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PHYSIOLOGY

# Melatonin and Epithalamin Inhibit Free-Radical Oxidation in Rats

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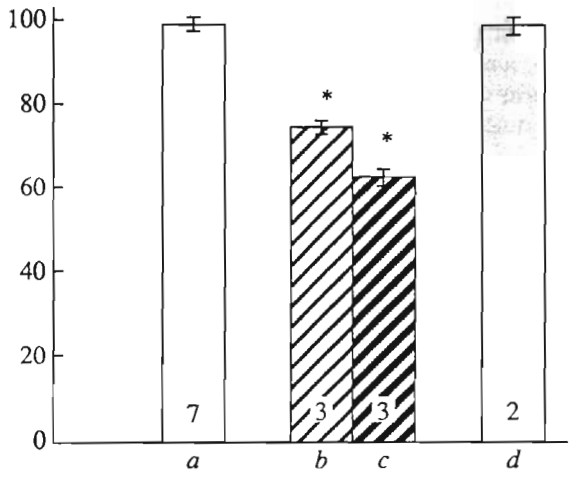
The free-radical theory of aging is one of the most extensively developed fundamental theories in gerontology. This theory suggests that superoxide ($O_2^{\cdot-}$), $H_2O_2$, hydrogen peroxide, hydroxyl radical ($HO^{\cdot}$), and, possibly, singlet oxygen ($^1O_2$) damage cell macromolecules (DNA, proteins, and lipids) and cause mutations and genome instability as a whole. These processes result in aging and in the development of pathologies including cancer, disorders of the cardiovascular system, age immunodepression, brain disorders, cataracts, etc. [1 - 3]. Superoxide dismutase, glutathione peroxidase, catalase, β-carotene, α-tocopherol, and ascorbic and uric acids are the most important endogenous factors that protect macromolecules from the damaging effects of free radicals [1 - 3].

Recently, melatonin, a metabolite of tryptophan, was suggested to be the most potent inhibitor of free-radical processes in an organism [4, 5]. This hormone is produced by epiphysis (the pineal gland). The inhibitory effect of melatonin on the generation of hydroxyl radical was 5 to 14 times stronger than that of other inhibitors known to date, including glutathione and mannitol [6]. Moreover, melatonin inhibits the formation of carcinogen adducts [7]. Melatonin and other natural and synthetic antioxidants prolong the life span of and suppress the development of neoplasms in animals [8, 9]. Additionally, twenty-year studies of epithalamin, a peptide isolated from bovine epiphysis, demonstrated that it effectively prolonged the life span, slowed down the aging of reproductive and immune systems, and suppressed the development of spontaneous neoplasms and tumors induced by various chemical carcinogens or radiation [10, 11]. It is reasonable to assume that the effects of epithalamin are mediated by melatonin, because the injection of epithalamin stimulated the biosynthesis and secretion of melatonin by epiphysis [12].

In this work, we compared the antioxidant activity of melatonin and epithalamin *in vitro* and *in vivo*.

Two- to three-month-old male rats were obtained from the vivarium of the Petrov Research Institute of Oncology. Rats were distributed into three groups. The first group was injected subcutaneously with 0.2 ml of 0.9% NaCl every morning for 5 days; melatonin solution (20 μg/ml; Sigma) in 0.01% ethanol was given instead of drinking water from 6 p.m. to 8 a.m. [9]. The second group was injected subcutaneously with 0.5 mg of epithalamin (Lenmyasokombinat Production Association, St. Petersburg) in 0.2 ml of 0.9% NaCl every morning for 5 days; 0.01% ethanol solution was given instead of drinking water from 6 p.m. to 8 a.m. [9]. The third group was injected subcutaneously with 0.2 ml of 0.9% NaCl every morning for 5 days; 0.01% ethanol solution was given instead of drinking water [9] from 6 p.m. to 8 a.m. On day 5, food was removed from the cages at 6 p.m. On day 6, the rats were sacrificed by decapitation between 10 and 11 a.m. Blood was collected and centrifuged at 1500 rpm for 15 min. Blood serum was stored at −20°C.




Antioxidant activity of melatonin and epithalamin *in vitro*: *y*-axis, chemiluminescence, conventional units; (*a*) control; (*b*) melatonin, 25 μg/ml; (*c*) melatonin, 50 μg/ml; (*d*) epithalamin, 0.5 μg/ml. Figures in bars indicate the number of experiments; asterisk, significant differences, $P < 0.05$.

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The intensity of free-radical oxidation *in vitro* and *in vivo* was studied by $H_2O_2$-induced chemiluminescence of egg lipoproteins [13] and peroxide chemiluminescence of blood serum [14], respectively. Chemiluminescence was measured using an Emilite-EL 1105 luminometer (BioKhimMak, Russia) at 37°C for 2 min and expressed in arbitrary units.

Melatonin (but not epithalamin) produced a pronounced antioxidant effect *in vitro* (see the figure). Daily injections of both melatonin and epithalamin for one week considerably inhibited the intensity of peroxide chemiluminescence in rats (the table). Hence, we demonstrated that melatonin inhibited free-radical processes both *in vivo* and *in vitro*, whereas epithalamin produced an inhibitory effect only *in vivo*.

Our data on *in vitro* effects of melatonin correlated with the reported inhibitory effect of this hormone on the generation of hydroxyl anions *in vitro* [6]. This effect was revealed by measuring the extent of UV-induced photolysis of hydrogen peroxide, which was estimated from the formation of the adduct of 5,5-dimethylpyrroline N-oxide with hydroxyl radical. In *in vitro* experiments, we used an extremely sensitive detection system. The total antioxidant activity was estimated from the extent of peroxide oxidation of yolk lipoproteins [13]. Considering high lipophilicity of melatonin, its penetration through biomembranes, and its accumulation in cell nucleus [5, 6], we can suggest that melatonin not only binds hydroxyl radicals but protects lipids of cell membranes from peroxide oxidation and, probably, eliminates the damaging effects of free radicals on DNA. The latter suggestion is in agreement with the data on the ability of melatonin (taken at physiological and pharmacologic concentrations) to inhibit the formation of DNA adducts with the chemical carcinogen safrole [7]. We demonstrated for the first time that melatonin exhibits antioxidant activity *in vivo*. This correlated well with the fact that epithalamin produced similar effects only *in vivo*. Earlier, we found that a single injection or daily injections for five days of epithalamin stimulated the synthesis of melatonin by epiphysis and its secretion into the blood in rats [11, 12]. It is interesting that syngeneic grafting of epiphyses (taken from young animals) into or instead of thymuses of aging animals increased their life span [9, 15].

These data allowed us to suggest that melatonin stimulates the immune response in animals and inhibits the development of neoplasms, thereby increasing the life span [8, 9]. The effects of melatonin can be largely mediated by the inhibition of free-radical processes in the organism. Our results indicate that various effects of epithalamin occur by the same mechanism, i.e., they are mediated by the high antioxidant activity of endogenous melatonin whose secretion is stimulated by epithalamin injections.

Effects of melatonin and epithalamin on peroxide chemiluminescence of rat blood serum

| Variant | Number of rats | Chemiluminescence, arbitrary units | Inhibition, % | *P* |
|---|---|---|---|---|
| Control | 8 | 7.40 ± 0.99 | | |
| Melatonin | 6 | 4.53 ± 0.61 | 38.8 | <0.05 |
| Epithalamin | 10 | 2.64 ± 0.61 | 70.3 | <0.001 |

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