# Effect of the Hydra Peptide Morphogen on Posthypoxic Disorders in Rats Exposed to Prenatal Hypoxia

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Lipid peroxidation in the lungs and blood are activated while DNA synthesis in the tracheal epithelium and hepatocytes is inhibited during the first five days of postnatal life in rat pups after severe prenatal hypoxia. Intraperitoneal injection of the undecapeptide pGlu-Pro-Pro-Glu-Glu-Ser-Lys-Val-Ile-Leu-Phe, a peptide morphogen isolated from the hydra, before hypoxia normalizes lipid peroxidation in the lungs and blood of the five-day-old pups. A compensatory activation of DNA synthesis occurs in tracheal epithelium and hepatocytes.

**Key Words:** *lipid peroxidation; DNA synthesis; hydra peptide morphogen; hypoxia*

Previously, we showed that prenatal hypoxia leads to inhibition of DNA synthesis in the tracheal epithelium of newborn rats and to activation of lipid peroxidation (LPO) in their lungs and blood [5]. Injection of pGlu-Pro-Pro-Glu-Glu-Ser-Lys-Val-Ile-Leu-Phe, a peptide morphogen isolated from the hydra head, into pregnant females before hypoxia prevents posthypoxic disturbances in newborn pups [6]. The purpose of the present study was to evaluate proliferative processes and LPO in lingual epithelium, hepatocytes, trachea, lungs, and blood of 5-day-old rat pups after prenatal hypoxia.

## MATERIALS AND METHODS

Newborn rats ($n$=394) were separated into four groups: intact rats (control, group 1), rats exposed to prenatal hypoxia (group 2), rats born to females given hydra peptide morphogen (HPM) (group 3), and rats born to females given HPM before hypoxia sessions. Hypoxia was produced by placing the females in a pressure chamber (9000 m, $Po_2$=42 mm Hg) for 4 h daily from days 14 to 19 of pregnancy. The peptide was synthesized at the Laboratory of Peptide Chemistry (Cardiology Research Center, Russian Academy of Medical Sciences). It was injected into females of groups 3 and 4 intraperitoneally in a dose of 10 μg/kg body weight in 0.15 ml normal saline. Females of groups 1 and 2 were given the same volume of normal saline.

The pups were euthanazed by rapid decapitation on day 5 after birth (7 days after the last exposure to hypoxia). For the evaluation of DNA synthesis, rat pups were injected with $^3$H-thymidine (1 μCi/g body weight, specific activity 84 Ci/mmol) 1 h before euthanasia. Immediately after euthanasia, the trachea, liver, and tongue of the animals were fixed in a 3:1 ethanol:acetic acid mixture and embedded in paraffin, after which sections were prepared and coated with type M photoemulsion. Autoradiographs were prepared as previously [4]. The nuclear labeling index (NLI) was determined by counting 2500 nuclei in the tracheal proliferative zone and 3000 nuclei in liver and tongue tissues and expressed as the percentage ratio of labeled nuclei to the total number of nuclei. Nuclei were considered as labeled if they contained at least 5 silver grains. Levels of malonic dialdehyde [8], α-tocopherol [10], lipid hydroperoxides [2], and total lipids (using Lachema test kits) were measured after decapitating the animals and treating the tissues with liquid nitrogen. The results were analyzed by Student's $t$ test.

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## RESULTS

In rats exposed to prenatal hypoxia, abnormal proliferative processes were observed in tracheal epithelium and hepatocytes on day 5 of postnatal development (day 7 after the last exposure to hypoxia). The NLI in tracheal epithelium and liver was more than 2 times and 1.3 times lower, respectively, than in the control group (Table 1). In lingual epithelium, however, cell division was stimulated, NLI being significantly higher than in the control. This stimulation is of a compensatory nature [1]. C. Niedenzu *et al.* [11] showed that chronic hypoxia stimulates but not inhibits DNA synthesis in the lungs and liver of adult rats. This discrepancy may be due to different experimental conditions: a medium hypoxia (an "altitude" of 4250 m) was employed by these researchers.

The content of lipid hydroperoxides in the lungs of experimental rats was 1.4-fold higher, while that of α-tocopherol was 1.5-fold lower than in the control (Table 2). Blood levels of lipid hydroperoxides and malonic dialdehyde in experimental rats were 1.4- and 1.6-fold higher, respectively, than in the control, while blood level of α-tocopherol was 1.6 times lower (Table 3). Activation of LPO and weak of antioxidant defense (AOD) may play an important role in the inhibition of proliferative processes [7]. The breakdown of AOD caused by prenatal hypoxia was not compensated on the 7th day after barocamera.

Administration of HPM to pregnant females led to inhibition of DNA synthesis in the tracheal epithelium of 5-day-old rats, which was manifested in a significant 1.9-fold decrease in NLI compared with the control. A similar proliferative response was observed in hepatocytes of 5-day-old rats (NLI was 1.5 times lower than in the control group), whereas proliferative activity in their lingual epithelium did not differ from that in the control. It should be noted that in our previous studies this morphogen stimulated cell proliferation in a wide dose range and in various tissues (cornea, thymus, and tongue) of adult rats [9]. The elucidation of the causes of changes in cell proliferation in rat pups whose mothers had received HPM during pregnancy requires a separate investigation.

In 5-day-old rats, we observed no significant response of blood LPO—AOD system to HPM noted if their mothers had not been exposed to hypoxia. However, significant changes occurred in lung lipid hydroperoxides and total lipids: the content of total lipids (LPO substrate) was lower (1.6-fold) while that of lipid hydroperoxides (an LPO product) was higher compared with the control, indicating intensification of LPO. Lipids serve as a substrate of active oxidation and a source of tissue antioxidant activity. Hence, a decrease in the total lipid content indicates the suppression of AOD. Previously, we showed that glutathione peroxidase activity is decreased in the lungs of newborn rats of HPM-treated mothers without exposure to hypoxia [6]. Presumably, the inhibited proliferative activity in the tracheal epithelium of newborn and 5-day-old rats born to such mothers is associated with LPO processes and is due to the effect of HPM on the thiol mechanisms involved in the regulation of cell division.

In 5-day-old rats whose mothers had received HPM before exposure to hypoxia, the NLI values were significantly higher than in the control group. In the lingual epithelium of these rats, the DNA synthesis was activated, as evidenced by higher NLI (1.5-fold) than in the control group. Administration of HPM to pregnant rats before exposure to hypoxia prevented the DNA synthesis impairment in the liver of 5-day-old pups: NLI in their hepatocytes did not differ from that in the control group. Thus, post-hypoxic compensatory stimulation of DNA synthesis in tracheal epithelium under the influence of HPM occurred at earlier stages of ontogeny than in hepatocytes. Regulatory peptides exert opposite effects depending on the conditions of their use [3]. The finding that HPM acts as an inhibitor of cell division in the trachea and liver if maternal oxygen supply during embryogeny was normal does not argue against its ability to normalize or stimulate cell division in these tissues following exposure to hypoxia. In this

**TABLE 1.** Effect of the Hydra Peptide Morphogen (HPM) on DNA Synthesis in Tracheal and Lingual Epithelia and Hepatocytes of Five-Day-Old Rats

| Group | Nuclear labeling index, % | | |
|---|---|---|---|
| | trachea | tongue | liver |
| 1st: control group | 0.97±0.04 | 7.28±0.25 | 6.77±0.36 |
| 2nd: hypoxia | 0.42±0.04* | 8.44±0.19* | 5.10±0.26* |
| 3rd: HPM | 0.49±0.04* | 6.72±0.17 | 4.51±0.32* |
| 4th: HPM+hypoxia | 1.20±0.09* | 11.10±0.46* | 7.54±0.43 |

**Note.** Here and in Tables 2 and 3: *$p<0.001$ compared with the control group.

**TABLE 2.** Effect of the Hydra Peptide Morphogen (HPM) on the LPO—AOD System in the Lungs of Five-Day-Old Rats

| Group | Total lipids, mg/g tissue | Lipid hydroperoxides, mmol/g lipid | Malonic dialdehyde, fl. units/g lipid | α-Tocopherol, μg/g lipid |
|---|---|---|---|---|
| 1st: control group | 2.29±0.11 | 0.508±0.028 | 566.7±30.4 | 31.70±2.39 |
| 2nd: hypoxia | 2.14±0.13 | 0.697±0.033* | 638.5±36.2 | 21.62±1.43* |
| 3rd: HPM | 1.87±0.12* | 0.690±0.037* | 659.6±39.8 | 29.64±1.98 |
| 4th: HPM+hypoxia | 2.03±0.12 | 0.574±0.040 | 591.8±44.4 | 40.34±3.15* |

**TABLE 3.** Effect of the Hydra Peptide Morphogen (HPM) on the LPO—AOD System in the Blood of Five-Day-Old Rats

| Group | Total lipids, mg/g tissue | Lipid hydroperoxides, mmol/g lipid | Malonic dialdehyde, fl. units/g lipid | α-Tocopherol, μg/g lipid |
|---|---|---|---|---|
| 1st: control group | 5.37±0.24 | 0.103±0.007 | 52.54±3.02 | 33.13±2.56 |
| 2nd: hypoxia | 5.32±0.27* | 0.151±0.017* | 85.93±4.76* | 24.47±1.51* |
| 3rd: HPM | 5.82±0.31 | 0.095±0.008 | 55.85±3.94 | 36.35±1.94 |
| 4th: HPM+hypoxia | 6.29±0.29* | 0.124±0.008 | 47.85±2.78 | 36.56±1.90 |

case, a modulatory effect of this morphogen on tissue homeostasis is plausible.

When HPM was injected into pregnant females with oxygen deficiency, the lipid hydroperoxide content in the lungs of newborn pups did not differ from that in the control, while the α-tocopherol content was significantly higher. HPM also corrected post-hypoxic abnormalities in the blood. Owing to normalization of α-tocopherol content or a significant increase in total lipids, the levels of lipid hydroperoxides and malonic dialdehyde did not differ from those in the control group.

Our results indicate that the consequences of prenatal hypoxia in newborn rats persist for at least 5 days of postnatal development. Antenatal administration of HPM prevents the occurrence of post-hypoxic disturbances in the LPO—AOD system of the lungs and blood of 5-day-old rats, leading to normalization of proliferative processes.

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