Effect of Melatonin on Cyclic Nucleotide Content and Intensity of Lipid Peroxidation in the Hippocampus and Habenula of Rats Exposed to Acute Hypoxia

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Single intraperitoneal injection of melatonin in a dose of 1 mg/kg prevented accumulation of cGMP and intensification of lipid peroxidation in the hippocampus and habenula of rats exposed to acute hypobaric hypoxia (12,000 m). Changes in habenular content of cGMP suggest that melatonin prevents hypoxia-induced activation of heme-oxygenase.

Key Words: *melatonin; acute hypoxia; forebrain; cyclic nucleotides; heme-oxygenase*

Excessive production of free radicals is one of the main factors causing neuronal death under hypoxic conditions [13]. Antioxidants preventing overproduction of free radicals were shown to protect nerve cells against hypoxia [7]. Pineal hormone melatonin is an antioxidant directly interacting with free radicals (especially with the most toxic $OH^{\bullet}$ radical) and activating the synthesis of antioxidant enzymes glutathione peroxidase [10] and SOD [6], which effectively protect membrane lipids and proteins against hypoxic damage *in vitro* [7]. In the *in vitro* model, melatonin inhibits neuronal production of NO [9], which (under conditions of oxidative stress and in the presence of superoxide anion radicals $O_2^{\bullet-}$) is converted into highly toxic peroxinitrite $ONOO^{\bullet}$ promoting protein peroxidation [8] and damage to brain cell. Protection of cells from the toxic effect of $ONOO^{\bullet}$ provided by melatonin should also reduce hypoxic damage.

Cyclic nucleotide assay (in particular cAMP) is an obligatory test for evaluation of tissue sensitivity to hypoxia [1]. In addition, the level of cGMP allows to evaluate the formation of NO and CO stimulating soluble guanylate cyclase [15]. In most brain structures, the content of cGMP correlates with guanylate cyclase, rather than NO synthase activity. In some structures, for instance in habenular nuclei, NO synthase can not be detected and the content of cGMP is determined by the rate of CO generation, *i.e.* heme-oxygenase activity [15]. However, *in vivo* effects of melatonin on the formation of cyclic nucleotides, lipid peroxidation (LPO), and heme-oxygenase activity in different brain structures under hypoxic conditions are poorly studied. The aim of this study was to investigate the effect of melatonin on the level of cAMP, cGMP, and TBA-reactive LPO products (MDA) in the hippocampus and habenala of rats exposed to acute hypoxia.

MATERIALS AND METHODS

The study was carried out on immature male albino rats (65-75 g) growing up to the age of 5.5-6 weeks by the end of the experiments. The experiments were performed at natural illumination during spring- and summer-time. Two weeks before the experiments the rats (n=49) were tested for their resistance to acute hypobaric hypoxia and the animals with moderate resistance were selected for further experiments. During the experiments one group of rats received intraperitoneal melatonin (Sigma) in a dose of 1 mg/kg in 0.1% ethanol 30 min before acute hypoxia. The control group received an equal volume of vehicle. Acute hypobaric hypoxia was modeled by elevating the animals to an altitude of 12,000 m (50 m/sec) in a modi-

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fied pressure chamber. The rats were kept at this altitude until the second agonal inspiration, then returned to the zero altitude and allowed to recover. Thirty minutes after hypoxic exposure (1 h after melatonin injection) the animals were decapitated, the brain was rapidly removed and stored in liquid nitrogen before measurements.

The hippocampus (mostly the CA1 area) and habenula were isolated from forebrain sections according to the stereotaxic atlas for immature rats [12]. Tissue samples were homogenized in a phosphate buffer, pH 7.4 (9 mM KH_2PO_4, and 30 mM Na_2HPO_4), cyclic nucleotides were extracted on Amper SAX minicolumns (Amersham), eluted with 367 mM trichloroacetic acid, and assayed with cAMP and cGMP radioimmune kits (Immunotech). For MDA measurements, tissue specimens were homogenized in cold (2-4°C) 0.25 M Tris-HCl buffer, pH 7.4 (Sigma) and centrifuged for 15 min at 900*g*. MDA was assayed in the supernatant by the reaction with TBA (Sigma) [4]. The data were analyzed statistically by ANOVA, Student's *t* test and nonparametric Wilcoxon's test.

RESULTS

Acute hypoxia increased the content of cGMP in both the hippocampus and habenula (Table 1). Accumulation of cAMP after hypoxic exposure was observed only in the hippocampus, and therefore cAMP/cGMP ratio remained at the control level, while in the habenula this index decreased below the control. It was demonstrated that acute hypoxic exposure reduced cerebral content of cAMP in high-resistant animals, while the animals which did not survive hypoxia exhibited initially high level of cAMP [2]. Stimulation of cAMP production in the nervous tissue reduces the resistance to hypoxia, while a decrease in the cAMP/cGMP ratio is associated with by its increase [3]. Therefore, accumulation of cAMP in the hippocampus and the decrease of cAMP/cGMP ratio in the habenula indicate low resistance of hippocampal CA1 area cells to hypoxia which agrees with published data [13].

Soluble guanylate cyclase constituting about 85% total guanylate cyclase [5] is activated by CO• and NO•, free-radical second messengers produced after activation of NO synthase and heme-oxygenase, respectively. Habenular nuclei are characterized by very high activity of heme-oxygenase [15] and exhibited no NO synthase activity. It suggests that the concentration of cGMP in the habenular complex is completely determined by CO and heme-oxygenase activity. The increased content of cGMP in the habenula suggests that acute hypoxia activates heme-oxygenase. This conclusion agrees with the data reported for other brain structures: one of two heme-oxygenase isozymes, heme-oxygenase 1, was identified as a heat shock protein. Its expression increases under conditions of oxidative stress associated with acute oxygen deficiency [11,14].

Considerable heme-oxygenase activity was also found in the hippocampus characterized by high NO synthase activity. However, hippocampal heme-oxygenase is weakly stimulated by oxygen deficiency [14]. The level of hippocampal cGMP seems to be largely determined by NO synthase. In addition, guanylate cyclase can be activated by other free radicals [5]. The increased content of hippocampal MDA observed in our study (Table 1) suggests LPO activation with the

TABLE 1. Effect of Melatonin on cAMP, cGMP, and MDA Content and cAMP/cGMP Ratio in Forebrain of Rats Exposed to Acute Hypobaric Hypoxia (*M*±*m*, *n*=6)

Index	Control	Hypoxia	Melatonin	Melatonin and hypoxia
cAMP, nmol/g				
hippocampus	0.980±0.095	1.350±0.142*	1.130±0.115	1.09±0.11
habenula	1.190±0.116	1.390±0.127	1.390±0.115	1.040±0.087+°
cGMP, nmol/g				
hippocampus	0.390±0.037	0.590±0.065*	0.110±0.012	0.120±0.014*+
habenula	0.260±0.031	1.790±0.177*	0.270±0.025	0.360±0.043+
cAMP/cGMP				
hippocampus	2.70±0.28	2.30±0.25	11.2±1.2*	9.30±0.95*+
habenula	5.60±0.53	0.80±0.05*	4.70±0.43	2.90±0.32*+°
MDA, μmol/g				
hippocampus	45.50±1.35	52.90±2.36*	36.00±1.29*	35.6±3.9*+
habenula	47.80±4.07	51.60±4.29	39.70±2.24	44.80±4.36

Note. $p<0.05$: *compared to the control, +compared to hypoxia, °compared to the effect of melatonin.

formation of free radicals which can stimulate guanylate cyclase. In general, the accumulation of cGMP in the forebrain without significant changes in cAMP content should improve neuronal survival under conditions of acute hypoxia due to improvement of intracerebral circulation [5].

Intraperitoneal melatonin had no effect on the content of cAMP in the hippocampus and habenula of nonhypoxic animals, but reduced the hippocampal content of cGMP (Table 1), which agrees with published data [9]. It also reduced the production of MDA (Table 1), which attests to its pronounced antioxidant activity.

In the hypoxic rats, melatonin prevented accumulation of cAMP in the hippocampus and reduced the content of cGMP, which markedly increased the cAMP/cGMP ratio (Table 1). The content of cAMP and cGMP in the habenular complex decreased, while the cAMP/cGMP ratio increased. As a result, the content of cAMP and cGMP in melatonin-treated animals did not significantly differ from the control, while the cAMP/cGMP ratio decreased below the control and was lower than in melatonin-treated nonhypoxic animals. Melatonin considerably decreased the hippocampal content of MDA (Table 1) without affecting its level in the habenular complex. The latter suggests that low activity of habenular guanylate cyclase in melatonin-treated hypoxic animals could hardly be explained by antioxidant activity of this hormone and reduced production of free radicals.

Thus, administration of melatonin prevented the effects of acute hypoxia on the formation of cyclic nucleotides and LPO intensity in rat forebrain. Simultaneous decrease in the content of cGMP in the hippocampus and habenula implies that this hormone acts against both free radicals and NO synthase, and probably against heme-oxygenase. There is no doubt that antiradical and anti-NO synthase effects of melatonin play a positive role under hypoxic conditions. The role of anti-heme-oxygenase effect remains unclear: activation of heme-oxygenase during oxidative stress promotes neuronal survival and reflects their protective reaction [11,14], but it can also have negative consequences [11]. In general, our findings allow to refer melatonin to antioxidants reducing destructive effects of *in vivo* hypoxia and producing complex antihypoxic action.

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