Doklady Biological Sciences. Vol. 348, 1996, pp. 213–214. Translated from Doklady Akademii Nauk, Vol. 348, No. 2, 1996, pp. 265–267.
Original Russian Text Copyright © 1996 by Anisimov, Arutyunyan, Khavinson.

Melatonin and Epithalamin Inhibit Lipid Peroxidation in Rats

V. N. Anisimov, A. V. Arutyunyan, and V. Kh. Khavinson

Presented by Academician V.L. Sviderskii September 12, 1995

Received September 19, 1995

During the past few years, the concept of the key role of free radicals in aging and age-related pathologies has been intensively developed. Reactive oxygen species, such as superoxide ($O_2^{\dot{-}}$), hydroxyl radical ($HO^{\cdot}$), H_2O_2, and singlet oxygen ($\uparrow O_2$), which are generated during breathing and in various metabolic reactions, damage cell macromolecules (DNA, proteins, and lipids) and either result in aging or cause various pathologies (cancer, atherosclerosis, ischemia, age-related immunosuppression, cataract, etc.) [1, 2].

Melatonin, an indole hormone of epiphysis and a natural antioxidant, has recently drawn considerable attention. The inhibitory effect of melatonin on *in vitro* generation of hydroxyl radical was 5–14 times higher than that of glutathione or mannitol [3, 4]. We found that melatonin efficiently inhibits *in vitro* chemiluminiscence of rat serum peroxides [5]. As well as other natural and synthetic antioxidants, melatonin suppresses tumor growth and increases the life span in animals [1, 6]. Epithalamin, a peptide preparation from bovine epiphysis, which stimulates the synthesis and secretion of melatonin, inhibits free-radical processes [7], increases the life span, slows aging of reproductive and immune systems, and suppresses tumor formation [7, 8].

Here, we describe the results of comparative studies of *in vivo* effects of melatonin and epithalamin on lipid and protein peroxidation.

Eighty 20- to 30-day-old male rats (line LIO, Petrov Institute of Oncology) were used in our experiments. The rats were randomly subdivided into three groups. The rats of the first group were injected subcutaneously with 0.5 mg epithalamin (Plant of Medical Preparations, St. Petersburg Meat-Packing Plant), dissolved in 0.2 ml 0.9% NaCl, every morning for 5 days. The second group was given melatonin solution (20 μg/ml; Sigma) in 0.01% ethanol instead of drinking water [6]. Animals of control groups were (1) injected subcutaneously with 0.2 ml of 0.9% NaCl every morning for 5 days or (2) given 0.01% ethanol solution instead of drinking water [9]. The results obtained for the two control groups were the same; both sets of control data were combined for analysis. At day 5, food was removed from the cells at 6 p.m. At day 6, the rats were sacrificed by decapitation between 10 a.m. and 11 a.m. The collected blood was centrifuged at 1500 rpm for 15 min; the serum was stored at –20°C. The level of lipid peroxidation (LPO) was estimated from the accumulation of its primary and end products (conjugated dienes and Schiff's bases, respectively) in the serum. Conjugated dienes were extracted with an isopropanol–heptane (1 : 1) mixture containing 0.5% butylated hydroxytoluene as an antioxidant, and their content in the heptane phase (diluted with ethanol (1 : 5)) was determined. The concentration of the dienes was determined spectrophotometrically at 232 nm and expressed in nmol/mg protein. The concentration of Schiff's bases was estimated from the fluorescence intensity (excitation, 370 nm, emission, 450 nm) [10] and expressed in arbitrary units/mg protein. The fluorescence intensity of quinine sulfate (30 ng/ml 0.1 N/H_2SO_4) was taken as 100%. The level of protein peroxidation was determined from the content of amino acid carbonyl derivatives in the serum [11]. This method is based on the reaction of 2,4-dinitrophenylhydrazine with proteins (precipitated with trichloroacetic acid and treated with a 1 : 1 ethanol–ethyl acetate mixture). The concentration of the carbonyl derivatives was determined spectrophotometrically (after dissolving the protein precipitate in 8 M urea) at 37°C and expressed in mmol/mg protein, using the molar extinction coefficient of 21 mM^{-1} cm^{-1}. Protein was determined by the method of Lowry [12]. The results were processed statistically using Student's *t*-test and Wilcoxson–Mann–Whitney's test.

Melatonin significantly inhibited LPO (Table 1). It decreased the content of conjugated dienes by a factor of 3.5 and the content of Schiff's bases, by 30.4%. Epithalamin decreased the content of the dienes by a factor of 4.1 and sightly decreased the content of Schiff's bases (by 14.4%, $p > 0.05$). These results are indicative of a specific inhibitory effect of epithalamin on the initial stages of LPO. It cannot be excluded that the observed low content of conjugated dienes was due to the formation of aliphatic hydrocarbons (ethane, butane, etc.), in addition to the formation of Schiff's bases from carbonyl compounds [13].

Neither melatonin nor epithalamin affected the peroxidation of blood serum proteins. However, the

Petrov Research Institute of Oncology, Russian Ministry of Medical Industry, St. Petersburg, Russia

Parameters of lipid and protein peroxidation in blood serum of rats treated with melatonin and epithalamin

Parameter	Control	Melatonin	Epithalamin
Conjugated dienes, nmol/mg protein	0.99 ± 0.12 (12)	0.28 ± 0.36* (9)	0.24 ± 0.04* (10)
Schiff's bases, arbitrary units/mg protein	4.87 ± 0.36 (12)	3.39 ± 0.35** (10)	4.17 ± 0.38 (11)
Amino acid carbonyl derivatives, 10^{-2} mmol/mg protein	8.58 ± 0.34 (12)	9.59 ± 0.29*** (9)	8.02 ± 0.23 (11)

Note: The number of samples is shown in parenthesis. Each sample contained pooled blood sera from 2–3 animals. Significant difference: * $p < 0.01$; ** $p < 0.1$; *** $p < 0.05$.

content of amino acid carbonyl derivatives in proteins slightly increased in rats that were given melatonin with water (by 11.8%, $p < 0.05$). The known inhibitory effect of melatonin on LPO makes it difficult to interpret this finding without data on the influence of melatonin on the generation of reactive oxygen species. It is known, for example, that the highly active hypochlorite anion $HClO^-$, formed by leukocyte myeloperoxidase from hydrogen peroxide (which is generated in free-radical reactions) in the presence of chloride ions, preferentially attacks proteins (not lipids) [14].

Our results correlate well with the reported data on the antioxidant activity of melatonin [3, 4] and epithalamin [5]. It is known that many natural and synthetic antioxidants display a broad spectrum of biological activities. In particular, these substances improve some functions of the immune system, act as efficient geroprotectors, and can be used for preventing atherosclerosis and cancer [1, 2, 13, 15]. Both melatonin and epithalamin increase the life span, stimulate the immune response, and inhibit the development of serially transplantable, carcinogen-induced, and spontaneous tumors [4, 7, 8]. Moreover, suppression of the functions of epiphysis (epiphysectomy, exposure to continuous illumination) decreases the life span, suppresses the immune response, and favors the development of atherosclerosis and tumors [4, 8]. Considering that (1) epithalamin stimulates the biosynthesis and secretion of melatonin by epiphysis and (2) the level of melatonin secretion decreases with aging [4, 7, 8], we infer that the effects described above be underlain by melatonin-induced inhibition of free-radical processes. The fact that both melatonin and epithalamin inhibit LPO provides an additional argument in support of this conclusion.

ACKNOWLEDGMENTS

This work was supported by the Institute of Bioregulation and Gerontology, St. Petersburg (project no. 03-95).

REFERENCES

1. Harman, D., *Proc. Natl. Acad. Sci. USA*, 1981, vol. 78, pp. 7124–7128.
2. Shigenaga, M.K., Hogen, T.M., and Ames, B.N., *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 10771–10778.
3. Poeggeler, B., Reiter, R.J., Tan, D.-X., *et al.*, *J. Pineal Res.*, 1993, vol. 14, pp. 151–168.
4. Reiter, R.J., Poeggeler, B., Chen, L., *et al.*, *Acta Gerontol.*, 1994, vol. 44, pp. 92–114.
5. Anisimov, V.N., Khavinson, V.Kh., and Prokopenko, V.M., Abstracts of Papers, *I Int. Meet. Clinical Neuroimmunomodulation*, Monza, 1995, p. 28.
6. Pierpaoli, W. and Regelson, W., *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 787–791.
7. Anisimov, V.N., Bondarenko, L.A., and Khavinson, V.Kh., *Ann. N. Y. Acad. Sci.*, 1992, vol. 673, pp. 53–57.
8. Anisimov, V.N., Khavinson, V.Kh., and Morozov, V.G., *Ann. N.Y. Acad. Sci.*, 1994, vol. 719, pp. 483–493.
9. Stal'naya, I.A., *Sovremennye metody v biokhimii* (Contemporary Methods in Biochemistry), Moscow, 1977, pp. 63–66.
10. Chio, K.S. and Tappel, A.L., *Biochemistry*, 1969, vol. 8, pp. 2821–2827.
11. Levine, R.L., *Methods Enzymol.*, 1990, vol. 186, pp. 464–485.
12. Lowry, O.H., *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.
13. Armin, M., Peter, G., Wendel, A., and Sies, H., *FEBS Lett.*, 1981, vol. 4/5, pp. 743–755.
14. Lin, H.M., Louie, S., Cross, C.E., *et al.*, *Lab. Clin. Med.*, 1993, vol. 121, pp. 257–262.
15. Voskresenskii, O.N., *Itogi nauki tekh. Obshchie problemy biologii* (Achievements of Science and Technology. General Problems of Biology), Moscow: VINITI, vol. 5, pp. 163–201.