MORPHOLOGY AND PATHOMORPHOLOGY

Nitrergic Neurons in Respiratory Organs

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NADPH-diaphorase colocalized with nitric oxide synthase in neurons of respiratory organ was studied. Sensory pseudounipolars and Dogiel types I and II multipolars expressing nitric oxide were identified.

Key Words: *nitric oxide synthase; nitric oxide; nitrergic neurons*

Nitric oxide (NO) participates in the regulation of bronchial reactions [3]. NO belongs to neurotransmitters of bronchodilatory nerves of nonadrenergic and noncholinergic innervation [10]. Some NO-ergic axons of submucous plexuses belong to the vagus nerve and others are processes of local neurons [5]. However, expression of NO by neurocytes of the respiratory tract has never been reported. The purpose of our study was to identify nerve cells with NO-ergic function.

MATERIALS AND METHODS

Respiratory organs of 12 albino rats (150-180 g) and 5 adult humans were examined. Rat trachea and bronchial tree were examined *in toto;* 1-cm fragments were cut from human trachea, main bronchi, and lungs.

NO-synthase was located by the histochemical test for NADPH-diaphorase [7]. Material was fixed in cold 4% paraformaldehyde on 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. Of all diaphorases, only NADPH-diaphorase retains activity under such conditions [7]. The material was washed for 24 h in 15% sucrose at the same temperature. Cryostat sections (10 μ) were placed onto slides and put into medium containing (in mM): 50 Tris buffer (pH 8.0), 1 NADPH (Sigma), 0.5 NBT (Sigma), and 0.2% Triton X-100 (Serva). The sections were incubated for 60 min at 37°C, washed in distilled water, dehydrated, and embedded in balm. Activity of NADPH-diaphorase was evaluated on an M-85 densitometer (Vickers).

RESULTS

Diaphorase-positive neurons containing equivalent amount of NO synthase [1] were detected in the airways (trachea and bronchi). This constitutive form, or type I enzyme, is characteristic of nerve cells [8].

Two types of neurons differing by shape, topography, and NO synthase activity were identified.

One type are typical pseudounipolar cells (Fig. 1, *a, b*) characteristic of cerebrospinal nodes. An initial process originates from a pear-shaped neuronal body; this process divides in a T-shaped manner into two branches (Fig. 1, *b*). Activity of NO synthase is low in the majority of cells (8.8±0.7 opt. density units), in few cells it increased twofold (14.6±1.2 opt. density units), which is in line with published reports about enzyme content in protoneurons of spinal ganglia [9].

Pseudounipolars are permanently located in the adventitium of the upper portion of the trachea, where they are assembled into microganglia (Fig. 1, *a*). Each nodule is covered by a thin connective capsule and has its own microcirculatory bed. Solitary cells lie along and inside the vagus nerve and their processes are incorporated in it. Associations of pseudounipolars are characteristic of rats. In man, such groups contain no more than 3 neurons.

These heterotypical cells are traditionally considered as sensory neurons [2]. Recently these cells were

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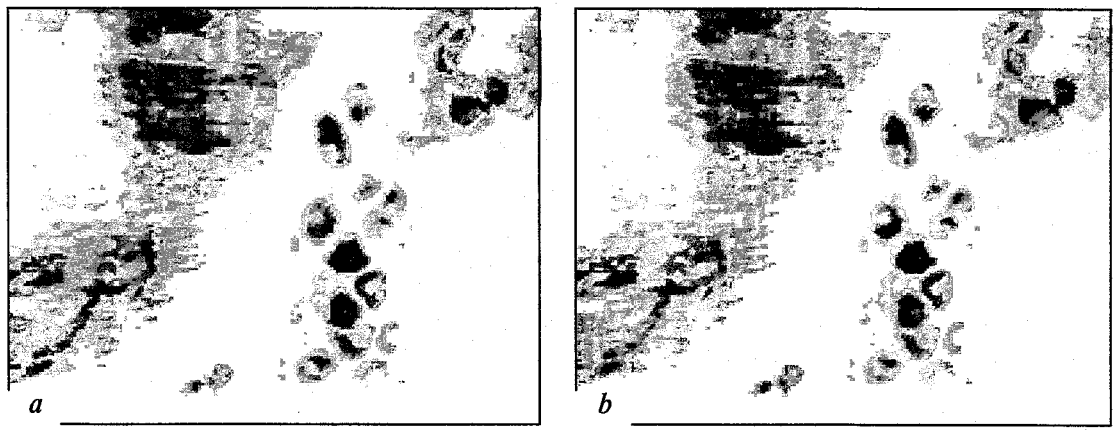

Fig. 1. NO-ergic pseudounipolars (method [7]). *a*) microganglion in rat trachea, ×100; *b*) pseudounipolar in human trachea, ×400.

considered as effector neurons due to their capacity to produce NO. P. Barnes [4] considers that protoneurons of the nodular ganglion modulate the smooth muscle tone of the bronchi and bronchial arteries via NO-mediated axon-reflector effects. Therefore, pseudounipolars expressing NO are not only NO-positive, but also NO-ergic nerve cells.

Another morphological type includes multipolar neurons characteristic of the autonomic nervous system nodes. They are arranged in groups of 4-5 elements in the tracheal adventitium in rats but not in men (Fig. 2, *a*).

Solitary multipolars are located along the bronchial tree. Some lie in the submucosa and are found in the bronchi with a cartilaginous skeleton and in small bronchi without skeleton (Fig. 2, *c*), others are anatomically fixed on the bronchial arteries and their processes grow into the vascular wall (Fig. 2, *d*).

By their morphological signs, multipolars conform to classification of autonomic neurons proposed

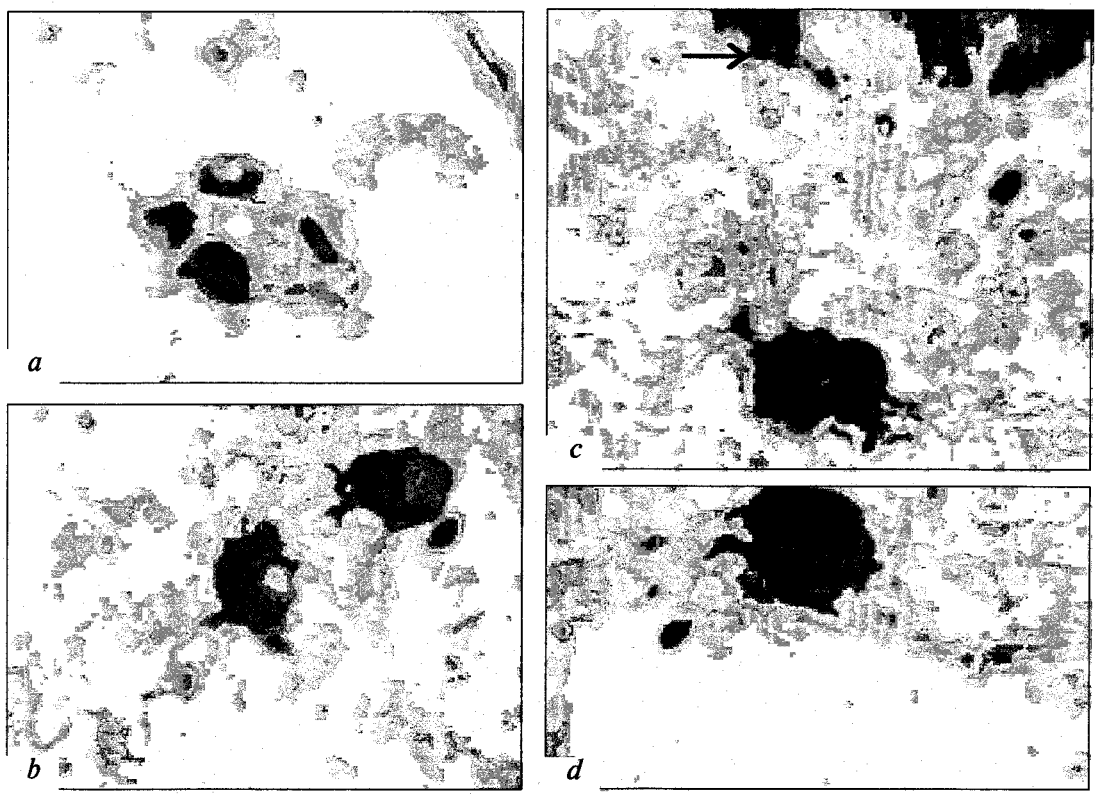

Fig. 2. Multipolar NO-ergic neurons (method [7]). *a*) microganglion in rat tracheal adventitium, ×100; *b*) type I neurons (Dogiel) in human tracheal adventitium, ×400; *c*) type II neuron (Dogiel) in the submucosa of the rat acartilagenous bronchus, ×400. Arrow shows NO-positive bronchial epithelium; *d*) multipolar neuron on the wall of rat bronchial artery, ×400.

by A. S. Dogiel [6]. Some of them possess short thick dendrites and clearly seen axon (Fig. 2, *b*). These are, no doubt, type I cells. Others are similar to type II neurons. Thin varicose nerve processes originating from the cell poles lie longitudinally along the bronchial walls (Fig. 2, *c*). Sensory function of these cells is now acknowledged. Hence, the respiratory tract has autonomic NO-ergic neurons which can be regarded as a substrate of local reflexes, but no direct connections between types I and II neurons were detected.

Activity of NO synthase in all multipolars is high (37.0±3.2 opt. density units), which distinguishes them from pseudounipolar neurons in the tracheal adventitium.

The most complete data on local NO-ergic neurons were obtained in rats: their respiratory organs were studied on a single section from trachea to the acinus. This helped more often observe local neurons, which was ruled out in studies of human airways, because sections from small sites of the trachea and bronchi rarely contained NO synthase-positive neurons.

Not all neurons express constitutive enzyme. For example, similarly to tracheal pseudounipolars, only 10-20% protoneurons of the cerebrospinal nodes are NO synthase-positive [9]. Apparently, not all local neurons of the respiratory organs can secrete NO.

Hence, sensitive unipolars and types I and II multipolars (according to Dogiel) positively reacting to NO synthase were detected in human and rat airways. These nerve cells should be regarded as a part of nonadrenergic and noncholinergic nerve system of respiratory organs.

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