BIOGERONTOLOGY

Effect of Peptide Preparation Epithalamin on Circadian Rhythm of Epiphyseal Melatonin-Producing Function in Elderly People

O. V. Korkushko, V. Kh. Khavinson*, V. B. Shatilo, and L. V. Magdich

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Circadian rhythm of plasma melatonin concentrations in healthy elderly subjects was studied before and after a course treatment with Epithalamin (peptide preparation from the pineal gland). Epithalamin modulated the melatonin-producing function of the pineal gland. During the dark period plasma melatonin concentration increased in subjects with initially lowered activity of the pineal gland, while in subjects with normal epiphyseal function plasma melatonin concentration tended to decrease.

Key Words: *elderly age; pineal gland; melatonin; Epithalamin*

Recent studies demonstrated an important role of the pineal gland in the regulation of various functions (sleeping, wakefulness, circadian and seasonal biological rhythms, body temperature, activities of the pituitary and peripheral endocrine glands, arterial pressure, immunity, antioxidant status, *etc.*) [1,11]. The melatonin-producing function (MPF) of the pineal gland in humans and animals decreases with age. According to published data, plasma level of melatonin in elderly people is 10-50% lower than in young people [7,9-11,13]. Urinary excretion of 6-sulfatoxymelatonin (6-SM) also decreases with age [6,12].

Decreased production of melatonin by the pineal gland causes disorders in biological rhythms of the organism, promote the development of pathological processes, and probably can lead to preterm aging [1,8,9]. On the other hand, experiments with pineal gland transplantation from young to old animals, long-term treatment with melatonin or preparation of the pineal gland (Epithalamin) demonstrated geroprotective effects and prolongation of the life span due to these treatments [2,3,8]. Therefore, the search for agents restoring MPF of the pineal gland in elderly subjects is an important and perspective problem.

Experimental studies showed that single injection or course therapy with Epithalamin increased nocturnal production of melatonin in old animals [3]. However, these studies were never carried out in humans, which became the object of our present work.

MATERIALS AND METHODS

Elderly people (nonsmokers, without alcohol abuse) aged 60-74 years were selected on the basis of the results of clinical, laboratory, and instrumental examinations. All of them gave written consent to participation in the study. The examinees had no pathologies of the CNS, respiratory or endocrine system, malignant tumors, and clinically significant cardiovascular diseases. Selection, examinations, and treatment were carried out in Institute of Gerontology of Academy of Medical Sciences of Ukraine. The examinees received standard diets and had free regimen without

Institute of Gerontology, Academy of Medical Sciences of the Ukraine, Kiev; *St. Petersburg Institute of Bioregulation and Gerontology, North-West Division of Russian Academy of Medical Sciences. ***Address for correspondence:*** admin@geront.kiev.ua. Shatilo V. B.

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changes in normal level of daily physical activity, with 8-h night sleep (22.30-6.30).

Initial circadian rhythms of MPF were studied in 31 elderly subjects, after which the examinees were randomly divided into 2 age- and sex-matched groups similar by their initial functional status.

Subjects of the main group (n=12) received Epithalamin (peptide preparation from the pineal gland) dissolved in 2 ml normal saline: 5 intramuscular injections every 3 days at 10.00. Controls (n=10) were injected with normal saline according to the same protocol.

None of the examinees received β-adrenoblockers, soporifics, tranquilizers, nonsteroid antiinflammatory drugs, caffeine, and alcohol during examination and treatment.

Circadian rhythms of melatonin concentrations in the blood plasma were measured in winter-spring (December-April) before Epithalamin or placebo treatment and on the next day after completion of the treatment protocol.

Venous blood (5 ml) was collected through a minicatheter inserted into surface forearm vein at 9.00, 15.00, 21.00, and 3.00. At 3.00 blood was collected at poor illumination (red lantern). Blood was centrifuged for 15 min at 3000 rpm, plasma was transferred into tubes, frozen, and stored at -20°C for up to 3 months. Plasma melatonin concentration was measured by radioimmunoassay using standard kits (DPC) in accordance with the manufacturer's instruction.

RESULTS

Circadian rhythm of melatonin concentration in the plasma of elderly subjects had a minimum at 15.00 (4.9±0.7 pg/ml) and maximum at 3.00 (46.8±13.5 pg/ml). The mean amplitude of the rhythm was 41.9±13.2 pg/ml, the ratio between hormone concentrations at night and during the day was 9.55±2,31. Plasma melatonin level at 9.00 was significantly higher than at 15.00, but appreciably lower than at 21.00.

Analysis of individual data showed 2 types of circadian rhythms of plasma melatonin concentrations:

1) low-amplitude rhythm (in 22 of 31 examinees, 71%) was characterized by the absence of appreciable nocturnal rises of plasma hormone concentration (<40 pg/ml) and night/day ratio of melatonin concentrations about 2-7;
2) high-amplitude rhythm (in 9 of 31 examinees, 29%) was characterized by a significant increase in melatonin concentration at night (more than 40 pg/ml) and the night/day ratio of the hormone concentration >7.

Young healthy people usually have high nocturnal peak of plasma melatonin concentration and high level of nocturnal excretion of 6-SM. In elderly and senile people plasma hormone concentration not much increased during the dark hours and, as a result, the excretion of 6-SM is lower [4,6,7,10-13].

Our data indicate that in less than one-third of healthy elderly subjects MPF of the pineal gland is preserved at a high level (comparable to that in young people). In the majority of elderly people functional activity of the pineal gland markedly decreased and needs correction.

The effect of course Epithalamin treatment on MPF clearly depended on the initial concentration of melatonin in the plasma before treatment. Therefore, the effect of preparation was evaluated separately in

TABLE 1. Plasma Melatonin Concentrations (pg/ml) at Different Hours during Treatment with Epithalamin and Normal Saline (M±m)

Time of the day, h	Period of measurement	Subgroup with retained MPF (n=4)	Subgroup with lowered MPF (n=8)	Placebo
9:00	Before treatment	15.5±6.1	4.9±1.1	10.9±3.7
	After treatment	20.6±12.3	12.1±5.8	11.3±4.0
	Changes	+5.2±7.6	+7.1±6.3	+0.4±1.2
15:00	Before treatment	6.7±1.8	4.1±1.1	3.9±0.8
	After treatment	6.8±1.2	4.9±1.0	4.3±1.1
	Changes	+0.1±0.8	+0.8±1.2	+0.4±0.8
21:00	Before treatment	66.1±20.5+	15.3±3.3	14.8±4.1
	After treatment	30.3±11.5	16.3±4.8	12.3±3.6
	Changes	-35.8±19.0	+1.0±2.6	-2.5±4.3
3:00	Before treatment	149.6±42.2+	24.2±5.1	32.4±6.8
	After treatment	75.0±37.6	59.0±12.6*	28.1±5.9
	Changes	-74.7±40.2+	+34.8±13.2	-4.3±6.3

Note. p<0.05 compared to *the level before treatment, +subgroup with impaired MPF.

subgroups of subjects with preserved (n=4) and reduced (n=8) MPF.

In subjects with preserved MPF of the pineal gland the amplitude of circadian rhythm before treatment was 142.9±35.4 pg/ml and the ratio between the night and day concentrations of the hormone was 22.3. Epithalamin treatment decreased plasma melatonin concentrations at 21.00 and 3.00 in all subjects of this group (Table 1).

In elderly people with reduced MPF the amplitude of circadian rhythm was 20.1±4.6 pg/ml and the ratio of night/day hormone concentrations was 5.9. Epithalamin treatment increased nocturnal melatonin concentration in this subgroup more than 2-fold (Table 1).

Placebo treatment led to negligible changes of melatonin concentrations in the plasma at different time of the day (Table 1).

Previous experimental studies on rats showed that single injection of Epithalamin and a course of injections at 10.00 increased melatonin concentration in the pineal gland and plasma during the dark hours [3].

In our study Epithalamin or normal saline were also injected to elderly subjects at 10.00. Functional activity of the pineal gland changed differently in volunteers treated with Epithalamin. Plasma melatonin concentration increased during the dark period in subjects with initially low functional activity of the pineal gland. In subjects with retained MPF plasma hormone concentration tended to decrease, which confirmed the modulatory effect of Epithalamin on MPF in elderly people. On the other hand, melatonin concentration in controls did not change under the effect of normal saline. On the basis of these results we recommend Epithalamin for the correction of age-associated disorders in circadian rhythm of MPF of the pineal gland in elderly subjects.

REFERENCES

1. V. N. Anisimov, *Ros. Fiziol. Zh.*, **83**, No. 8, 1-10 (1998).
2. V. N. Anisimov, *Uspekhi Gerontol.*, No. 4, 55-74 (2000).
3. V. N. Anisimov, L. A. Bondarenko, and V. Kh. Khavinson, *Ann. NY Acad. Sci.*, **673**, 53-57 (1992).
4. J. D. Bergiannaki, C. R. Soldatos, T. J. Paparrigopoulos, *et al.*, *J. Pineal Gland*, **18**, No. 3, 159-164 (1995).
5. P. Cugini, Y. Touitou, A. Bogdan, *et al.*, *Chronobiol. Int.*, **18**, No. 1, 99-107 (2001).
6. J. C. Hendrick, M. Crasson, M. T. Hagelstein, *et al.*, *Ann. Endocrinol.* (Paris), **63**, No. 1, 3-7 (2002).
7. Y. Ohashi, N. Okamoto, K. Uchida, *et al.*, *Biol. Signals*, **6**, Nos. 4-6, 301-306 (1997).
8. W. Pierpaoli and W. Regelson, *Proc. Natl. Acad. Sci. USA*, **91**, No. 2, 787-791 (1994).
9. Y. Touitou, A. Bogdan, A. Auzeby, and B. Selmaoui, *Therapie*, **53**, No. 5, 473-478 (1998).
10. Y. Touitou, *Exp. Gerontol.*, **36**, No. 7, 1083-1100 (2001).
11. F. Waldhauser, J. Kovacs, and E. Reiter, *Ibid.*, **33**, Nos. 7-8, 759-772 (1998).
12. L. Wetterberg, J. D. Bergiannaki, T. J. Paparrigopoulos, *et al.*, *Psychoneuroendocrinology*, **24**, No. 2, 209-226 (1999).
13. Z. Y. Zhao, Y. Xie, Y. R. Fu, *et al.*, *Chronobiol. Int.*, **19**, No. 6, 1171-1182 (2002).