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PHYSIOLOGY

Effects of Melatonin and Epithalamin on the System of Antioxidant Defence in Rats

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At present, free radicals are believed to play a key role in aging and age pathologies. Reactive oxygen species and nitric oxide and its derivatives, generated upon breathing and in various metabolic enzymatic reactions, damage cell macromolecules and cause aging and various pathologies (cancer, atherosclerosis, ischemia, age-related immunodepression, cataracts, etc.) [1, 2].

During the past few years, melatonin, an indole hormone of the pineal gland (epiphysis), has attracted considerable attention. The inhibitory effect of melatonin on the *in vitro* generation of the highly toxic hydroxyl radical is 5–14 times higher than those of glutathione and mannitol [3, 4]. We found that melatonin efficiently inhibited peroxidative chemiluminescence of rat serum *in vitro* [5]. This hormone also activates glutathione peroxidase [3], an enzyme metabolizing H_2O_2 and other hydroperoxides, thereby preventing generation of the hydroxyl radical [5]. Similar to other natural and synthetic antioxidants, melatonin suppresses the development of tumors and cataracts and increases life-span [3, 6]. Epithalamin, a peptide preparation isolated from bovine epiphysis, was found to stimulate the synthesis and secretion of melatonin and to inhibit free-radical processes. It also increases life-span, slows the aging of reproductive and immune systems, and suppresses tumor formation [4, 7]. However, there are no data on the effects of melatonin and epithalamin on the activities of an organism's antioxidant system and its components, specifically Cu,Zn-superoxide dismutase (SOD) and ceruloplasmin.

Here, we describe the results of comparative studies of the *in vivo* effects of melatonin and epithalamin on the activity of Cu,Zn-superoxide dismutase (SOD), ceruloplasmin level, total antioxidant activity, and nitrite content in blood plasma.

Fifty 20–30-day-old male rats (line LIO, Petrov Institute of Oncology) were used in the experiments. The rats were randomly subdivided into three groups. Every morning for five days, the rats of the first group were injected subcutaneously with 0.5 mg of epithalamin (Plant of Medical Preparations, St. Petersburg Meat-processing Plant) dissolved in 0.2 ml of 0.9% NaCl. The second group was given melatonin solution (20 mg/ml; Sigma) in 0.01% ethanol instead of drinking water [6]. One subgroup of the control animals was injected subcutaneously with 0.2 ml of 0.9% NaCl every morning for 5 days; the other subgroup was given 0.01% ethanol solution instead of drinking water [9]. The results obtained for both control groups (the levels of lipid and protein peroxidation) were the same; hence, they were combined for analysis. On day 5, food was removed from the cells at 18 p.m. Between 10 and 11 a.m. on day 6, the rats were euthanized by decapitation. The collected blood was centrifuged at 1500 rpm for 15 min; the serum was stored at –20°C. The antioxidant activity of blood serum was determined by the riboflavin chemiluminescence method [8]. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 9.0), 10 mM riboflavin, 25 mM Fe_2SO_4, and 0.1 ml of blood serum. The reaction was initiated by addition of 0.2 ml 0.1% H_2O_2. The control sample contained no blood serum. Chemiluminescence was measured with an Emilitte EL-1105 chemoluminometer (BioChemMac, Moscow) for 2 min at 37°C. The antioxidant activity was determined as the extent of the chemiluminescence inhibition in the presence of blood serum and expressed in arbitrary units per mg protein. The SOD activity was determined from the inhibition of Nitro Blue Tetrazolium oxidation [9]. The content of ceruloplasmin was determined from oxidation of *p*-phenylenediamine [10]. The nitrite concentration was determined by the Griess method. Before the reaction, proteins were precipitated with 5% zinc sulfate [11]. The results were processed statistically using Student's *t*-test and Wilcoxon–Mann–Whitney's test.

Both melatonin and epithalamin significantly (by 35%, $p < 0.01$) increased the total antioxidant activity (table). A week after initiation of melatonin injection, the SOD activity in the blood serum decreased by 36.5%, whereas epithalamin increased the SOD activity by 19.7% ($0.05 < p < 0.1$). Similar effects were

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Parameters of the antioxidant activity and the content of nitrites in the blood serum of rats injected with melatonin or epithalamin

Parameter	Control ($n = 7$)	Melatonin ($n = 6$)	Epithalamin ($n = 6$)
Antioxidant activity, arbitrary units/mg protein	0.82 ± 0.03	1.12 ± 0.03*	1.12 ± 0.04*
SOD activity, units/mg protein	1.93 ± 0.03	1.32 ± 0.07*	2.31 ± 0.24*, **
Ceruloplasmin, mg %	34.7 ± 3.9	29.0 ± 2.1	36.8 ± 3.0***
Nitrites (NO_2^-), nmol/mg protein	16.4 ± 1.7	20.8 ± 1.2	21.4 ± 2.3

Note: Each sample contained blood sera from 2 to 3 animals. Significant difference with the parameters obtained for control rats: *$p < 0.01$; for rats injected with melatonin: **$p < 0.01$; ***$p < 0.05$.

observed for ceruloplasmin; however, the content of this compound varied within normal limits. The levels of the SOD and ceruloplasmin activities in rats injected with epithalamin were 75.0 ($p < 0.01$) and 26.9% ($p < 0.05$) higher, respectively, than those in rats injected with melatonin. The melatonin-induced decrease in the SOD activity was accompanied by an increase in the content of nitrites generated by oxidation of nitric oxide, which is produced by vessel epithelium.

These results confirmed the data obtained earlier on the antioxidant activities of melatonin [3, 4] and epithalamin [4]. Despite the fact that both compounds displayed a pronounced antioxidant activity, the mechanisms of their action appeared to be different. Thus, epithalamin activated SOD and ceruloplasmin, whereas melatonin decreased their activities. The antioxidant properties of melatonin appeared to be due to its antiradical activity, i.e., its capacity for binding free radicals generated from molecular oxygen or by lipid peroxidation, e.g., the hydroxyl radical ($^{\cdot}OH$) and peroxyl radical ($ROO^{\cdot}$) [3, 12]. We suggested that epithalamin regulates the enzymes of the antioxidant defence system. This suggestion is confirmed by the fact that melatonin exhibited its antioxidant activity both *in vivo* and *in vitro* [3, 4, 12], whereas epithalamin displayed its activity only *in vivo* [4]. Further studies of the difference between the mechanisms of action of melatonin and epithalamin on the content of low-molecular-weight antioxidants (ascorbic acid, α-tocopherol, and uric acid) and thiol-containing compounds, especially glutathione, are of considerable interest.

It is known that many natural and synthetic antioxidants display a broad array of biological activities; in particular, they activate the functioning of the immune system. These antioxidants are efficient gerontoprotectors and can be used for prevention of atherosclerosis and cancer [1, 2]. Both melatonin and epithalamin increase life-span, stimulate immune response, and inhibit tumor development [3, 6, 7]. Moreover, suppression of epiphysis functioning (epiphysis ectomy, twenty-four-hour illumination) decreases life-span, suppresses immune response, and favors the development of atherosclerosis and tumors [3, 6]. Considering that epithalamin stimulates the biosynthesis and secretion of melatonin by epiphysis (the level of melatonin secretion decreases with aging [7]), the effects described may be mediated by the interaction of melatonin with free radicals. However, epithalamin may be more effective than melatonin, because, in addition to the antioxidant activity, it directly stimulates various antioxidant enzymatic systems (in particular, SOD and ceruloplasmin).

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