Nitric Oxidergic Function of Chromaffin Cells of Respiratory Organs

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We examined NADPH-diaphorase co-localized with nitric oxide synthase in chromaffin cells of microparaganglia of rat respiratory organs. Nitric oxidergic function of chromaffinocytes and participation of nitric oxide in exocytosis of biogenic monoamines are established.

Key Words: *nitric oxide synthase; nitric oxide; chromaffinocytes; exocytosis*

Many cells of mammalian respiratory organs produce nitric oxide (NO) [3,4]. However, there are no data on NO production by chromaffin cells (CAC) widely distributed in the lungs [5]. The aim of the present study was to examine the possibility of NO synthesis by CAC and to establish the role of NO in CAC functioning on the model of exocytosis of biogenic monoamines.

MATERIALS AND METHODS

The study was carried out with 20 albino male rats (150-180 g) kept under standard vivarium conditions. The animals were decapitated and lung specimens were obtained.

Topochemistry and activity of NADPH-diaphorase co-localized with NO-synthase [9] were determined. Lung fragments were fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. Only NADPH-diaphorase retaines its activity under these conditions. Tissue fragments were washed for 1 day in 15% sucrose at the same temperature, frozen in a cryostat, and cut into 10-μ thick sections. The sections mounted on glass slides were incubated in a medium containing 1 mM NADPH (Sigma) in 50 mM Tris-buffer (pH 8.0), 0.5 mM nitroblue tetrazolium (Sigma), and 0.2% Triton X100 (Serva) at 37°C during 60 min, washed with distilled water, dehydrated, and embedded into a balsam. NADPH-diaphorase activity was determined on an M-85 microdensitometer (Vickers).

Biogenic monoamines (catecholamines) were revealed using the Falck technique. Luminescence was measured on a Lyumam I-2 microscope with a PMEL-1A packing and 0.1 mm probe at 480 nm [6]. In some experiments (n=6) NO-synthase agonist acetylcholine was injected into the pleural cavity in a therapeutic dose [2]. The tissue was examined after 5 and 10 min (3 rats per point) postinjection.

RESULTS

NO is formed from L-arginine after oxidation of a nitrogen atom by NO-synthase co-localized and depending on flavin enzyme NADPH-diaphorase. Activity of these coupled enzymes changes similarly and synchronously [1]. These enzymes are co-localized in NO producing cells and their activity is directly related to the amount of released NO [9].

Examination of lung specimens revealed NO-synthase in epithelial cells of the trachea and bronchi, alveolar macrophages, mast cells, and alveolocytes which agreed with published data [3,4].

CAC are less abundant in the lungs than other cell elements. Individual CAC primarily occur along vagus branches innervating the tracheobronchial tree (Fig. 1, *a*). Local CAC aggregates organized in microparaganglia can be revealed in the adventitia between the trachea and larynx more frequently than in other lung regions.

Each microparaganglion isolated from the surrounding tissue represents a 0.3-0.5 mm long and

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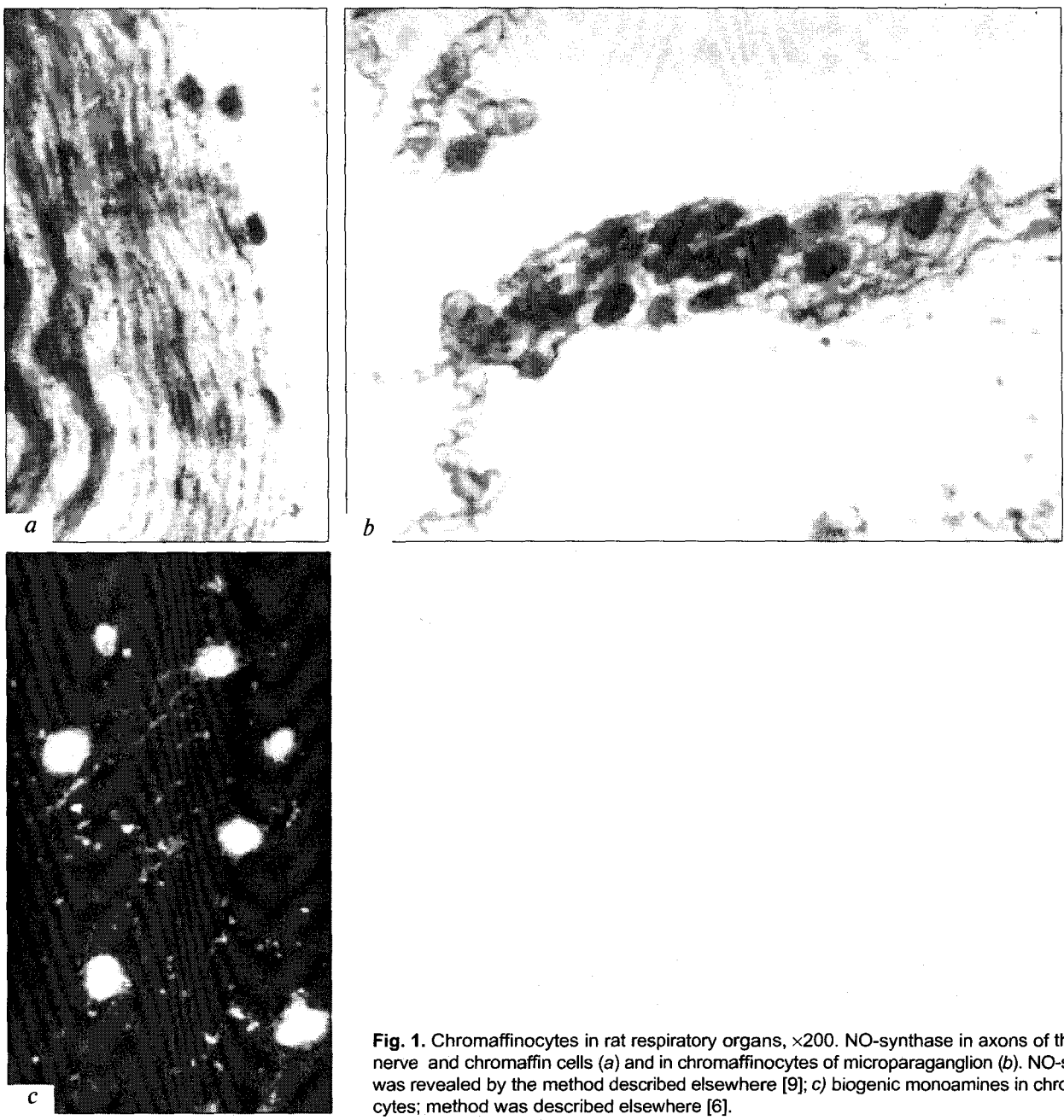

Fig. 1. Chromaffinocytes in rat respiratory organs, ×200. NO-synthase in axons of the vagus nerve and chromaffin cells (*a*) and in chromaffinocytes of microparaganglion (*b*). NO-synthase was revealed by the method described elsewhere [9]; *c)* biogenic monoamines in chromaffinocytes; method was described elsewhere [6].

0.15-0.25 mm thick structure (Fig. 1, *b*). It consists of 17-38 oval or round cells 14-16.4 μ in diameter. Microparaganglion possesses an arteriole 6-8 μ in diameter which forms capillaries between CAC and an efferent venule with a 2-fold wider diameter. The density of diformazan formed in CAC cytoplasm as a result of histochemical reaction and characterizing activity of NO-synthase reached 27.5-34.0 arb. units as compared to 31.7±1.3 arb. units as standard level.

There are contradictory data on NO producing capacities of CAC localized in other body regions. Thus, CAC of the adrenal medulla are known to produce NO [7,11], while no NO-synthase is identified in glomus cells of the carotid body [8].

We assume that CAC localized in the lungs of healthy animals express constitutive NO-synthase and its genes are induced by specific spatial information in the early ontogeny. The envinonment as the sourse of information differs in each body region. That is why chromaffin tissue does or does not produce NO under various physiological conditions.

CAC are known to secrete mainly catecholamines (dopamine and noradrenaline; Fig. 1, *c*), though serotonin production by CAC was also reported [10,12].

Intrapleural injection of acetylcholine significantly ($p<0.01$) decreased CAC luminescense to 4.1±0.1 *vs.* 9.2±1.3 arb. units in the control. At the same time, NO-synthase activity in these cells significantly increased from 31.7±1.3 to 38.8±1.7 ($p<0.01$).

These synchronous changes in NO-synthase activity and the content of biogenic monoamines suggest that NO is involved in exocytosis of monoamines. The NO-synthase agonist acetylcholine enhances its activity, which results in short-term hyperproduction of NO and cGMP-mediated potentiation of biogenic monoamines excretion.

Thus, our findings confirm the presence of NO-synthase in CAC of the respiratory organs and the role of NO in exocytosis of biogenic monoamines.

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