Effect of Epithalamin on Circadian Relationship between the Endocrine Function of the Thymus and Melatonin-Producing Function of the Pineal Gland in Elderly People

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The effect of epithalamin on circadian rhythms of thymic serum factor titers and melatonin concentrations in the blood of elderly people was studied. Course treatment with epithalamin modulated the rhythm of the thymic endocrine function. The increase in the titer of thymic serum factor at night coincided with the increase in blood melatonin concentration and shift of its acrophase to 3.00, which was characteristic of young people. In elderly people with preserved nocturnal peak of the thymic serum factor titer before therapy the nocturnal (3.00) concentration of melatonin was high and did not change after drug therapy. The correlation between melatonin concentration and titer of thymic serum factor increased after epithalamin treatment.

Key Words: *biorhythms; age; epithalamin; melatonin; thymic serum factor*

Impairment of the endocrine function of the thymus is an important factor of age-associated changes in the immune system [2]. We found that the circadian rhythm of the thymic function is also disordered during aging [4,11]. Biorhythms ensure adaptation of the immune system to fluctuations of environmental conditions [8], and the search for approaches to the correction of disorders in the rhythm of the function of its central organ (the thymus) is a very important problem.

The pineal gland is the main synchronizer of many functions of the body and is linked with the thymic-lymphatic system [6,13,14]. The pineal gland produces bioactive substances of not only indole (melatonin), but also of peptide nature [9]. A pineal peptide epithalamin produced a positive effect on age-associated changes in the functions of the thymus and immune system under clinical conditions [3,9]. Moreover, the drug improved their rhythm and stimulated the melatonin-producing function of the pineal gland in old animals [1,5].

Here we studied the possibility of restoring the circadian rhythm of the thymic endocrine function with epithalamin through modulation of the melatonin-producing function of the pineal gland in elderly subjects.

MATERIALS AND METHODS

Circadian fluctuations of the studied parameters were evaluated in 14 normal elderly men and women aged 60-79 years hospitalized at Institute of Gerontology, Academy of Medical Sciences of Ukraine. All examinees gave informed consent to participation in the study. The blood was collected from the ulnar vein at 6-h intervals: at 9.00, 15.00, 21.00, and 3.00 (at red

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dim light during the dark hours). The study was carried out in September-April. The plasma was stored at -20°C for 2 months.

Epithalamin was prescribed in accordance with our protocol designed with consideration for age-specific sensitivity of the immune system to its effect [3]. The course consisted of 5 intramuscular injections (every 3 days), single dose 10 mg, total dose 50 mg. Examinations were carried out before and 1-2 days after the end of the course.

The endocrine function of the thymus was evaluated by the titer of thymic serum factor (TSF), one of the true hormones of the gland [10]. The results were expressed in TSF titer log.

Melatonin concentration in the plasma was determined by radioimmunoassay using DPC kits.

The results were statistically processed using Student's *t* test. The relationships between the parameters were measured using coefficient of correlations (η): 0.3-0.5 for moderate correlation, 0.5-0.7 considerable, and 0.7-0.9 strong correlation [7].

RESULTS

The titers of TSF in young people (20-29 years) at 9.00, 15.00, 21.00, and 3.00 were 4.3±0.3, 3.6±0.9, 6.9±0.7, and 7.3±0.9, respectively [5]. The titers at 21.00 and 3.00 were higher than at 9.00 and 15.00 ($p<0.05$). Rhythmic organization of the endocrine function of the thymus was disordered in the majority of examined elderly people. TSF titer did not increase at night in 6 of 12 examinees, and in some cases was even lower than in the morning (monotonous or inverted rhythm). In 6 other elderly subjects the nocturnal peak of TSF titer was preserved, but was lower than in young people ($p<0.05$). Since the modulating effect on the immune system is characteristic of epithalamin [3,9], we evaluated the efficiency of its modulation of the TSF titer rhythm with consideration for the initial rhythm (Table 1). The titers at 21.00 and 3.00 appreciably increased after epithalamin treatment in elderly people, who had no nocturnal peaks of TSF titer before therapy. In subjects whit nocturnal rises of TSF titer before treatment, the treatment appreciably increased the morning and night titers, the difference between the values was retained.

In 2 elderly subjects TSF titers at 21.00 were 4-fold higher than at 9.00. Only the morning titers increased after epithalamin course in these subjects. Hence, epithalamin modulated the rhythm of the thymic endocrine function in elderly subjects (induced the appearance of the nocturnal amplitude of TSF titer, if it was absent before treatment and preserved the existing nocturnal peak of the parameter).

At the next stage of the study we evaluated the effect of epithalamin on plasma melatonin concentration in elderly people with different initial circadian rhythms of the endocrine function of the thymus (with and without nocturnal peaks of TSF titer). Previously we showed that in young people melatonin concentrations at 9.00, 15.00, 21.00, and 3.00 were 112.2±32.7, 169.4±33.1, 273.9±55.5, and 553.4±162.9 pmol/liter. The concentrations at 21.00 and 3.00 were higher than at 9.00 and at 15.00 lower than at 3.00 ($p<0.05$).

In elderly people without nocturnal TSF peaks before treatment the concentrations of melatonin in the plasma at 9.00, 15.00, 21.00, and 3.00 were 44.3±23.2, 20.2±0.9, 133.3±52.4, and 71.8±17.6 nmol/liter, respectively. The concentrations at 21.00 and 3.00 were higher than at 15.00 ($p<0.05$). The concentration at 3.00 was lower than in young people ($p<0.05$). After treatment the plasma melatonin concentrations in this subgroup of elderly subjects at 9.00, 15.00, 21.00, and 3.00 were 70.1±42.6, 23.2±3.0, 69.7±22.8, and 251.1±105.2 pmol/liter, respectively. The level at 3.00 was higher than before treatment and higher than at 15.00. It should be noted that after epithalamin treatment the acrophase of melatonin concentration was recorded at 3.00, like in young people.

In elderly subjects with nocturnal peaks of TSF titers before therapy (n=5), the initial melatonin concentrations at 9.00, 21.00, and 3.00 were 32.3±5.6,

TABLE 1. Effect of Epithalamin on Circadian Rhythm of TSF Titer ($\log_2$) in Elderly People with Different Initial Rhythms of This Parameter (n=6; $M±m$)

Time of the day, h	Elderly people with monotonous or inverted rhythm of TSF titer		Elderly people with nocturnal increase of TSF titer	
	before treatment	after treatment	before treatment	after treatment
9:00	5.8±0.7	6.5±0.5	2.3±0.5	5.0±1.0*
15:00	4.0±0.5	4.3±0.9		
21:00	3.0±0.7⁺	7.7±0.9*°	3.7±0.5⁺	6.3±0.9*⁺
3:00	3.8±0.5⁺	5.8±0.7*	3.4±0.2⁺	6.0±0.9*

Note. $p<0.05$ compared to *titer before treatment (by the sign test), ⁺titer at 9.00 (by the sign test), °titer at 15.00.

150.1±99.8, and 234.1±100.8 pmol/liter, respectively; after treatment 32.7±9.0, 110.5±52.0, and 231.0±83.6 pmol/liter, respectively. The difference between the parameters at 9.00 and 3.00 before and after treatment was significant ($p<0.05$).

In elderly people coefficient of correlations η between melatonin concentrations and TSF titer before and after epithalamin treatment was 0.48±0.10 and 0.58±0.15 ($p<0.05$). Before treatment this coefficient was lower than in young people (0.86±0.06; $p<0.05$).

Thus, changes in the function of the pineal gland in elderly people manifested in decreased blood melatonin concentration at night and a shift of its acrophase. Treatment with epithalamin activated the melatonin-producing function of the pineal gland and improved its rhythm. Our clinical data on increased blood melatonin concentration under the effect of peptide preparation from the pineal gland are in line with experimental data [1,6]. Epithalamin modulated not only the nocturnal amplitude of melatonin concentration, but also its acrophase. These data suggest that peptides of the pineal gland can be used for chronotherapy of conditions associated with disturbed rhythms of melatonin-producing function. A possible mechanism of epithalamin effect on the rhythm of epiphyseal function in elderly individuals is recovery of the neuromediator balance in cerebral structures and restoration of gland sensitivity to the peripheral regulatory signals, specifically to glucocorticoids [6, 14]. Preliminary results of our morphological studies indicate that age-associated changes in the hypothalamic suprachiasmatic nucleus containing melatonin receptors [13] were decelerated in aging mice after long-term epithalamin treatment. It was previously shown that circadian rhythms of adrenocortical function also improved in these animals [6].

Since melatonin coordinates the fluctuations of thymic hormone concentrations and illumination [12], and the rhythms of the pineal gland and thymus functioning are interrelated not only before epithalamin treatment, but also after it, the observed changes in the rhythm of melatonin concentration under the effect of epiphyseal peptides is a component of their normalizing effect on the circadian rhythm of the endocrine function of the thymus in elderly subjects. The increase in TSF titer at night was observed only in subjects with improved phase relationships between melatonin and hydrocortisone concentrations [5]. Epithalamin can be used for restoring the disturbed rhythm of thymus function.

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