Activity of NADPH Diaphorase in Rat Lungs during Respiratory Distress Syndrome

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Activity of NADPH diaphorase colocalized with NO synthase in the epithelium of bronchi, alveoli, and alveolar macrophages increased in rats with experimental distress syndrome suggesting the involvement of NO in lung injury.

Key Words: *respiratory distress syndrome; acute lung injury; nitric oxide; NADPH diaphorase*

Respiratory distress syndrome (RDS) is a severe and dangerous form of acute pulmonary insufficiency associated with sepsis, aspiration, and polytrauma. The pathogenesis of RDS and the role of NO-ergic mechanisms in acute lung injury are poorly understood. Some authors reported that NO concentration in expired air increases in patients with RDS [1,2,6]. A wide range of functional peculiarities of NO [3,4] can be used for evaluation of the state of airways and prediction of the severity and outcome of the inflammatory process. The understanding of the role of NO-ergic mechanisms and pathophysiology of RDS opens up new prospects for elaboration of new methods of pathogenetic therapy.

Here we performed histochemical identification and quantitative analysis of NADPH diaphorase involved in NO synthesis in the bronchial epithelium, alveolar cells, and alveolar macrophages in animals with experimental acute lung injury.

MATERIALS AND METHODS

Experiments were performed on 25 male outbred rats weighing 150-180 g and kept under standard vivarium conditions. Experimental RDS was induced as described by N. G. Kharlamova and E. A. Bardakhch'yan (1991). Lipopolysaccharide (LPS) from *Escherichia coli* (serotype O 111) was administered into the caudal vein in a dose of 2 mg/100 g. The rats (5 animals per group) were euthanized with Nembutal 1, 24, 48, and 72 h or 7 days after the injection. Intact animals (n=5) served as the control.

Localization of NO synthase (NOS) was determined histochemically by visualizing NADPH diaphorase [7]. The material was incubated in 4% cold paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Among all diaphorases, only NADPH diaphorase retained its activity in paraformaldehyde. The material was fixed at 4°C for 2 h and then washed with 15% sucrose at the same temperature for 1 day. Cryostat sections (10 μ) were mounted on slides and placed in a medium containing (in mM): 50 Tris buffer (pH 8), 1 NADPH (Sigma), 0.5 nitroblue tetrazolium (NBT, Sigma), and 2% Triton X-100 (Serva), incubated at 37°C for 60 min, washed with distilled water, dehydrated, and embedded into balsam as described previously. Enzyme activity was measured on a Vickers M-85 microdensitometer (550 nm, ×400, mask 2) and expressed in optical density units (U).

Morphology of the lungs was examined on slices stained with hematoxylin and eosin.

RESULTS

NAPH-diaphorase converted NBT into diformazan colored blue to indigo. Activity of NOS colocalized with NADPH diaphorase was estimated by the color intensity [7].

Bronchial epithelium was stained with diformazan formed in the cytochemical reaction. The epithelium

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looked like a blue or indigo zone around the lumen. All cells including ciliated, intercalary, and goblet cells were stained (Fig. 1). In alveolar cells, the preparation was concentrated at the periphery, but not in the central or perinuclear zones. Lung macrophages were stained more intensively than alveolar cells.

Densitometry of diformazan density in lung cells of control animals showed that NOS activity was maximum in epitheliocytes of large bronchi with cartilaginous walls and diameter of 0.3-0.5 mm (18.28±0.75 U) and in small noncartilaginous bronchi with a diameter of 0.1-0.2 mm (10.73±0.61 U). Types 1 and 2 alveolar cells had the same density of precipitate (5.37±0.46 U), while in alveolar macrophages this parameter was 8.51±0.62 U. NOS activity in these cells differed significantly ($p<0.05$).

In rats with experimental RDS, the highest NOS activity was detected in the epithelium of small bronchi 24 h after administration of LPS (78.41±1.85 U). In large bronchi, alveolar macrophages, and alveolar cells the content of diformazan and NOS activity were lower (54.19±2.06, 50.97±1.68, and 37.94±0.99 U, respectively). Enzyme activity in control animals and rats with experimental RDS differed significantly ($p<0.001$).

NOS activity in all cells underwent phasic changes. One hour after injection of LPS (against the background of endotoxin shock), NOS activity in alveolar cells 5-6-fold surpassed that in the control ($p<0.001$). In the epithelium of small and large bronchi and alveolar macrophages, NOS activity increased to a lesser extent, but 1.5-2-fold surpassed the control value ($p<0.001$).

Activity of NADPH diaphorase peaked 24 h after administration of LPS (Fig. 2). Forty-eight hours postinjection against the background of severe respiratory disturbances, patchy areas of atelectasis and pulmonary emphysema, pneumonia foci with lymphocyte and histiocyte infiltration, edema of the interalveolar septa, and epithelium desquamation, the activity of NOS decreased by 25-30%, but remained higher than in the control ($p<0.01$). The content of diformazan in all cells began to increase 72 h after injection of LPS. If the animal did not die, NOS activity continued to increase and reached maximum on day 7 (Fig. 1).

Thus, NOS activity in lung cells considerably increased during RDS. Our results are consistent with previous data on increased NO concentration in the expired air in patients with RDS. This is probably due to activation of NOS induced by kappa-B transcription factor [2,3,7].

During RDS, NOS activity in small bronchi, alveolar cells, and macrophages increased to a greater extent than in large bronchi, which was probably due

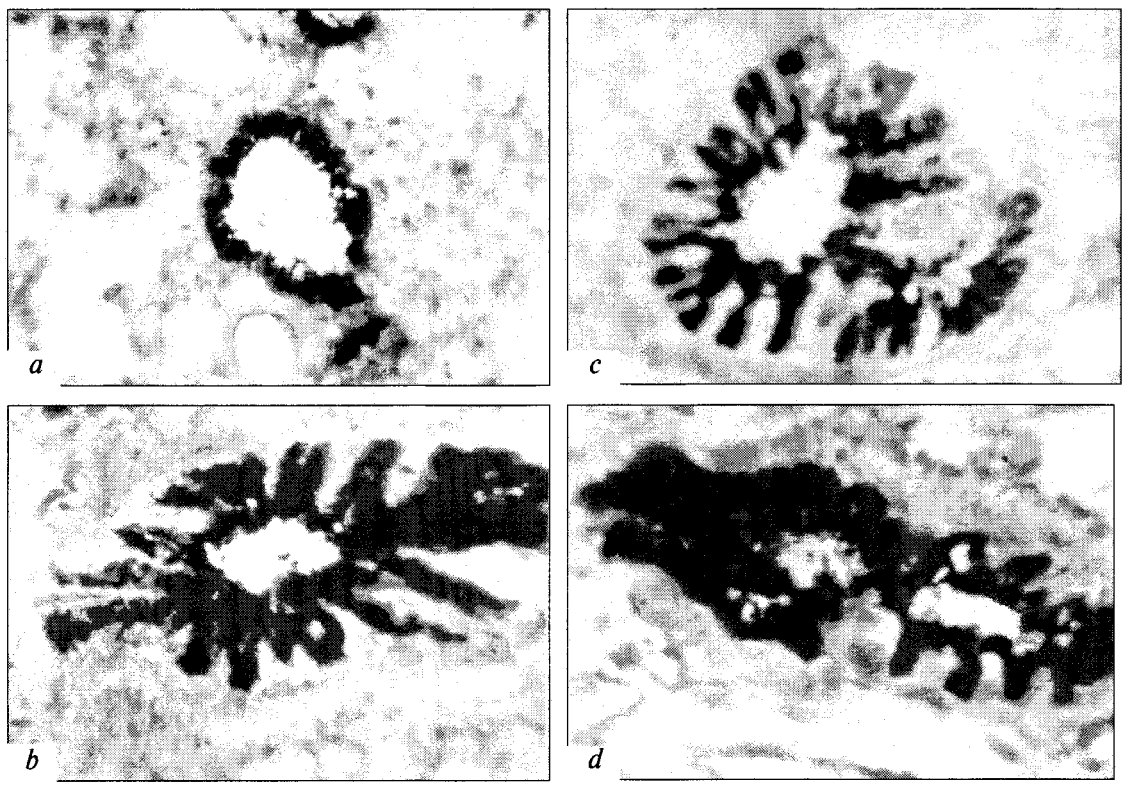

Fig. 1. NADPH diaphorase in small bronchi (×100) of control animal (*a*) and rat with respiratory distress syndrome after 24 h (*b*), 48 h (*c*), and 7 days (*d*).

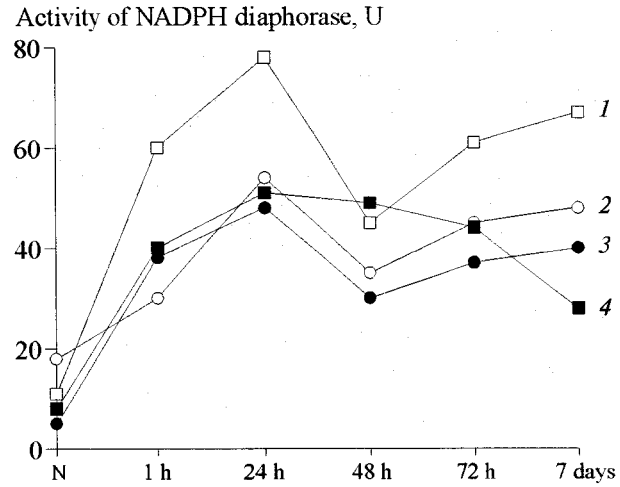

Fig. 2. Activity of NADPH diaphorase in epithelial cells of small (*1*) and large (*2*) bronchi, alveolar cells (*3*), and macrophages (*4*) under normal conditions (N) and during experimental respiratory distress syndrome.

to expression of type II NOS (inducible NOS) [4]. Enhanced production of NO by alveolar macrophages contributes to mechanisms aggravating damages to the lung parenchyma.

High activity of NOS in animals with experimental acute lung injury is probably related to hypoxia, which is associated with disturbances of external respiration. Profound morphological changes and severe edema of lung tissue reduce lung ventilation and blood oxygenation. Oxygen deficiency impairs mitochondrial functions, decreases the efficiency of oxidative phosphorylation, and activates more ancient mechanisms of energy production involving NOS [1]. Extensive destructive changes in the bronchial epithelium, activation of macrophages, and degranulation of mast cells are accompanied by the release of various cytokines activating NOS. Interferon-γ, interleukins, tumor necrosis factor, and LPS complexes stimulate NO synthesis via activation of constitutive and inducible NOS in human lung epithelium [3,5].

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