EXPERIMENTAL BIOLOGY

Effect of the Hydra Peptide Morphogen on Cell Proliferation in the Thymus of Newborn Rats Exposed to Prenatal Hypoxia

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Female rats were exposed to high-altitude hypoxia on days 14-19 of pregnancy. Thirty min before hypoxia the animals were injected with the hydra peptide morphogen in a dose of 10 μg/kg intraperitoneally. Prenatal exposure to hypoxia suppressed proliferative processes in thymic cortex and medulla in newborn rats and decreased the lymphocyte count in the female—newborn rat pairs and the percent of full-term deliveries. Injection of hydra peptide morphogen prevented the development of posthypoxic disorders in newborn rats.

Key Words: *thymic cortex and medulla; DNA production; lymphocytes; percentage of full-term deliveries; hydra peptide morphogen; newborns*

The immune status is a basic criterion for assessing the health status. Therefore, studies of immunogenesis in newborns exposed to prenatal hypoxia and the search for new means of correcting posthypoxic disorders are an important task [1].

Previously, we reported that it is possible to correct posthypoxic disorders of the respiratory system in newborn rats by the hydra peptide morphogen (HPM) [4].

This study was aimed at correcting by HPM the disorders in the thymus, the central organ of immunogenesis, in newborn rats whose mothers were exposed to hypoxia during pregnancy.

MATERIALS AND METHODS

Seventy-two female rats with a known pregnancy period were divided into 4 groups: 1) intact females; 2) females exposed to hypoxia on days 14-19 of pregnancy; 3) females injected with HPM in a dose of 10 μg/kg on days 14-19 of pregnancy; and 4) females injected HPM in a dose of 10 μg/kg 30 min before exposure to hypoxia on days 14-19 of pregnancy.

Hypoxia was simulated by a 4-h exposure in an SBK-48 pressure chamber (altitude 9000 m, partial oxygen pressure 42 mm Hg) on days 14-19 of pregnancy.

The percent of full-term deliveries was calculated as the ratio of the total number of pregnant females (100%) to the number of females whose pregnancies eventuated in delivery.

The studies were carried out on 397 newborn rats divided into 4 groups corresponding to the experimental groups of females. The newborn rats were decapitated 24 h after birth. Body weight and relative and absolute weights of the thymus were measured.

For assessing the DNA production, ^{3}H-thymidine was injected to newborn rats 1 h before euthanasia in a dose of 1 μCi/g (specific activity 84 Ci/mmol). The thymus was fixed in ethanol:acetic acid (3:1), embedded in paraffin, and cut into slices which

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TABLE 1. Effect of HPM on Body Weight and Thymus Weight of Newborn Rats Exposed to Prenatal Hypoxia and on the Percent Content of Lymphocytes in the Female—Newborn Pairs and the Percentage of Full-Term Deliveries (*M±m*)

Parameter	Group			
	control	hypoxia	HPM	hypoxia+HPM
Body weight, g	7.69±0.13	7.12±0.18	7.67±0.10	7.20±0.12
Absolute weight of thymus, mg	14.07±0.56	12.46±0.88	12.42±0.42	11.83±0.46
Relative weight of thymus, mg/g	1.78±0.06	1.55±0.08	1.57±0.06	1.61±0.06
Percentage if lymphocytes:				
female	70.85±1.07	63.71±2.23*	87.28±0.83*	74.71±1.42
newborn rat	72.44±0.79	60.68±0.48*	90.15±1.83*	74.51±0.74
Percentage of full-term deliveries	66.3	73.68	100	57.8

Note. Here and in Table 2: *$p<0.05$ *vs.* the control.

were then coated with type M photoemulsion. Radioautographs were examined by the method developed in our laboratory.

The index of labeled nuclei (ILN) was calculated from analysis of 2500 nuclei in thymic cortex and medulla as the percent ratio of labeled to total count of nuclei. The nuclei with at least 5 silver grains were considered labeled. The label intensity (LI) was expressed as the mean number of silver grains above the nucleus.

For estimating the percent content of peripheral blood lymphocytes in the female—newborn pair, smears were prepared and stained by the method of May—Grunwald.

RESULTS

Our data indicate that HPM affects the course of pregnancy and outcome of labor. In the control, the percentage of full-term deliveries was 66.3%. In the HPM-treated females this value was 100%. In the females injected HPM and exposed to hypoxia this value was 73.6%. In the "hypoxia" group it was 57.8% (Table 1).

Body weight and relative and absolute weights of the thymus were virtually the same in all experimental groups. Rats were exposed to hypoxia on days 14-19 of pregnancy, therefore, there were no classical small-for-date fetuses. These results are confirmed by other scientists [3].

Analysis of DNA production showed suppression of cell proliferation in the thymus of newborn rats exposed to prenatal hypoxia. ILN in the cortex and medulla decreased by 1.6 and 1.7 times, respectively, in comparison with the control. The decrease in the number of DNA-producing nuclei was associated with a significant decrease in LI (by 1.4 and 1.3 times, respectively) in comparison with the control. This indicates a decrease in the rate of DNA production (Table 2).

Injection of HPM on the 14th-19th day of pregnancy led to a significant increase in ILN in the cortex and medulla thymus (by 1.7 and 1.4 times, respectively) *vs.* the control; LI also increased (Table 2).

Injection of HPM to pregnant females prevented the inhibitory effect of hypoxia on the proliferation of thymocytes. The ILN in the cortex and medulla and LI in the medulla surpassed the reference values by 1.5, 1.3, and 1.1 times, respectively. LI in the thymic cortex was virtually the same (Table 2). Activation of DNA production in this group can be indicative of the post-stress compensatory stimulation, triggered by injection of HPM. This was confirmed by our results [6]. In addition, HPM might exert a modifying effect, which is characteristic of all neuropeptides [2,8,10].

TABLE 2. Effect of HPM on DNA Production in Thymic Cortex and Medulla in Newborn Rats Exposed to Prenatal Hypoxia (*M±m*)

Group	Cortex		Medulla	
	ILN	LI	ILN	LI
Control	14.13±0.78	16.41±0.19*	9.64±0.69	14.71±0.17
Hypoxia	8.48±0.17*	11.35±0.16*	5.42±0.30*	10.94±0.19*
HPM	24.51±0.65*	17.94±0.29*	14.29±0.60*	16.02±0.21*
HPM+hypoxia	22.40±1.98	16.91±0.20	12.63±1.19	15.91±0.26*

Injection of HPM led to a significant increase in the percentage of lymphocytes in both newborns (90.15±1.8) and females (87.28±0.8). It was 1.2 and 1.2 times higher than in the control, respectively. In the group with HPM injected in parallel with exposure to hypoxia, the percentage of lymphocytes was almost normal (Table 1).

When analyzing the corrective effect of HPM on the percentage of full-term deliveries, the thymus of newborn rats, and percentage of peripheral blood lymphocytes in the newborn—female pairs, one should not neglect the report [3] about activation of antioxidant defense (enzymatic and nonenzymatic).

The cyclic nucleotide (cAMP and cGMP) system is involved in the realization of HPM effect on cell proliferation [7]. Changes in the level of intracellular messengers is a triggering mechanism in the realization of the mitogen signal of HPM. The correctness of this assumption for immunogenesis has been proven [5].

The results of our experiments show the possibility of correcting posthypoxic disorders of cell proliferation in the thymus by HPM.

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