2. S. Fujita, G. Miuazono, and N. Ohba, *Jpn. J. Ophthalmol.*, **31**, No. 3, 412-424 (1987).
3. P. Holzer, *Neuroscience*, **24**, No. 3, 739-768 (1988).
4. P. Holzer, *Physiol. Pharmacol.*, **121**, No. 1, 49-146 (1992).
5. G. G. Knyazev, G. B. Knyazeva, and A. F. Nikiforov, *Acta Physiol. Hung.*, **75**, No. 1, 29-34 (1990).
6. G. G. Knyazev, G. B. Knyazeva, and Z. S. Tolochko, *Neuroscience*, **42**, No. 2, 555-560 (1991).
7. J. F. R. Konig and R. A. Klippel, in: *The Rat Brain*, Baltimore (1963), p. 161.
8. I. Sulakvelidze, P. Baluk, and D. M. McDonald, *J. Appl. Physiol.*, **76**, No. 2, 701-707 (1994).
9. J. G. Tian and E. T. Wei, *Drug Chem. Toxicol.*, **12**, No. 1, 61-66 (1989).
10. W. Zeman and J. R. M. Innes, in: *Graigie's Neuroanatomy of the Rat*, New York (1963), p. 230.

NADPH-Diaphorase in the Lungs of Rats with Experimental Bronchial Asthma

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Distribution and activity of NADPH-diaphorase colocalized with NO-synthetase are studied in bronchial epithelium, pneumocytes, and alveolar macrophages of rats with experimental bronchial asthma. Increased activity of NO-synthetase in these structures indicates that nitric oxide is involved in allergic inflammation.

Key Words: *asthma; nitric oxide; NADPH-diaphorase; macrophages; pneumocytes*

Recent studies show that nitric oxide (NO) is formed in the respiratory system [5,9,15]. Special attention has been focused on the role of NO in pulmonary pathologies, including bronchial asthma (BA) [3,6, 13]. There is evidence that the concentration of NO in the air exhaled by patients with BA is increased [3,13]; however, the role of NO in BA is not fully understood.

Our objective was to identify and quantitate NADPH-diaphorase, an enzyme participating in the synthesis of NO, in bronchial epithelium, pneumocytes, and lung macrophages of intact rats and rats with experimental BA.

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 180-200 g. The animals were maintained under standard vivarium conditions. Experimental BA was produced as described [4] to ensure changes in the bronchopulmonary system typical of this disease [14]. The rats were sensitized for 2 days by subcutaneous infection of 10 μg ovalbumin dissolved in 0.5 ml of solution containing 100 mg $Al(OH)_3$. Three weeks after sensitization, the rats inhaled the resolving dose of ovalbumin in a concentration of 0.8 ml/min and were decapitated at the peak of bronchospasm. Intact rats served as the control. Morphological examination of the lungs of sensitized rats revealed changes typical of BA: deformation of bronchial wall and narrowing of the bronchi, hyperplasia of goblet cells, focal desquamation of the epithelium, and moderate hypertrophy of smooth muscles.

NADPH-diaphorase was identified by the method [12]. Pieces of lungs (1×0.5 cm) were fixed for 2 h at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), which preserves the activity only of NADPH-diaphorase. The pieces were then washed at the same temperature in 15% sucrose. Cryostat sections (10 μ) were mounted on glass slides and incubated for 60 min at 37°C in a medium containing 50 mM Tris-HCl (pH 8.0), 1 mM NADPH (Sigma), 0.5 mM Nitro Blue Tetrazolium (Sigma),

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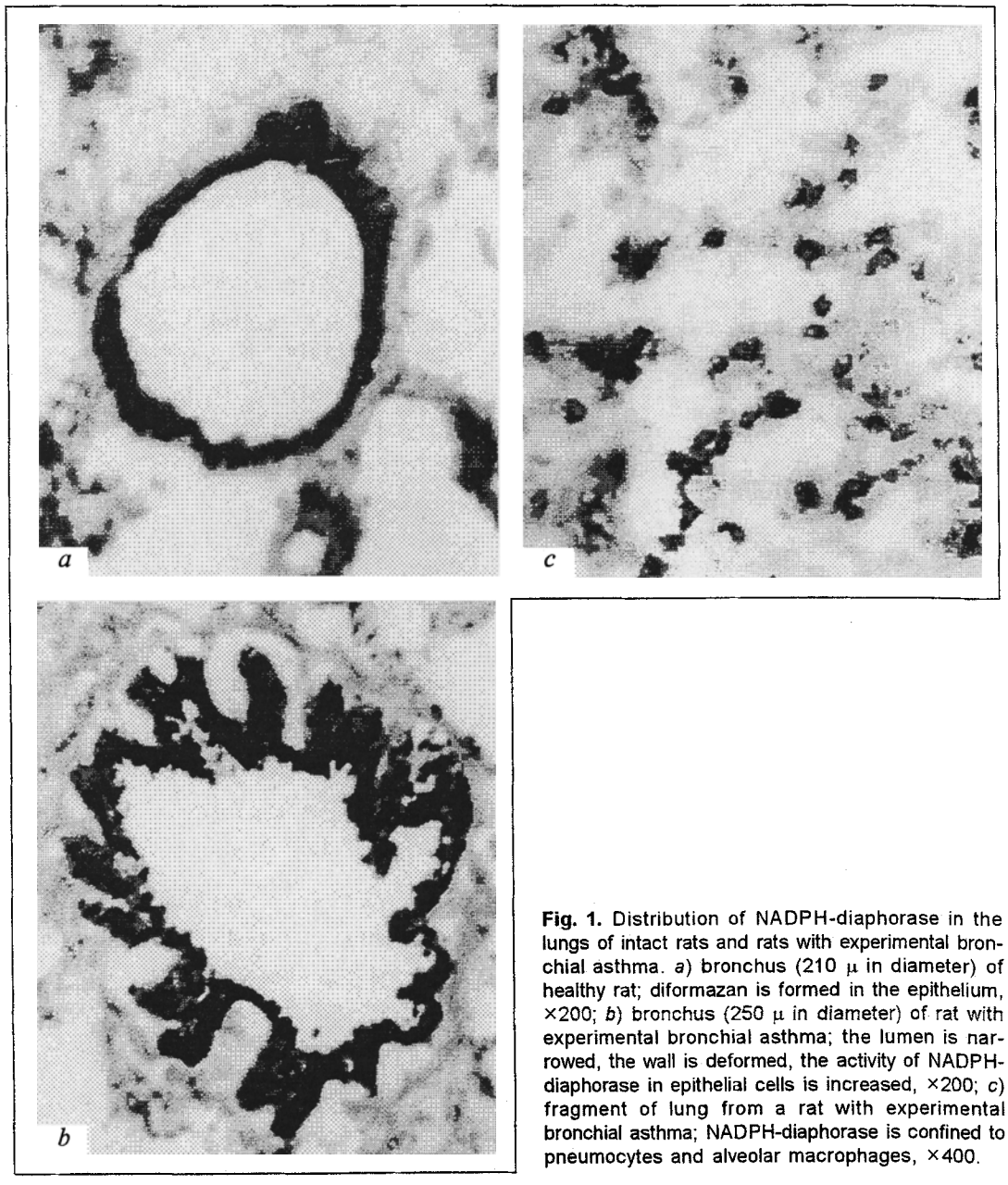

Fig. 1. Distribution of NADPH-diaphorase in the lungs of intact rats and rats with experimental bronchial asthma. *a*) bronchus (210 μ in diameter) of healthy rat; diformazan is formed in the epithelium, ×200; *b*) bronchus (250 μ in diameter) of rat with experimental bronchial asthma; the lumen is narrowed, the wall is deformed, the activity of NADPH-diaphorase in epithelial cells is increased, ×200; *c*) fragment of lung from a rat with experimental bronchial asthma; NADPH-diaphorase is confined to pneumocytes and alveolar macrophages, ×400.

and 0.2% Triton X-100 (Serva), rinsed in distilled water, dehydrated, and embedded in balm oil by the standard method. The enzyme activity was measured in an M-85 densitometer (Vickers)

RESULTS

NADPH-diaphorase converts Nitro Blue Tetrazolium into diformazan, yielding blue or dark blue color the intensity of which reflects the activity of NADPH-diaphorase and colocalized NO-synthetase [8,11].

NADPH-diaphorase was identified in ciliated, goblet, and basal cells of respiratory epithelium (Fig. 1, *a*, *b*). A blue ring corresponding to the epithelium was formed in bronchial lumen. In type I and II pneumocytes, diformazan staining was observed at the periphery of the cytoplasm, the center and nucleus remaining free of the precipitate. The intensity of the staining of lung macrophages was higher than that of pneumocytes (Fig. 1, *c*).

In control rats, the highest activity of NADPH-diaphorase was revealed in bronchial epithelium and the lowest in type I and II pneumocytes (Fig. 2). The enzyme activity did not depend on the diameter of bronchus and was equal in type I and II pneumocytes. Alveolar macrophages exhibited intermediate activity of NADPH-diaphorase, suggesting that the intensity of NO synthesis in them was higher than

in pneumocytes and lower than in bronchial epithelium ($p<0.001$).

In rats with experimental BA the highest activity of NADPH-diaphorase was observed in the epithelium of small bronchi ($p<0.001$). In order of decreasing NADPH-diaphorase activity the other structures can be arranged as follows: alveolar macrophages, type II pneumocytes, and type I pneumocytes.

Our observations indicate that the activity of bronchoepithelial, macrophagal, and pneumocyte NADPH-diaphorase and, consequently, of colocalized NO-synthetase increases in bronchial asthma (Fig. 2). In should be noted that in small bronchi the activity of NADPH-diaphorase was 1.5-fold higher than in the control, judging from the intensity of diformazan staining. The enzyme activity also increased in the epithelium of large bronchi, alveolar macrophages, and pneumocytes. This may be due to the fact that epithelial cells in large bronchi express predominantly constitutive NO-synthetase that provides formation of small amount of NO during a short time period. In BA, the epithelium of small bronchi, pneumocytes, and macrophages express inducible NO-synthetase that markedly enhances the NO synthesis [1,7]. Presumably, high expression of inducible NO-synthetase plays an important role in the formation of nonspecific reactivity of airways in BA [7]. Increased production of NO by alveolar macrophages may aggravate allergic inflammation [2,10].

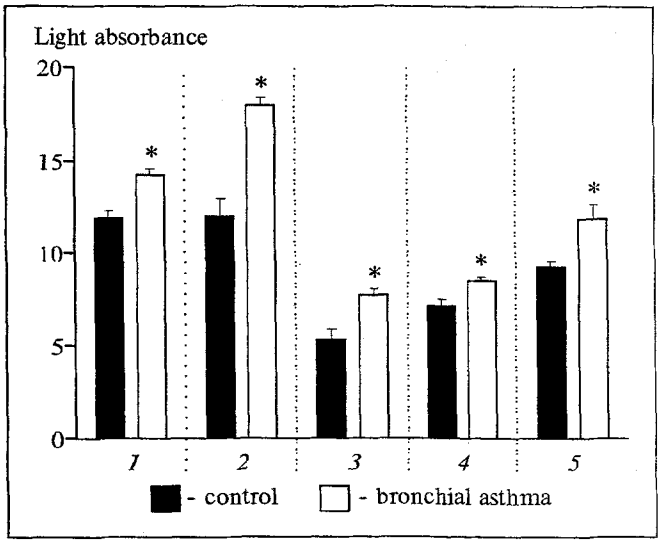

Fig. 2. Activity of NADPH-diaphorase in bronchial epithelium, pneumocytes, and macrophages in intact rats and rats with experimental bronchial asthma. Large (*1*) and small (2) bronchi, type I (*3*) and type II (*4*) pneumocytes, and alveolar macrophages (*5*). *$p<0.001$ compared with the control.

REFERENCES

1. Kh. M. Markov, *Pat. Fiziol.*, No. 1, 34-39 (1996).
2. J. E. Albina, J. A. Abate, and L. H. J. William, *J. Immunol.*, **145**, 8-12 (1991).
3. K. Alving, E. Weitzberg, and J. M. Lundberg, *Eur. Respir. J.*, **6**, 1368-1370 (1993).
4. P. Andersson, *Allergy*, **35**, 65-71 (1980).
5. P. J. Barness, *Ann. Med.*, **27**, No. 3, 389-393 (1995).
6. P. J. Barness and F. V. Liew, *Immunol. Today*, **16**, 128-130 (1995).
7. C. Bassoulet, M. Lonchampt, and E. Canet, *Eur. Respir. J.*, **9**, 182 (1996).
8. T. M. Dawson, D. C. Bredi, M. Fotuhi, *et al.*, *Proc. Natl. Acad. Sci. USA*, **88**, 7797-7801 (1991).
9. L. E. Gustafsson, A. M. Leone, M. G. Persson, *et al.*, *Biochem. Biophys. Res. Commun.*, **181**, 852-857 (1991).
10. R. A. Hoffman, J. M. Langrehr, T. R. Billiar, *et al.*, *J. Immunol.*, **144**, 2220-2226 (1990).
11. V. T. Hope, G. J. Michael, K. M. Knigge, and S. R. Vinsent, *Proc. Natl. Acad. Sci. USA*, **88**, 2811-2814 (1991).
12. V. T. Hope and S. R. Vinsent, *J. Histochem. Cytochem.*, **37**, 653-661 (1989).
13. S. A. Kharitonov, B. J. Oconnor, D. J. Evans, and P. J. Barness, *Am. J. Respir. Crit. Care Med.*, **151**, 1894-1899 (1995).
14. P. Peretti, L. Ballati, S. Evangelista, *et al.*, *Pulm. Pharmacol.*, **8**, 21-30 (1995).
15. M. G. Persson, N. P. Wiklund, and L. E. Gustafsson, *Am. Rev. Respir. Dis.*, **148**, 1210-1214 (1993).