Regulation of NO-Producing Function of the Lungs with Salmeterol

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The state of epithelial nitric oxide synthase, bronchomotor reaction, and concentration of NO metabolites (NO_2^- and NO_3^-) in bronchoalveolar lavage fluid were studied in healthy rats and rats with experimental bronchial asthma induced by inhalations of long-acting β_2-agonist salmeterol. The effects of salmeterol on NO-producing function of the lung in healthy animals and in animals with bronchial asthma were studied.

Key Words: *nitric oxide; salmeterol; bronchial asthma*

The application of long-acting β_2-adrenoreceptor agonists in bronchial asthma (BA) is theoretically based on their ability to provide long-term relaxation of bronchial smooth muscles, to improve external respiration, and on their bronchoprotective and antiinflammatory activities [5,6,14]. However, the mechanisms underlying the action of β-adrenoreceptor agonists during bronchial obstruction remain poorly understood. There are no experimental data on morphological basis of the regulation of bronchial patency with long-acting β-agonists.

Here we studied the dynamics of NO synthase activity in the bronchial epithelium and measured the content of NO metabolites in bronchoalveolar lavage fluid (BALF) in healthy rats and in rats with experimental BA treated with long-acting β-agonist salmeterol.

MATERIALS AND METHODS

The study was carried out on 60 outbred white male rats weighing 180-200 g kept under standard vivarium conditions. BA was modeled in 30 rats by subcutaneous injections of 10 μg ovalbumin in 0.5 ml solution containing 100 mg $Al(OH)_3$ for 2 succesive days. Permissive dose of ovalbumin (0.03%, inhalation 0.8 ml/min) was administered 3 weeks after sensitization until the development of bronchospasm (acrocyanosis, tachypnea, and whistling rales).

Healthy rats and animals with BA were placed in a polyethylene chamber connected to an ultrasonic nebulizer and inhaled salmeterol (0.002 μg/l/min) for 30 min. The rats were decapitated under thiopental narcosis immediately and 3, 6, 9, and 12 h after inhalation (5 rats from each group per point). Five healthy rats and 5 rats with BA receiving physiological saline served as the control.

Morphometry of the bronchi was performed on strictly transverse sections under a Karl Zeiss microscope equipped with MOV-1. The degree of constriction and relaxation was determined as the ratio of lumen to bronchus diameter and was expressed as relaxation quotient (RQ).

NADPH-diaphorase activity was determined as described elsewhere [7]. Lung specimens (1×0.5 cm) were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 2 h and washed in 15% sucrose for 24 h at the same temperature. Cryostate sections (10 μ) were mounted on glass slides and incubated in a medium containing 50 mM Tris buffer (pH 8.0), 1 mM NADPH (Sigma), 0.5 mM NBT (Sigma), and 0.2% Triton X-100 (Serva) for 60 min at 37°C. After that the sections were rinsed in distilled water, dehydrated, and embedded into a balsam. Computer images of micropreparations were visualized

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with the help of a videotape system mounted on a Vickers M-85 microdensitometer. Enzyme activity was expressed in optical density units. The contents of NO_2^- and NO_3^- were measured as described earlier [8,12]. Supernatant (1 ml) was mixed with 0.3 g copper-impregnated cadmium dust, heated in a water bath (100°C) for 60 min, cooled, and centrifuged at 1200 rpm. The total content of NO metabolites was measured in the supernatant. To this end, supernatant was transferred to multiwell plates (100 μl per well) and mixed with 50 μl 5% NH_4Cl and 50 μl 2% N-(1-naphthyl)ethylenediamide in 5% H_3PO_4. Optical density was measured at 540 nm on a Dynatech spectrophotometer. The contents of NO_2^- and NO_3^- were expressed in nanomoles per ml BALF.

RESULTS

In the lungs of healthy animals NO is produced in large and small bronchi. All epithelial cells in these bronchi are NO-positive [2]. Normally, bronchi are relaxed (RQ=0.62) and their lumens are free (Fig. 1, *a*).

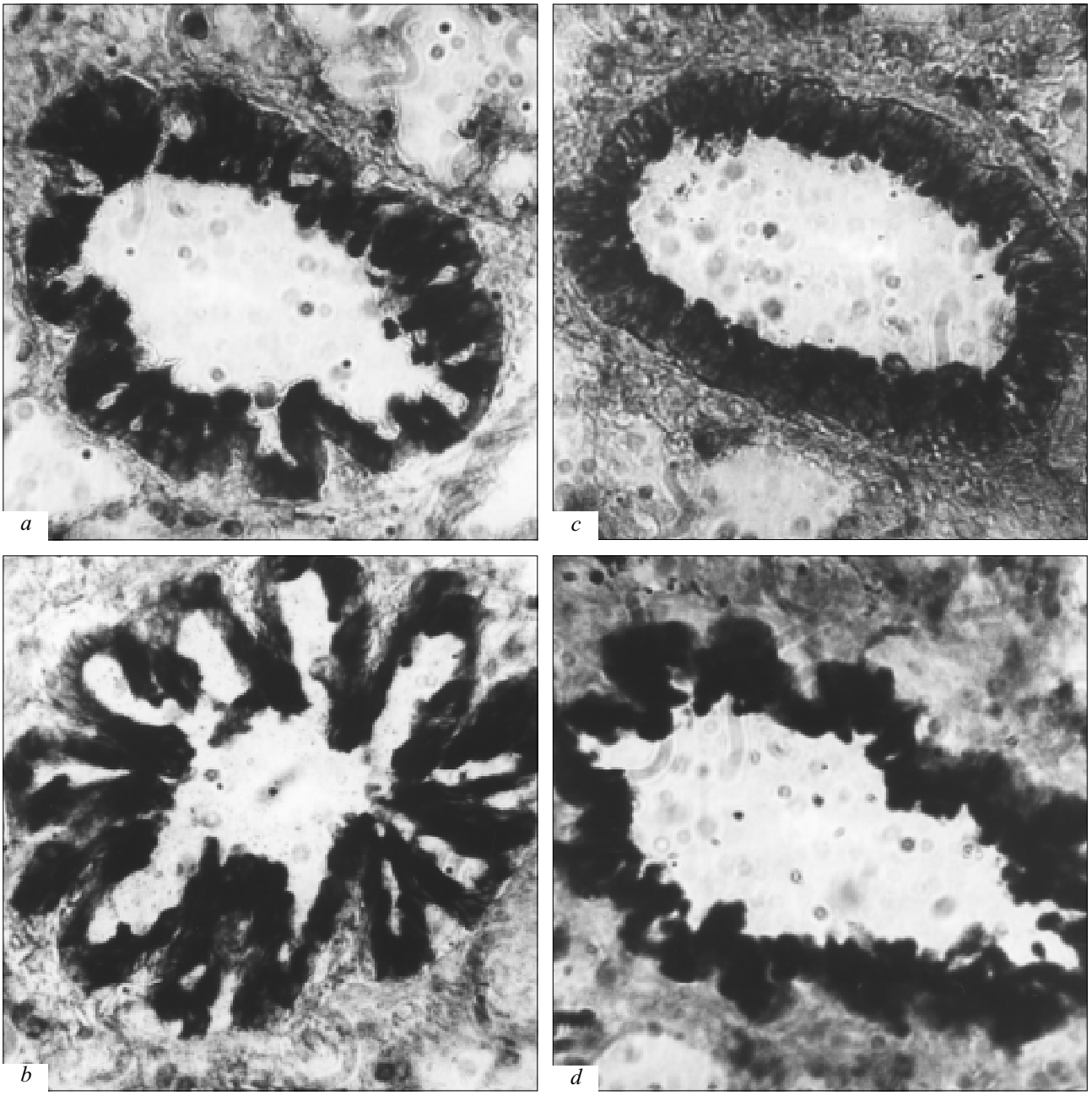

Fig. 1. Bronchus of a healthy rat (*a, c*) and a rat with experimental bronchial asthma (*b, d*) in the control (*a, b*) and after salmeterol administration (*c, d*). Hope—Vincent method, ×200.

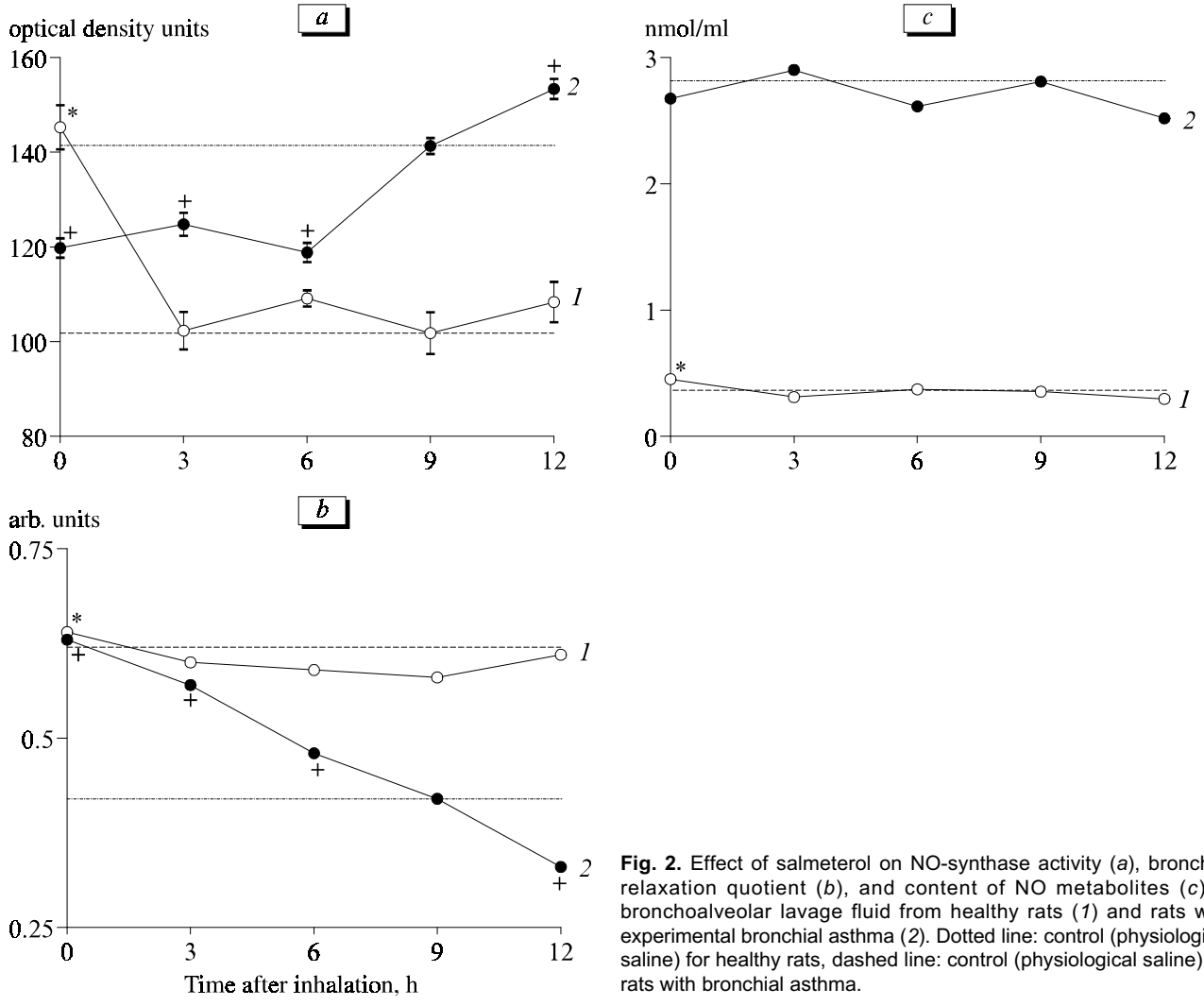

Fig. 2. Effect of salmeterol on NO-synthase activity (*a*), bronchial relaxation quotient (*b*), and content of NO metabolites (*c*) in bronchoalveolar lavage fluid from healthy rats (*1*) and rats with experimental bronchial asthma (*2*). Dotted line: control (physiological saline) for healthy rats, dashed line: control (physiological saline) for rats with bronchial asthma.

In animals with experimental bronchial obstruction (model of human BA) the lungs were characterized by typical histological changes involving mainly small airways [1-3]. Their diameter decreased (RQ= 0.42), and the lumens were obturated with mucus, eosinophils, and desquamated epithelial cells. NO-synthase activity was enhanced in all epithelial cells including desquamated cells. In rats with experimental BA, positive reaction for NO-synthase was found in hypertrophic bronchial smooth muscle cells, alveolar macrophages, and peribronchial mast cells.

Salmeterol enhanced expression of NO-synthase in healthy animals and potentiated relaxation of the bronchi immediately after application (Fig. 1, *d*), which was accompanied by an increase in the content of NO metabolites in BALF. However, 3 h after inhalation NO-synthase activity, motor reaction of the bronchi, and the level of NO metabolites did not differ from the initial values (Fig. 2). Thus, application of β_2-agonists to normal animals cause transient and similarly directed changes in NO-synthase activity, bronchial relaxation, and NO metabolite level.

In animals with experimental BA salmeterol caused phasic changes in NO-synthase activity (Fig. 2, *a*): enzyme activity considerably decreased immediately after inhalation, 6 h after inhalation this parameter increased and after 12 h it surpassed the control level. NO-synthase activity and bronchomotor reaction underwent opposite changes: the higher enzyme activity of epitheliocytes, the lower relaxation index (Fig. 2, *a, b*).

The concentration of NO metabolites in rats with BA was 7.5-fold higher than in controls and did not decrease after salmeterol inhalations. No correlation between NO-synthase activity and the concentration of NO metabolite was noted, but their content remained extremely high throughout the experiment (Fig. 2, *c*). Dose dependent increase in the content of NO_2^- and NO_3^- probably results from ovalbumin-stimulated expression of inducible NO-synthase [10]. It was found that in animals with BA, increased resistance of the

respiratory tract during early asthmatic response is accompanied by enhanced NO expiration [9]. The absence of a further increase in NO metabolite content attests to maximum functional strain of the NO-producing systems.

Decreased density of the precipitate in epithelial cells from salmeterol treated animals against the background of high content of NO metabolites can be explained by suppression of constitutive NO-synthase in epithelial cells [11] and by salmeterol-induced inhibition of the presynaptic release of acetylcholine, a functional agonist of NO-synthase [13].

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