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ANTIOXIDANT PROPERTIES OF GEROPROTECTIVE PEPTIDES OF THE PINEAL GLAND

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SUMMARY

It was shown that peptide preparations from the pineal gland (epithalamin and epitalon) possess antioxidant properties exceeding in some cases the effects of the well-known scavenger of reactive oxygen species (ROS), the melatonin, which is also produced by the pineal gland. The methods used in our experiments in old rats included determination of total antioxidant and antiradical activities, as well as those of antioxidant enzymes (superoxide dismutase = SOD, glutathione peroxidase, glutathione-S-transferase, etc.) in blood serum, liver and brain. It has been revealed that epithalamin (polipeptide preparation from bovine brain) and its active fragment, epitalon (Ala-Glu-Asp-Gly) along with their ability to stimulate melatonin production, have an antioxidant mechanism that is quite different from the action of melatonin. Epithalamin can be more beneficial than melatonin because the former not only produces direct antioxidant effects, but also is able to stimulate the expression of SOD, ceruloplasmin and other antioxidant enzymes. The possibility of oxidation chains by their interaction with different ROS by means of binding of transition metals (Fe^{2+}) cannot also be excluded. Thus, the results of our experiments testify that the pineal gland peptides enhance the antioxidant defense system, which can contribute to their geroprotective properties.

Keywords: pineal gland peptides, epithalamin, epitalon, reactive oxygen species (ROS)

INTRODUCTION

One of the most fruitful theoretical advances in basic gerontology is the free-radical theory of aging. According to this theory, the so-called ROS, including superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$) free radicals, hydrogen peroxide (H_2O_2) and, possibly, singlet oxygen ($^1O_2$) generated as byproducts of cellular respiration and other metabolic processes, damage cellular macromolecules (DNA, proteins, and lipids) culminating in mutations and genome instability, leading to the development of age-related pathological phenomena, including cancer, circulatory diseases, immunodepression, brain dysfunction, cataract, and others (Cutler, 1991; Harman, 1994; Shigenaga et al., 1994; Yu and Chung, 2006). It is still not clear how free radical reactions cause aging. They are supposed to damage cell membranes, intercellular matrix, chromatin and DNA, as well as to impair calcium

homeostasis (Harman, 1994; Papa and Skulachev, 1997). Aging is associated with the retardation of processes that result in free-radical generation. However, antioxidant defenses become less reliable with aging, which can facilitate the damage made by free radicals to tissues (Shigenaga et al., 1994; Papa and Skulachev, 1997).

Many natural and synthetic antioxidants possess a wide range of biological activities including the improvement of some immune functions, geroprotective effects, and preventing atherosclerosis and cancer (Harman, 1994; Shigenaga et al., 1994). An important role among them belongs to biologically active compounds of pineal gland, melatonin, epithalamin and epitalon. Epithalamin is a low molecular weight preparation obtained from the pineal gland and studied in the St. Petersburg Institute of Bioregulation and Gerontology.

Melatonin (Reiter, 1997) and epithalamin (Anisimov et al., 1994) showed an increase of animal lifespan, stimulation of immune responses, and inhibition of tumor development. Effects of epithalamin are mediated, at least partly, by its ability to stimulate synthesis and secretion of melatonin by the pineal gland and by its modulating effects on immune and metabolic functions, which suggests an important homeostatic role of epiphysis peptides (Anisimov et al., 1994; Anisimov, 1998). Recently we found geroprotective and antioxidant effects of the tetrapeptide epitalon (Ala-Glu-Asp-Gly) synthesized on the basis of an analysis of amino acids of epithalamin and probably being its active fragment.

MATERIALS AND METHODS

The effects of epithalamin and melatonin on free radical oxidation intensity and antioxidant activity in rat plasma as well as the effect of melatonin and epitalon on free radical processes in brain, liver and blood serum of CBA mice, were studied. Epithalamin was injected subcutaneously to rats in the morning for 5 days in the dose of 0.5 mg per animal. Melatonin was given to other animals for the same period of time with drinking water (20 mg/l). Epitalon was administered to mice for 5 consecutive days subcutaneously in the dose of 0.1 μg of the peptide in 0.1 ml of NaCl isotonic solution per animal.

RESULTS AND DISCUSSION

Data showed that epithalamin significantly suppressed blood serum chemoluminescence (3.4-fold) and lipid peroxidation, manifesting, in particular, as a pronounced reduction of diene conjugates (4.1-fold), whereas Schiff bases revealed only a trend to decrease (by 14.4%, $p > 0.05$). These observations suggest that treatment with epithalamin

affects the initial stages of lipid peroxidation (LPO). Melatonin also suppressed LPO as suggested by the decrease of either diene conjugates or Schiff bases. Treatment with melatonin resulted in an increase of carbonylated protein derivatives (by 12%, $p < 0.05$), whereas epithalamin did not influence protein oxidation. Treatment with either epithalamin or melatonin was associated with a significant increase (35%, $p < 0.01$) of the total antioxidant activity (TAA). One week after the onset of treatment with epithalamin, SOD activity in blood serum increased (19.7%; $0.05 < p < 0.1$), whereas treatment with melatonin suppressed the activity by 36.5%. The same trends were displayed by serum ceruloplasmin, albeit within the normal range. However, in rats treated with epithalamin, the levels of SOD and ceruloplasmin in blood serum were significantly higher (75%, $p < 0.01$, and 27%, $p < 0.05$, respectively) than in rats treated with melatonin. Treatment with melatonin was also associated with a trend to a decrease in the content of nitrites generated by oxidation of nitric oxide produced by blood vessel endothelium.

The above results confirm the antioxidant effect of melatonin. It should be stressed that, though either products of the pineal gland exhibit pronounced antioxidant effects, their mechanisms seem to be different. Epithalamin increases SOD and ceruloplasmin, whereas melatonin does the opposite. Probably, the antioxidant effects of melatonin, at difference from those of epithalamin, are associated with the antioxidant activity of the former, i.e., its ability to directly react with free radicals generated in the organism from molecular oxygen and lipids, in particular hydroxyl ($OH^{\bullet}$) and peroxyl ($OOR^{\bullet}$) radicals (Reiter, 1997). According to some studies, melatonin is not a direct peroxyl radical trapping antioxidant but can inhibit metal ion-catalyzed oxidation processes, forming coordinated bonds between metal ions and nitrogen atoms of the indole ring (Antunes et al., 1999). By contrast, antioxidant effects of epithalamin seem to be mediated by enzymatic antioxidant defenses. This suggestion is in agreement with data about comparable antioxidant effects of melatonin in vivo and in vitro (Anisimov et al., 1999), whereas epithalamin appears to be a much more potent antioxidant in vivo than in vitro. Further studies involving the identification of low molecular antioxidants (ascorbic acid and α-tocopherol) as well as thiol-containing compounds (glutathione, in the first place) are needed to elucidate differences between the mechanisms of in vivo antioxidant effects produced by melatonin and epithalamin.

With regard to the fact that epithalamin stimulates the synthesis and secretion of melatonin whose level decreases with aging (Reiter, 1997; Anisimov, 1998), the above effects of epithalamin are likely to be mediated to some extend by the antioxidant potential of melatonin. However, the use of epithalamin may appear to be more beneficial compared

to that of melatonin, because the former produces not only direct antioxidant effects but, also, stimulates enzymatic antioxidant systems exemplified by SOD and ceruloplasmin.

Results of the study of the effects of epitalon and melatonin on free radical processes in CBA mice showed that ROS are most actively formed in blood serum in which they are produced more than twice as intensely compared to the same values for liver and brain. Meanwhile, TAA was much lower in blood serum compared to liver and brain. Epitalon effectively suppressed ROS formation in brain tissue and peroxidation of lipids in liver and brain, its effect being directed mostly to the last step of the latter process in the brain (Schiff bases) and to the first step in the liver (diene conjugates). Administration of melatonin and epitalon resulted in a significant increase in TAA in blood serum in mice and suppressed both steps of lipid peroxidation in liver and brain. The profound antioxidant effect of epitalon is conferred to the one of melatonin. We obtained similar results showing the suppression of peroxidation processes in experiments with old rats, which showed, that alongside with the inhibition of lipid peroxidation, epitalon also suppresses the peroxidation of proteins in the brain and blood serum. The presented data justify the use of pineal gland geroprotective peptides as antioxidants and means of preventing the development of age-related pathologies.

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