbar, thalamic, and cortical structures [1,3]. Dysfunction of MNS modules may be a component of the pathogenesis of asthma.

Intense epithelial exfoliation occurring in the trachea and bronchi is regarded as specific manifestation of bronchial asthma [6,11-15]. These symptoms can be documented by bronchial biopsy at the beginning of the disease [9]. Epithelial cell exfoliation and destruction in the bronchi are accompanied by increased secretion of spasmogenic factors (for example, vasoactive intestinal peptide), similar to the events occurring in vascular endothelium [13]. As a result of epithelium damage, receptor terminals become exposed to the histamine-like inflammatory transmitters of the MNS sensory neurons. During inflammation, plasma kininogen is transformed into kinin, which selectively stimulates C-fibers. Moreover, pulmonary mast cells release kininogenase which may induce bradykinin synthesis. Bradykinin causes a powerful bronchial constriction reflex in patients with asthma and has practically no effect on intact isolated bronchi [9,13]. The effect of bradykinin is abolished by the substance P antagonists. This implies that in addition to reflex action bradykinin initiates the release of sensory neuropeptides [3] from C-fiber endings exposed as a result of epithelial damage (Fig. 2).

Inflammatory products stimulate the MNS nerve fibers and their sensory endings exposed due to epithelial exfoliation, which results in spastic constriction of tracheal muscles. The lower respiratory tract may be involved in the process via viscero-visceral reflex mechanisms associated with MNS neural modules. Thus, the reaction can be amplified by these modules. Generally, long-term local reflex

facilitates the transmission in interneuronal and effector parts of the reflex, lowering the thresholds for the initiation of local reaction.

This mechanism may have practical implications. In animals, corticosteroids reduce the sensitivity of bronchial receptors and enhance bronchial constriction induced by bradykinin [9]. The effect is due to blockade of neuropeptides released by sensory neurons. Interestingly, some anesthetics (chloroform) are effective even if antiasthmatic therapy is ineffective [6]. Although much has to be clarified in this phenomenon, it obviously results from the inhibition of local regulatory mechanisms associated with MNS modules.

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