Effect of Prenatal Hypoxia on DNA Synthesis in the Tracheal Epithelium and LPO-AOD System in the Lungs of Newborn Rats

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UDC 612.273.2:616.153.915.39:576.355]-053.31-092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 5, pp. 531-533, May, 1994
Original article submitted January 25, 1994

Newborn rats euthanized 24 h after birth were examined. The rats were born to females exposed to chronic hypobaric hypoxia on days 14-19 of gestation. The index of nuclei labeled with ^{3}H-thymidine in the tracheal epithelium of newborn rats exposed to prenatal hypoxia was 3 times lower than in the control. The LPO level was higher in posthypoxic animals than in intact rats. Prenatal hypoxia led to the suppression of antioxidant defense in the lungs of newborn rats.

Key Words: *hypoxia; newborns; DNA synthesis; lipid peroxidation; antioxidant defense*

The majority of injurious agents mediate their effects in the mother-placenta-fetus system via hypoxia [3]. Activation of lipid peroxidation (LPO), in its turn, is an essential component of posthypoxic cellular alteration. The processes of DNA synthesis determine the normal development of an organ and maintain tissue homeostasis. There are reports about an inverse correlation between DNA synthesis and LPO processes in various experimental situations [4]. Abnormalities of the respiratory organs are among the most prevalent perinatal injuries induced by hypoxia [7]. In the present work the processes of DNA synthesis and the system of LPO - antioxidant defense (AOD) in the lungs of newborn rats exposed to prenatal hypoxia were studied.

MATERIALS AND METHODS

Experiments were carried out with 199 newborn rats. Euthanasia was carried out 24 h after birth (3 days after the final experimental intervention). The newborn rats were divided into 2 groups: 1) intact and 2) exposed to prenatal hypoxia. The state of hypoxia was simulated by a daily 4-hour enclosure of females in an SBK-48 pressure chamber (altitude 9000 m, partial pressure of oxygen 42 mm Hg) from day 14 to 19 of gestation. The animals were "elevated" at an average rate of 13-20 mm Hg/h. For assessment of DNA synthesis, the animals were administered ^{3}H-thymidine, 1 μCi/g body weight (specific activity 84 Ci/mmole) 1 h before euthanasia. The tracheas were fixed in 3:1 ethanol:acetic acid mixture and embedded in paraffin, after which slices were prepared and coated with type M photoemulsion. Radioautographs were made after a method used in our laboratory [5]. The index of labeled nuclei was estimated on the basis of scintillation of 2500 nuclei in the zone of tracheal proliferation and expressed by the ratio of labeled nuclei to the total number of nuclei in percent. Nuclei with at least 5 silver grains above them were considered labeled. The choice of tracheal proliferation as the subject of study was dictated by the difficulties encountered in identifying the morphological origin of cells in radioautography of newborn rat

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0007-4888/94/0005-0530$12.50

TABLE 1. Effect of Prenatal Hypoxia on Gravimetric Parameters and DNA Synthesis in Tracheal Epithelium of Newborn Rats

Group	Index of labeled nuclei, %	Body weight, g	Absolute mass of lungs, mg	Relative mass of lungs, mg/g
Control	1.60±0.10	4.8±0.1	123.6±1.3	26.1±0.6
Hypoxia	0.50±0.05*	4.9±0.1	122.3±1.4	25.3±0.6

Note. Here and in Table 2 an asterisk indicates $p<0.001$ in comparison with the control.

lungs. Moreover, there are reports that alveocytes and epitheliocytes of airways generally have a unidirectional response to various proliferative stimuli. After the animals had been decapitated and the tissues treated with liquid nitrogen, malonic dialdehyde (MDA) [9] and α-tocopherol [11] were measured by fluorometry; lipid hydroperoxides [2] and the AOD enzymes catalase [10], superoxide dismutase (SOD) [14], and glutathione peroxidase (GPO) [6] by spectrophotometry; total lipids were measured by photocolorimetry using Lachema kits in lung homogenates. The enzyme activities were reduced to protein concentrations in the samples and determined after Lowry [12]. Body weight and lung mass of newborn rats were measured as well. Results were statistically processed using the Student t test.

RESULTS

Measurements of absolute values of body weight and of absolute and relative values of lung mass revealed no noticeable changes in the experimental animals in comparison with the controls (Table 1). Analysis of DNA synthesis processes showed a marked reduction of the index of labeled nuclei in the tracheas of rats exposed to hypoxia *in utero* (Table 1). In the controls this index was 1.60±0.10%, whereas in the experimental animals it was more than three times lower, 0.50±0.05 ($p<0.001$).

It is noteworthy that in adult rats experimental exposure to chronic hypoxia stimulated DNA synthesis in the lungs, liver, and kidneys, which was manifested in an increase of the index of labeled nuclei [13]. This difference may be due to the fact that in this experiment the rats were "elevated" to an altitude of 4250 m. Such an exposure is characterized as medium-severity hypoxia. However, Vdovenko *et al.* [1], who simulated hypoxia in their experiments similarly as we did, observed activation of proliferative processes in the corneal and glossal epithelium as well. This stimulation was regarded as a compensatory one, directed at the maintenance of tissue homeostasis under conditions of repeated stress. We speculate that the most probable cause of the differences observed in our experiments in comparison with Vdovenko's findings consists in age-specific reactions of proliferative processes of newborn rats to hypoxia. It should be mentioned here that in our previous experiments repeated prenatal hypoxia induced in newborn rats suppressed hepatocyte proliferation, which manifested itself in a reduction of the index of labeled nuclei and of the mitotic index [8].

Studies of the processes in the LPO-AOD system help understand the mechanism of proliferative disturbances observed in our experiments. A reliable (vs. the analogous parameters in intact rats) increase of the level of lipid hydroperoxide by 1.3 times, reduction of the α-tocopherol level by 1.2 times, and decrease of the total lipid level by 1.4 times were detected in the lungs of animals exposed to prenatal hypoxia. Measurements of AOD enzymes in experimental animals revealed a reliable decrease of SOD and catalase activities by 1.2 times and of GPO by 2 times in comparison with the control. The MDA level showed an evident tendency ($p<0.07$) to increase in posthypoxic lungs as compared to its control value (Table 2). Reduction of the level of total lipids (LPO substrates) in the presence of an increased content of lipid hydroperoxides, one of the LPO products, indicates

TABLE 2. Effect of Prenatal Hypoxia on the LPO–AOD System in the Lungs of Newborn Rats

LPO-AOD system parameter	Control	Hypoxia
Total lipids, mg/g tissue	1.71±0.07	1.26±0.09*
Lipid hydroperoxide, mM/g lipids	0.188±0.015	0.234±0.012*
MDA, U fl/g lipids	504.4±46.1	618.9±45.1
α–Tocopherol, μg/g lipids	12.68±0.68	10.64±0.51*
Catalase, μM H_2O_2/min per mg protein	13.20±1.00	9.80±0.57*
SOD, nM diformasan/min per mg protein	1.17±0.10	0.91±0.06*
GPO, nM NADPH/min per mg protein	10.34±0.10	5.23±0.40*

intensification of LPO processes. The adaptation reaction of the antioxidant system in response to oxidative factors is known to manifest itself in the mobilization of fat-soluble antioxidants and in a rise of their level in the lungs [15]. According to our findings, prenatal hypoxia does not entrain an increase of the μ-tocopherol content in the lungs of newborn rats, which fact should be regarded as a failure of the compensatory potential of the antioxidant system. The reduced functional activity of the enzymatic component of AOD of newborns' lungs (the SOD-catalase-GPO system) is further evidence of the failure of biooxidant defense in prenatal hypoxia. Since the LPO/AOD ratio is a physiological constant, the shift of the equilibrium in this system toward the intensification of peroxidation processes causes destabilization and disorders of homeostasis. Depressed DNA synthesis was an indicator of disordered tissue homeostasis in our experiments.

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