The Use of a Nonopiate Leu-Enkephalin Analog and of Hydra Peptide Morphogen for the Correction of Proliferation Disturbances in Tracheal Epithelium and of LPO Processes in Lungs of Newborn Rats Exposed to Prenatal Hypoxia

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Newborn rats were born of females exposed to high-altitude hypoxia. Pregnant females were injected i.p. either nonopiate leu-enkephalin analog or hydra peptide morphogen 10 μg/kg 30 min prior to being placed in a pressure chamber. Prenatal hypoxia causes the inhibition of the proliferative processes in tracheal epithelium and activation of lipid peroxidation (LPO) in lungs of newborn rats. The administration of nonopiate leu-enkephalin analog prevents the development of posthypoxic alterations in newborn rats. The administration of hydra peptide morphogen inhibits the proliferation of tracheal epithelium and lowers the activity of the antioxidant defense of the lungs in newborn rats.

Key Words: *lipid peroxidation; DNA synthesis; nonopiate leu-enkephalin analog; hydra peptide morphogen; newborns*

In previous investigations we established that prenatal hypoxia causes inhibition of DNA synthesis in tracheal epithelium as well as activation of LPO processes in lung tissue of newborn rats. At the present time regulatory peptides and their synthetic analogs are being assessed as potential correctors of various pathological processes. In this connection we decided to use representatives of different neuropeptide groups, such as nonopiate analog of leu-enkephalin (NALE) and hydra peptide morphogen (HPM). NALE has the structural formula Phe-D-Ala-Glu-Phe-Leu-Arg. The leu-enkephalin itself is considered an endogenous antihypoxant, while its synthetic analog dalargin possesses a pronounced antioxidant activity. NALE was used in the present study because opiates are contraindicated during pregnancy. HPM is from another regulatory peptide group, with the structural formula pGlu-Pro-Pro-Glu-Glu-Ser-Lys-Val-Ile-Leu-Phe. The physiological role of this regulatory peptide is now being actively studied. The stimulatory effect of HPM on proliferative processes is well known [9].

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MATERIALS AND METHODS

Experiments were carried out on 479 newborn rats. The animals were decapitated 24 h after birth (3 days after the final experimental treatment). The newborn rats were divided into 6 groups: the 1st was intact; the 2nd underwent prenatal hypoxia; the offspring in the 3rd group were of females which had received NALE; the offspring in the 4th

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group were of females which had received NALE against the background of hypoxia; the mothers of the 5th group of pups had received HPM; the 6th group comprised offspring of females which had received HPM against the background of hypoxia. The state of hypoxia was modeled by placing the animals in an SBK-48 pressure chamber (altitude 9000 m, partial pressure of oxygen 42 mm Hg) for 4 h from day 14 to day 19 of gestation, as described previously. The peptides studied were administered to females i.p. at 10 μg/kg in 0.15 ml saline 30 min prior to placement in the pressure chamber. Females of the 1st and 2nd groups received an equivolume of saline. For the assessment of DNA synthesis the newborn rats were injected with ^{3}H-thymidine at 1 μCi/g (specific activity 84 Ci/mmol) 1 h prior to decapitation. The autoradiographs were prepared according to the laboratory method [2]. The index of labeled nuclei (ILN) was determined on the basis of the estimation of 2500 nuclei in the zone of proliferation in the trachea and expressed as the ratio of labeled nuclei to the total number of nuclei in percent. Fluorometric determination of the levels of malonic dialdehyde (MDA) [8] and α-tocopherol [11], spectrophotometric assay of hydroperoxides of lipids (HPL) [1] as well as of enzymes of antioxidant defence, such as catalase [10], superoxide dismutase (SOD) [13], and glutathione peroxidase (GPO) [3], and photocalorimetric determination of total lipids (with the use of Lachema kits) were performed in lung homogenates after the decapitation of animals and treatment of tissues with liquid nitrogen. The level of enzyme activity was reduced to the protein concentration in samples determined after Lowry. In addition, the gravimetric measurements of absolute body weight were performed as well as recording of the absolute and relative mass of the lungs in newborn rats. The data were processed statistically using the Student test.

RESULTS

No reliable changes of the absolute values of body weight as well as the absolute and relative values of lung mass were found in any of the experimental groups in comparison with the analogous indexes in intact animals.

In the assessment of DNA synthesis a threefold reliable decrease of ILN from 1.60±0.10% in intact animals to 0.50±0.05% in posthypoxic newborn rats was noted in tracheal epithelium, just as was found in our previous experiments. The administration of NALE to pregnant rats against the background of a normal oxygen supply did not cause significant ILN changes in tracheal epithelium of newborn rats. These findings are in agreement with previous results when NALE did not affect hepatocyte proliferation in newborn rats [7]. The administration of HPM to pregnant rats resulted in a reliable ILN decrease to 1.20±0.04% in tracheal epithelium of newborn rats as compared to the control level. It should be noted that in our preceding investigations HPM stimulated proliferative processes in a broad range of doses and in various tissues (cornea, thymus, tongue) [9]. The question as to whether the inhibition of DNA synthesis is due to the characteristics of regulation of proliferation in tracheal epithelium or whether in the present case we are dealing with peculiarities of the age reaction requires further experiments to clarify. However, the normalization of ILN took place in newborn rats of the "HPM-hypoxia" group. Not only was normalization of ILN found in offspring of females which had received NALE prior to hypoxia, but a reliable increase of this index to 2.00±0.08% as compared to the intact control.

Antenatal administration of NALE and HPM against the background of a normal oxygen supply of pregnancy did not cause changes in total lipids, HPL, functional activity of catalase, or SOD (Table 1). A varying response to administration of preparations was found in the GPO measurements: whereas NALE did not affect the enzyme's activity, HPM reliably lowered it 1.6-fold. The statistically reliable MDA decrease for NALE administration may be due to the significant 1.4-fold increase of α-tocopherol in lung tissue. Presumably, it can be mobilized under the influence of NALE from the liver, its main depot.

Thus, the result of NALE action against the background of normal pregnancy was activation of the nonenzyme component of antioxidant defense (AOD) which resulted in a decrease of the content of secondary LPO products in the lungs. However, the proliferative activity of tracheal epithelium was not affected by this shift of balance in the LPO-AOD system. Antenatal HPM administration resulted in an attenuation of the AOD enzyme component due to a decrease of GPO activity against the background of a normal oxygen supply of pregnancy. It may be assumed that the decrease of proliferative activity of tracheal epithelium in newborn rats in this case is associated with LPO processes and is due to the effect of HPM on thiolic mechanisms of cell division regulation.

Antenatal administration of NALE against the background of hypoxia leveled the negative posthy-

TABLE 1. Effect of NALE and HPM on the State of the LPO–AOD System in Lungs of Newborn Rats

Index of LPO-AOD system	Control	Hypoxia	NALE	NALE-hypoxia	HPM	HPM-hypoxia
Total lipids, mg/g tissue	1.71±0.07	1.26±0.09*	1.55±0.08	1.66±0.08	1.56±0.07	1.69±0.09
HPL, mM/g lipids	0.188±0.015	0.234±0.012*	0.179±0.016	0.184±0.014	0.176±0.013	0.191±0.011
MDA, unit of fluorescence/g lipids	504.4±46.1	618.9±45.1	344.3±53.6*	358.3±48.1*	488.3±42.3	498.9±42.9
α–Tocopherol, μg/g lipids	12.68±0.68	10.64±0.51*	17.45±1.37*	19.52±1.39*	13.08±0.68	16.62±0.86*
Catalase, nm H_2O_2/min/mg protein	13.20±1.00	9.80±0.57*	10.60±0.63	12.50±0.64	10.50±0.54	13.10±0.89
SOD, nM diformasan/min/mg protein	1.17±0.10	0.91±0.06*	1.21±0.07	1.15±0.07	1.19±0.08	1.12±0.07
GPO, nM NADPH/min/mg protein	10.34±0.10	5.23±0.40*	11.09±0.53	13.47±0.97*	6.60±0.46*	10.08±0.63

Note. Asterisk – $p<0.001$ in comparison with the control group.

poxic sequelae in the LPO-AOD system. This is confirmed by the increase, as compared to the control, both of the enzyme (a reliable increase of GPO activity) and of the nonenzyme (a reliable increase of the α-tocopherol content) AOD components. This probably caused the drop of HPL to the control level while the MDA content was significantly lower than the control indexes. The level of total lipids in lung tissue was not different from the control. When considering the significance of the stimulatory processes of DNA synthesis in the trachea, the idea about the compensatory nature of post-stressor activation of proliferation should be taken into account [4].

It should be stressed that under conditions of a normal oxygen supply the peptides of interest variously affected the nonenzyme AOD component, namely NALE reliably increased the α-tocopherol content, whereas HPM affected it insignificantly as compared to the control, but both substances reliably increased the α-tocopherol level in hypoxia.

HPM differs substantially from the leu-enkephalins according to its structural formula, biological activity, and the mode of interaction with the cell. HPM and NALE did, however, demonstrate some common properties under the studied conditions. Interesting in this context are the reports that a number of regulatory peptides may act on the cell by binding with receptors for other bioregulators [15], by directly affecting the activity of membrane-binding enzymes [5], or by interacting directly with the lipid matrix of plasma membrane of the effector cell [6,12].

The high efficiency of NALE in the prevention of posthypoxic disturbances as well as the data on cytoprotective properties of this preparation prompt further investigation of its biological properties.

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