Effects of Melatonin and Epithalamin on the Content of Protein and Lipid Peroxidation Products in Rat Cortex and Hippocampus under Conditions of Acute Hypoxia

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The effects of melatonin and epithalamin on the content of protein and lipid peroxidation products in the cortex and hippocampus of hypoxic rats were studied under conditions of acute hypobaric hypoxia equivalent to the altitude of 12,000 m. Pineal preparations reduced the intensity of free-radical processes in the brain exposed to acute hypoxia. Epithalamin more effectively than exogenous melatonin protected hippocampal neurons during acute hypoxia by reducing free-radical damage to lipids and proteins.

Key Words: *melatonin; epithalamin; oxidation-modified proteins; malonic dialdehyde; acute hypoxia*

Accumulation of oxidation-modified proteins (OMP) and lipid peroxides, specifically MDA, are important "markers" of tissue injury under conditions of unfolding acute stress [5,6,15]. Oxidative modification of proteins is the earliest and at the same time most reliable indicator of disease [3]. Accumulation of LPO and OMP products under conditions of oxygen deficit has been described not once [6,15], but just few means are offered for correction of protein peroxidation under conditions of hypoxia. On the other hand, endogenous regulators of physiological functions melatonin [14] and epithalamin [1,8] have been used as antioxidants. We studied the effects of pineal preparations (melatonin, epithalamin) on the intensity of OMP and LPO in brain structures, the target organ in acute hypoxia [11].

MATERIALS AND METHODS

The study was carried out on 97 young outbred male rats. The following groups were formed: control (group 1), melatonin injection (group 2), epithalamin injection (group 3), acute hypoxia modeling (group 4), melatonin injection before hypoxia (group 5), and epithalamin injection before hypoxia (group 6). Rat resistance to acute hypobaric hypoxia was evaluated 2 weeks before the study; only animals with medium resistance were then used in the study. Acute hypobaric hypoxia was simulated in a modified flow pressure chamber by "elevating" the rats to an altitude of 12,000 m at a rate of 50 m/sec. The animals were exposed at the "height plateau" until the second agonal inspiration, after which they were "returned" to the zero height by restoring normal atmospheric pressure and vital activity of animals. Pineal preparations were injected intraperitoneally 30 min before hypoxia simulation: melatonin (Sigma) in 0.1% ethanol solution in a dose of 1 mg/kg and epithalamin (Samson) in 0.9% NaCl solution in a dose of 2.5 mg/kg. Controls and animals of the hypoxia group received an equivalent volume of the solvent. Thirty minutes after acute hypoxic exposure or 1 h after injection of melatonin or epithalamin, the animals were decapitated, the brain was removed and stored in liquid nitrogen until further studies.

Brain structures, cortex (mainly, the frontal lobe) and hippocampus (mainly, the CA1 field), were taken for the study. For MDA and OMP assays, the samples

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were homogenized in cold (2-4°C) 0.25 M Tris-HCl (Sigma buffer (pH 7.4). Samples from two animals were pooled for preparing the suspension. Protein and lipid peroxidation products were measured in supernatant after 15-min centrifugation at 900*g* of the homogenates. The levels of OMP were evaluated by the content of aldehyde- and ketone-dinitrophenyl hydrazones; neutral products were measured at λ=370 nm and basic at 430 nm [2,7]; MDA content was evaluated by the reaction with TBA (Sigma). The data were processed by Statistica 5.0 software using parametric (Student's *t*) and nonparametric (Wilcoxon's) tests and ANOVA for evaluating the significance of differences between the groups.

RESULTS

Pineal preparations injected to normoxic animals modulated the levels of protein and lipid peroxidation products in the studied brain structures (Tables 1 and 2). Injection of epithalamin to normoxic animals caused no changes in the levels of protein peroxidation products, but reduced MDA content by 16.9% in the cortex and by 23.7% in the hippocampus. Melatonin increased OMP content in the hippocampus (neutral by 19.8%, basic by 15.2%) and decreased MDA level by 20.9%. Melatonin caused no changes in the cortical levels of OMP and MDA. This stimulation of oxidative modification of the hippocampal proteins after melatonin injection was to a certain measure in line with the data indicating an increase of plasma levels of protein peroxidation products after melatonin injection [1].

Significant accumulation of OMP in the studied brain structures was noted under conditions of acute hypoxia (Table 1). In the cortex, the level of neutral products increased by 38.3% in comparison with the control, of basic products by 58.8%; in the hippocampus, the levels of these products increased by 16.9 and 22.5%, respectively. The content of MDA (Table 2) in the cortex and in the hippocampus also increased under conditions of hypoxia (by 16.3%). These results were in line with published data [5,15].

Injection of melatonin before creation of acute hypoxia reduced the intensity of free-radical processes in the hemispheric cortex to the levels of control animals. In the hippocampus only LPO intensity normalized, while the levels of protein peroxidation products in the cells did not decrease, but continued to increase (by 25.3%) in comparison with hypoxic animals which received no melatonin. A lesser efficiency of melatonin protection of proteins from free radical attack after hypoxic exposure could be explained by lesser solubility of melatonin in water medium [10]. As a lipophilic molecule [13], melatonin more actively exhibited the free-radical detoxifying effects in the membranous lipid layer, and presumably for this reason did not always effectively protected the protein molecules. It was shown that it did not prevent free-radical inactivation of glucose-6-phosphatase [9] and peroxide modification of apolipoproteins [12]. Epithalamin injection before acute hypoxic exposure reduced significantly the levels of OMP and MDA in the hippocampus (to the levels of control animals), though did not modify the intensity of protein peroxidation in the cortex, normalizing MDA levels in it.

These results indicate that pineal preparations reduce the intensity of free-radical processes (at the expense of LPO and OMP) in the brain under conditions of acute hypoxia, thus inhibiting the development of oxidative stress. Epithalamin more effectively than exogenous melatonin protects the hippocampal neurons from acute hypoxia by reducing the intensity of lipid and protein peroxidation. Presumably, a more pronounced reduction of biomolecules oxidation in the

TABLE 1. Levels of Protein Oxidative Modification Products (mmol 2,4-dinitrophenyl hydrazones/g protein) in the Brain Cortex and Hippocampus of Young Rats in Acute Hypobaric Hypoxia and Melatonin and Epithalamin Treatment (*M*±*m*, *n*=7)

Exposure	Brain cortex		Hippocampus	
	neutral products	basic products	neutral products	basic products
Control	2.530±0.132	1.360±0.108	2.530±0.127	1.380±0.096
Melatonin	2.600±0.174	1.510±0.064	3.030±0.213*	1.850±0.125*
Epithalamin	3.030±0.219	1.650±0.168	2.690±0.133	1.590±0.112
Hypoxia	3.500±0.206*	2.160±0.136*	2.960±0.136*	1.690±0.104*
Melatonin and hypoxia	2.520±0.367+	1.410±0.207+	3.740±0.274+°	2.100±0.140°
Epithalamin and hypoxia	3.560±0.162*°	1.920±0.183*	2.290±0.205+	1.480±0.088

Note: Here and in Table 2: p<0.05 in comparison with: control; hypoxia; parameters after injection of melatonin or epithalamin to normoxic animals.

TABLE 2. Content of MDA (μmol/g tissue) in Hemispheric Cortex and Hippocampus of Young Rats in Acute Hypobaric Hypoxia and Melatonin or Epithalamin Treatment ($M \pm m$, $n=7$)

Exposure	Drain cortex	Hippocampus
Control	46.50±1.61	45.50±1.35
Hypoxia	54.10±1.83*	52.90±2.36*
Melatonin	43.10±1.46	36.00±1.29*
Epithalamin	38.60±1.29*	34.70±4.72*
Melatonin and hypoxia	38.80±1.34+	35.60±3.90*+
Epithalamin and hypoxia	38.50±1.31+	27.30±3.28*+

brain structures under conditions of oxygen shortage after injection of pineal peptide (epithalamin) is due to the stimulatory effect of the pineal peptides on the production of endogenous melatonin [4,8].

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