Effect of Melatonin on the Relationship between Lipid Peroxidation and Proteolytic Activity in Basal Nuclei of Rat Brain during Acute Hypoxia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 94-96, July, 2006
Original article submitted March 1, 2005

We studied the effect of melatonin on the relationship between LPO intensity and proteolytic activity in basal nuclei (caudate nucleus, globus pallidus, amygdaloid complex, and nucleus accumbens septi) of rat brain during acute hypobaric hypoxia. Acute hypoxia was accompanied by LPO activation and increase in proteolytic activity. It should be emphasized that the intensity of proteolysis was higher in structures responding by more pronounced LPO activation (nucleus accumbens septi and globus pallidus). Intraperitoneal injection of 1 mg/kg melatonin 30 min before acute hypoxia inhibited LPO and prevented the increase in proteolysis in basal nuclei of the brain. The effect of melatonin was most pronounced in basal nuclei highly sensitive to acute hypoxia.

Key Words: *melatonin; basal nuclei; lipid peroxidation; proteolysis; acute hypobaric hypoxia*

Previous studies showed that acute hypoxia is accompanied by LPO activation [5]. Increased production of LPO products results in activation of membrane enzymes [2], including proteolytic enzymes, while activation of proteolytic enzymes induces cell damage or their death [6].

A relationship exists between LPO and proteolysis. Therefore, melatonin holds much promise for the correction of these processes. Melatonin is a potent antioxidant [4,7] that directly modifies LPO and, probably, affects proteolysis.

Here we studied the relationship between LPO and proteolytic activity during acute hypoxia. Moreover, we evaluated whether melatonin modulates on this relationship in basal nuclei of the brain.

MATERIALS AND METHODS

The experiments were performed on 48 male outbred albino rats aging 5-6 weeks. The animals were divided into 3 groups (1 control group and 2 experimental groups). Experimental hypoxia was induced in rats of experimental group 1. The animals of experimental group 2 received melatonin before hypoxia. Acute hypoxic hypoxia was produced in a modified flow altitude chamber. The animals were "elevated" to a height of 12,000 m. Melatonin in a dose of 1 mg/kg in 0.1% ethanol was injected intraperitoneally 30 min before hypoxia. The rats were decapitated 30 min after the end of acute hypoxia.

The caudate nucleus, globus pallidus, nucleus accumbens, and amygdaloid complex (amygdala) were isolated. Homogenates were prepared in 0.05 M Tris-HCl buffer (pH 7.4). Samples from two animals were pooled.

The intensity of LPO was estimated by the concentration of primary (conjugated dienes, CD) and secondary products (malonic dialdehyde, MDA) [3]. Proteolytic activity (PRA) was determined in the reaction with nitrogen compounds. Proteolysis was assayed by the contents of azoalbumin, azocasein, and azocol [1].

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The results were analyzed by methods of variational statistics (Student's *t* test). The changes in test parameters were studied by calculating the correlation coefficients (Spearman correlation test, Statistica 5.1 software).

RESULTS

Acute hypoxia was accompanied by significant changes in LPO and PRA in basal nuclei (Tables 1 and 2). The concentrations of primary and secondary LPO products increased after hypoxia (Table 1). Changes in the concentration of CD varied from 21.7 to 55.0% (in the caudate nucleus this parameter remained unchanged), while the concentration of MDA (stable LPO product) in all brain structures increased by 1.5-1.9 times.

Acute hypoxia increased PRA in basal nuclei of the brain. Activity of collagen-degrading enzymes in the nucleus accumbens, caudate nucleus, and amygdala increased most significantly (by 65.1, 59.3, and 153.7%, respectively). Enzyme activity in the globus pallidus of posthypoxic animals increased by 5 times (9.28±2.01 *vs*. 1.810±0.177 E_{440}/g tissue/h in the control). Lysis of albumin and casein in some nuclei considerably increased after hypoxia. For example, activity of albumin-degrading enzymes in nerve cells of the nucleus accumbens, globus pallidus, and amygdala increased by 31.6-53.0%. The increase in PRA was estimated by casein hydrolysis and observed in the nucleus accumbens and globus pallidus (by 20.2 and 52.6%, respectively).

These data show that acute hypoxia is accompanied by activation of LPO and increase in PRA

TABLE 1. Effect of Melatonin on the Concentration of LPO Products in Basal Nuclei of Rat Brain during Acute Hypoxia (*M*±*m*, *n*=6-8)

LPO product, group of animals	Brain structure			
	nucleus accumbens	caudate nucleus	globus pallidus	amygdala
CD, μmol/g tissue				
control	231.30±24.55	254.20±21.59	175.10±14.05	195.50±14.72
hypoxia	358.50±32.74*	247.60±23.17	229.90±13.03*	237.80±26.46*
melatonin+hypoxia	308.60±20.14*+	297.20±22.73*+	285.00±23.64*+	277.4±14.1*+
MDA, μmol/g tissue				
control	140.10±4.85	131.20±6.53	126.90±9.01	96.20±4.49
hypoxia	261.70±10.94*	195.90±8.87*	187.30±5.87*	161.20±18.74*
melatonin+hypoxia	162.60±7.33*+	152.50±7.32*+	157.2±5.4*+	139.80±7.59*+

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to hypoxia.

TABLE 2. Effect of Melatonin on PRA in Basal Nuclei of Rat Brain during Acute Hypoxia (*M*±*m*, *n*=6-8)

Proteolysis, group of animals	Brain structure			
	nucleus accumbens	caudate nucleus	globus pallidus	amygdala
By albumin, E_{440}/g tissue/h				
control	102.57±16.50	106.47±11.83	79.78±8.63	55.107±3.720
hypoxia	135.34±12.23*	92.54±6.80*	122.08±10.64*	72.50±6.46*
melatonin+hypoxia	90.94±11.90*+	74.11±9.55*+	65.57±6.22*+	51.17±3.34+
By casein, E_{440}/g tissue/h				
control	108.50±10.08	78.04±9.74	65.250±3.021	50.73±6.24
hypoxia	130.37±10.62*	87.99±6.03	99.55±13.04*	47.68±4.19
melatonin+hypoxia	88.01±9.67*+	65.87±9.07+	47.36±4.95*+	41.36±3.11
By collagen, E_{440}/g tissue/h				
control	2.920±0.579	3.100±0.307	1.810±0.177	1.620±0.219
hypoxia	4.820±0.572*	4.930±0.721*	9.28±2.01*	4.11±0.64*
melatonin+hypoxia	4.210±0.235*	4.310±0.527*	3.620±0.311*+	2.920±0.171*+

in the basal nuclei of the brain. It should be emphasized that PRA was higher in structures exhibiting significant LPO intensification. Therefore, a strong relationship exists between LPO and activity of proteolytic enzymes. The correlations were found between changes in LPO and PRA in the basal nuclei of the brain during hypoxia. These correlations were positive. During hypoxia the increase in LPO was accompanied by activation of proteolytic enzymes. The correlation coefficients varied from 0.602 to 0.851. The mean correlation coefficient for MDA-PRA was higher compared to CD-PRA. For example, hypoxia-induced changes in MDA correlated with variations in albumin PRA in the nucleus accumbens (r=0.788, p=0.002), globus pallidus (r=0.775, p=0.003), and amygdala (r=0.851, p=0.0004).

Administration of melatonin 30 min before hypoxia attenuated changes in LPO and PRA. Some parameters in the basal nuclei returned to normal after melatonin treatment. MDA content in all brain structures surpassed the control by 16.1-45.3%, but was lower than in posthypoxic animals. The concentration of CD underwent various changes. After acute hypoxia CD content increased most significantly in the nucleus accumbens (Table 1). Melatonin slightly increased the concentration of CD (by 33.4% compared to the control). CD content in rats of the melatonin group was 21.6% lower than in posthypoxic animals. CD production in other brain structures of melatonin-treated rats increased compared not only to controls, but also to animals of the hypoxia group.

Pretreatment with melatonin prevented the increase in PRA by albumin and casein (Table 2). Collagenase activity was slightly surpassed the control. However, collagen hydrolysis by nerve cell enzymes in melatonin-treated rats was much lower compared to animals not receiving the hormone. These differences were revealed in the globus pallidus and amygdala (by 61.9 and 22.2%, respectively).

We conclude that administration of melatonin 30 min before experimental acute hypoxia significantly decreases the concentration of stable LPO products (MDA) in basal nuclei of the brain. The observed changes play an important role in the pathological process and probably prevent the increase in proteolysis.

These data are consistent with the results of the correlation test. The number of correlations between the test parameters decreased after melatonin administration. The correlations were mainly found in brain structures exhibiting a significant change in LPO and proteolysis during hypoxia. It should be emphasized that the effect of melatonin was most pronounced in these structures. For example, the number of correlations was maximum in the nucleus accumbens and globus pallidus. In the nucleus accumbens of melatonin-treated rats we found correlations between CD content and casein PRA (r=0.650, p=0.022), MDA content and albumin PRA (r=0.755, p=0.0045), and casein PRA and collagen PRA (r=0.692, p=0.013). After melatonin administration CD content in the globus pallidus negatively correlated with PRA by albumin (r=-0.729, p=0.007), casein (r=-0.753, p=0.005), and collagen (r=-0.806, p=0.002). A positive correlation was found between MDA content and albumin PRA (r=0.599, p=0.040).

Our results indicate that during acute hypoxia activity of proteolytic enzymes of nerve cells in basal nuclei of rat brain depends on LPO. Melatonin modifies LPO and proteolysis. Pretreatment with melatonin before hypoxia significantly decreases the concentration of stable LPO products (MDA), which probably serves as a key event prevent activation of proteolysis.

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