Nitric Oxide Synthase in Bronchial Epithelium and Nitric Oxide Metabolites in the Lungs of Rats with Bronchial Asthma after Fenoterol Inhalation

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Nitric oxide synthase of the bronchial epithelium and concentrations of nitric oxide metabolites (NO_2^- and NO_3^-) in bronchoalveolar lavage fluids were measured in rats with bronchial asthma after fenoterol inhalation. It was suggested that nitric oxide-ergic mechanisms can mediate the effects of inhaled β_2-adrenergic agonists.

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

Monitoring of the level of nitric oxide (NO) in expired air reflecting the severity of inflammation and the analysis of NO metabolites in bronchoalveolar lavage fluid (BALF) are widely used in clinical practice for evaluating the efficiency of therapy in patients with bronchial asthma (BA) [9]. The mechanism of the effects of inhaled glucocorticoids on NO synthase (NOS) expression in the lungs of patients with BA is well understood [6]. However, the effects of inhaled β-adrenergic agonists used for the correction of bronchial obstruction in BA received little attention [3].

Here we studied NOS in bronchial epitheliocytes and NO metabolites (NO_2^- and NO_3^-) in BALF of rats with BA inhaling fenoterol.

MATERIALS AND METHODS

Experiments were performed on 25 male outbred albino rats weighing 180-200 g with BA [5]. Fifteen rats were sensitized by repeated (for 2 days) subcutaneous injections of 10 μg ovalbumin dissolved in 0.5 ml solution containing 100 mg $Al(OH)_3$. Three weeks later, ovalbumin in a provocation dose (0.03%, 0.8 ml/min) was inhaled to the appearance of bronchospasm (acrocyanosis of limbs, ears, and tail, tachypnea, and whistling rales). Intact rats served as the control (n=10).

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Fenoterol was inhaled in a dose of 0.007 μg/liter/min for 30 min. The animals were placed into a polyethylene chamber equipped with an ultrasound inhaler. Control rats with BA (n=6) were treated with physiological saline.

Morphometry of bronchial slices was performed under a Carl Zeis microscope using an MOV-1 device. Constriction and relaxation were estimated by the ratio between the diameter of the lumen and the outer diameter of the bronchus.

NADPH-diaphorase was assayed as described elsewhere [8]. Lung specimens (1×0.5 cm) dissected with a blade were placed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), fixed at 4°C for 2 h, washed with 15% sucrose at 4°C for 24 h, and frozen in a cryostat. Slices (10 μ) were mounted on slides and incubated in a medium containing 50 mM Tris-buffer (pH 8.0), 1 mM NADPH (Sigma), 0.5 mM HCT (Sigma), and 0.2% Triton X-100 (Serva) at 37°C for 60 min, washed with distilled water, dehydrated, and embedded into a balsam. Enzyme activity was measured on an M-85 microdensitometer (Vickers).

The concentrations of NO_2^- and NO_3^- were estimated as described elsewhere [10,12]. Cadmium powder (0.3 g) was added into a tube with 1 ml supernatant. The sample was impregnated with copper, heated in a water bath at 100°C for 60 min, cooled, and centrifuged at 1200 rpm. NO metabolites were assayed

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in the supernatant: 50 μl 5% NH_4Cl and 50 μl 2% N-(1-naphthyl)ethylenediamine in 5% H_3PO_4 were added to 100 μl supernatant. Measurements were performed in a Dynatech spectrophotometer at 540 nm. The contents of NO_2^- and NO_3^- were expressed in nmol/ml BSA.

RESULTS

Intact airway epithelium serves as a barrier between bronchoconstrictor agents and smooth myocytes and regulates bronchial patency by production of various relaxants, including NO. Damages to epitheliocytes increase bronchial sensitivity to constrictor stimuli and attenuate their relaxation induced by adrenergic agonists [13]. Our experiments revealed an interrelation between bronchomotor tone and NOS activity in the epithelium (Table 1), which confirmed the ability of epitheliocytes to modulate bronchial patency during fenoterol inhalation.

The bronchial epithelium is NADPH-diaphorase-reactive (Fig. 1, *a*), which reflects NOS activity [7]. It is known that ciliated, goblet, and intercalated cells react with diaphorase [2] yielding diformazan colored violet to light blue depending on enzyme activity. The intensity of staining decreases from large to small bronchi. Densitometry showed that in healthy animals enzyme activity in large bronchi was higher than in small bronchi (Table 1). The content of NO metabolites in BALF of intact rats was 0.369±0.020 nmol/ml.

In animals with experimental BA, NOS activity was high in epitheliocytes of large and small bronchi

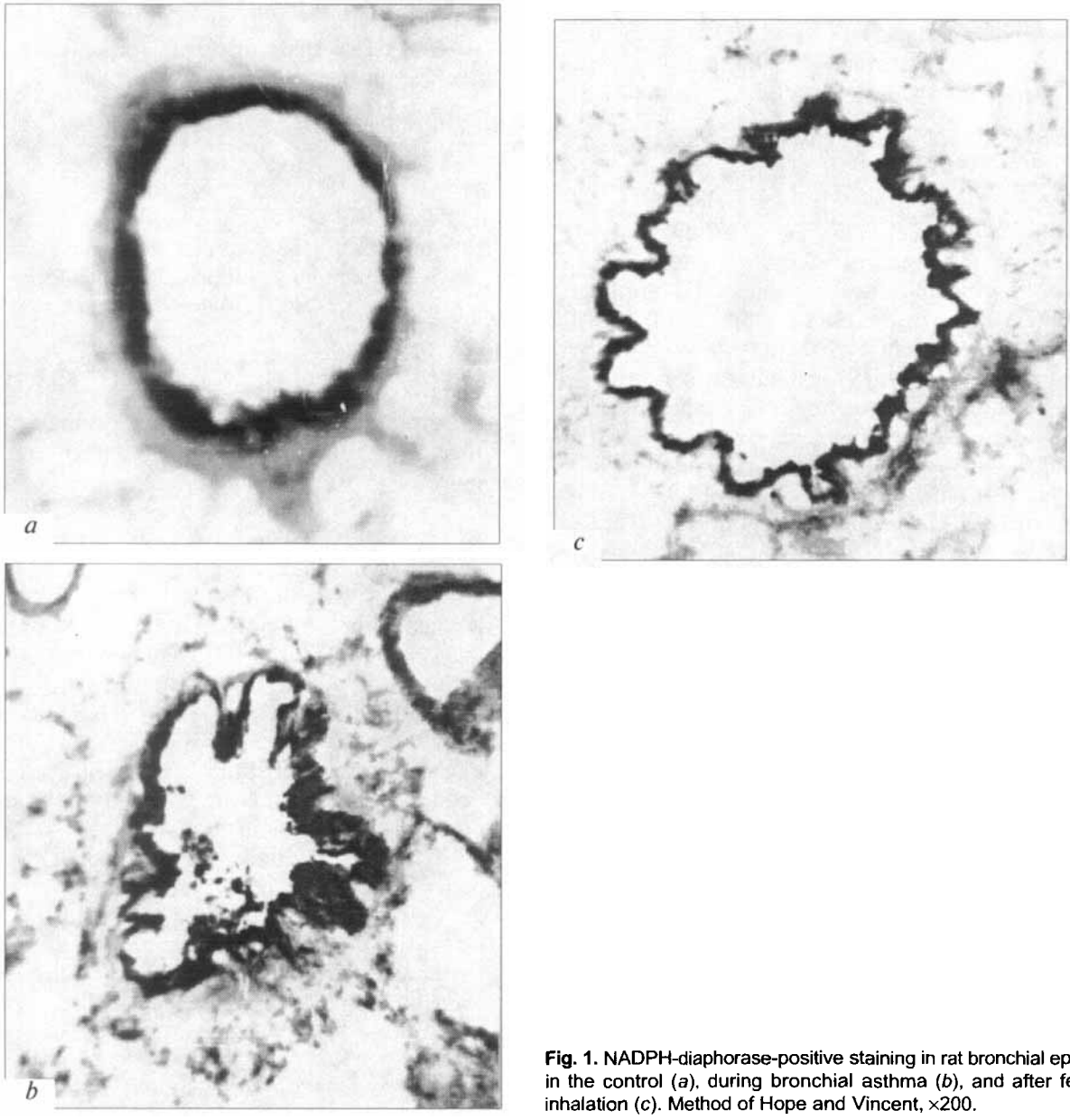

Fig. 1. NADPH-diaphorase-positive staining in rat bronchial epithelium in the control (*a*), during bronchial asthma (*b*), and after fenoterol inhalation (*c*). Method of Hope and Vincent, ×200.

TABLE 1. NOS Activity and NO Metabolism in Lung Epithelial Cells after Fenoterol Inhalation

Parameter		Control	BA	BA+fenoterol
NOS activity, rel. units	large bronchi	15.75±0.42	16.2±0.5	15.30±0.33
	small bronchi	11.35±0.38	19.90±0.61*	15.10±0.22+
Coefficient of bronchial relaxation		0.620±0.015	0.42±0.04*	0.62±0.03+
Content of NO metabolites in BALF, nmol/liter		0.369±0.020	2.789±0.001*	2.874±0.011+

Note. $p<0.05$: *compared to the control, +compared to BA.

(Fig. 1, *b*). Enzyme activity depended on the state of bronchi. The greater was the severity of damages, the higher was enzyme activity in epitheliocytes. NOS activity was maximum in small noncartilaginous bronchi with severe pathological changes, which correlated with a 7.5-fold increase in the concentration of NO metabolites in BALF compared to the control (Table 1).

Hence, in animals with BA-simulating bronchial obstruction we revealed not only characteristic changes in the lumen of bronchi, but also increased production of NO (pathognomonic sign of BA). High expression of NOS in airway epitheliocytes [10] and elevated concentration of NO in expired air [9] are also typical of patients with BA.

In animals with experimental BA inhaling fenoterol, NOS activity in the bronchial epithelium considerably decreased and the coefficient of bronchial relaxation increased (Fig. 1, *c*). However, the content of NO metabolites in BALF remained high (Table 1). Probably, inhalation of fenoterol does not inhibit NO production, which puts in doubt the antiinflammatory effects of this β-adrenergic agonist. It was shown that single administration of high doses of salbutamol (β_2-adrenergic agonist with short-lasting effects) and treatment with salmeterol (β_2-adrenergic agonist with prolonged action) do not change NO concentration in expired air, which indicates that these substances have no antiinflammatory properties [11]. β-Adrenergic agonists do not increase the concentration of NO in expired air. On the other hand, chemiluminescence and the method for measuring NO_2^- and NO_3^- (stable products of aerobic oxidation of NO) in the presence of Griess reagent may introduce some errors into the measurements. In all instances, rapid reaction between NO and O_2^- leads to the formation of $ONOO^-$ [1], which must be taken into account is analyzing the results of NO_2^- and NO_3^- measurements in BALF. The problem of antiinflammatory action of β-adrenergic agonists requires further detailed studies.

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