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PRIMATOLOGY

Regulatory Effect of Epithalon on Production of Melatonin and Cortisol in Old Monkeys

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The effect of Epithalon on melatonin and cortisol secretion in female rhesus monkeys of various ages was evaluated by enzyme immunoassay. Epithalon stimulated evening melatonin production and normalized circadian rhythms of cortisol production in old monkeys.

Key Words: *melatonin; cortisol; circadian rhythms; Epithalon; monkeys*

Previous studies showed that blood melatonin concentration in humans and animals markedly decreases during aging [11-13]. Melatonin plays a key role in the regulation of biological rhythms and modulates functions of the endocrine, nervous, and immune systems [12,13]. Age-related neurodegenerative disorders and other diseases are associated with decreased melatonin production [12,14]. Melatonin possesses geroprotective activity [2,5,9,12,13], but in some cases this hormone causes side effects (*e.g.*, neoplasms) [6]. The search for new stimulators of endogenous melatonin secretion is of considerable importance. Physiologically active preparations of the pineal gland, Epithalamin and Epithalon, hold much promise in this respect [1,6].

The pharmacopoeial preparation Epithalamin contains peptides isolated from the pineal gland. The tetrapeptide Epithalon (Ala-Glu-Asp-Gly) was synthesized on the basis of amino acid composition of Epithalamin at the Laboratory of Peptide Chemistry (St. Petersburg Institute of Bioregulation and Gerontology). Our previous studies showed pronounced regulatory effects of these preparations on various organs and systems [1,6].

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Here we studied the effect of Epithalon on melatonin and cortisol secretion in monkeys at various age periods.

MATERIALS AND METHODS

Experiments were performed on 6 young adult (6-8 years, average age 7.0±0.3 years) and 6 old (20-26 years, average age 22.8±1 years) female rhesus monkeys (*Macaca mulatta)* obtained from the Adler Primatology Center. The body weights of young and old monkeys were 5.1±0.9 and 4.8±0.2 kg, respectively. Young animals had normal menstrual cycles, while in old monkeys disturbances in the reproductive system (short-term amenorrhea or absence of menstruation) were noted.

The monkeys were kept in individual metabolic cages under natural light-dark cycles (8.00-19.00: daytime) and *ad libitum* food and water supply. The experiment was performed in summer (June-July).

After 3-week adaptation to experimental conditions and blood sampling, the animals were intramuscularly injected with Epithalon (10 μg in 1 ml physiological saline) or placebo (1 ml physiological saline). There were 2 control (placebo) and 2 experimental groups (Epithalon) of young and old monkeys (3 animals per group). Epithalon and physiological saline were injected at 9.00 for 10 days. The blood from the cubital or femoral vein was taken 2 times (9.00 and

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21.00) on day 10 after the start of injections. Heparin was used as an anticoagulant. The blood was centrifuged at 2000*g* for 15 min, and the plasma was collected and stored at -50°C.

Plasma contents of melatonin (Immuno Biological Laboratories) and cortisol (Alkor Bio) were estimated using enzyme immunoassay kits. The sensitivity of this method was 10 nmol/liter and above 3 pg/ml for cortisol and melatonin, respectively. The coefficients of correlation between melatonin and cortisol concentrations in industrial sera and control sera from rhesus monkeys did not exceed 12%.

The results were analyzed by Student's *t* test.

RESULTS

The content of melatonin in control old monkeys was 2 times lower than in young animals ($p<0.01$), especially in the evening time (Fig., *a*), which is consistent with published data on progressive decrease in blood melatonin concentration in humans and animals during aging [11-13].

Melatonin concentration in control young and old monkeys at 21.00 was higher than at 9.00 (young females: 30±9 and 17±7 pg/ml, respectively; old animals: 15±3 and 10±2 pg/ml, respectively), which is consistent with previous reports on activation of melatonin secretion in the evening time [11-13].

Epithalon increased melatonin concentration in old monkeys in evening (Fig. 1, *a*). Melatonin content in 20-26-year-old rhesus monkeys treated with Epithalon was more than 3 times higher than in control animals ($p<0.001$). However, Epithalon had no effect on blood melatonin concentration in young females.

There were considerable age-related differences between basal melatonin production and Epithalon-induced changes in melatonin synthesis. The selective stimulatory effect of Epithalon on melatonin production in old monkeys was probably related to modulation of an age-related decrease in the sensitivity of melatonin-synthesizing systems to environmental light-dark cycles. This is confirmed by lower basal melatonin content in old monkeys in the evening time. Moreover, stimulatory effect of Epithalon depended on the time of experiments: blood melatonin concentrations at 21.00 and 9.00 were 48±5 and 10±2 pg/ml, respectively ($p<0.001$). Our assumptions are confirmed by published data on the inhibitory effect of daylight on melatonin synthesis and secretion [7,8,12]. Moreover, previous studies demonstrated the absence of age-related changes in pinealocyte structure [10] and activity of key enzymes of melatonin synthesis in humans [15].

Adrenergic innervation of the pineal gland plays a key role in the regulation of melatonin secretion [12,13]. Taking into account decreased sensitivity of pineal β-adrenoceptors in aging rodents [4], it can be assumed that Epithalon increases the number and/or affinity of β-adrenoceptors on pinealocyte membranes for norepinephrine.

Epithalon not only stimulated melatonin synthesis in old monkeys, but also normalized circadian rhythms of peripheral blood cortisol concentration (Fig. 1, *b*). Cortisol content in old animals did not drop in evening (21.00), which indicated a decrease in the amplitude of its circadian rhythms (similarly to humans [12]).

Close relationships between the pineal gland and adrenals [7,12] suggest that Epithalon recovers circadian rhythms of cortisol in old animals by normaliz-

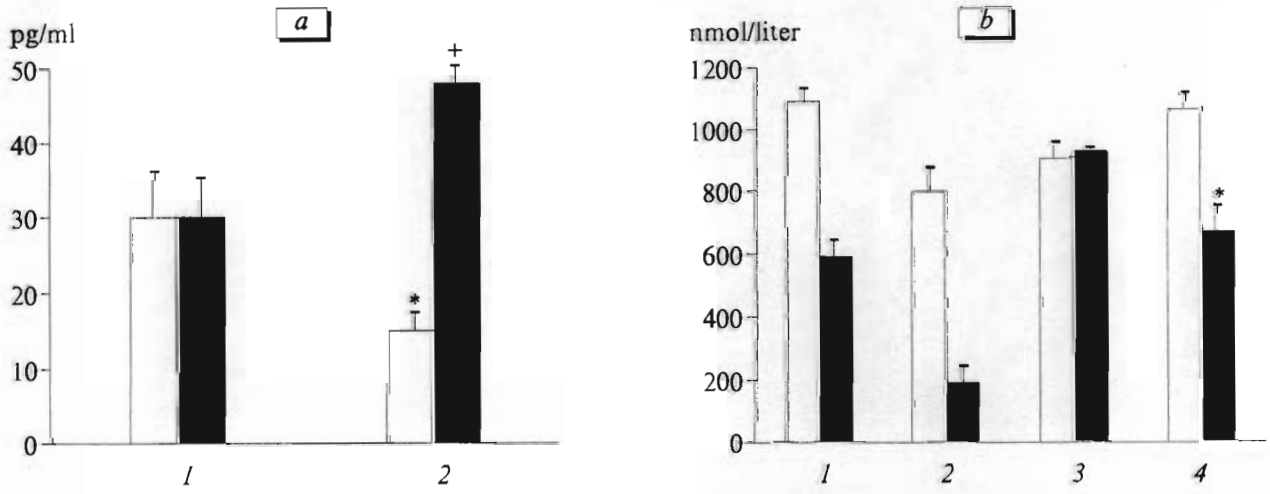

Fig. 1. Effects of Epithalon on production of melatonin (21.00, *a*) and cortisol (9.00 and 21.00, *b*) in monkeys of various ages. *a*) Light bars: control (placebo); dark bars: Epithalon. Age: 6-8 (*1*) and 20-26 years (*2*). *$p<0.01$ compared to control young animals; +$p<0.001$ compared to control old animals. *b*) Light bars: 9.00; dark bars: 21.00. Age: 6-8 (*1*, *2*) and 20-26 years (*3*, *4*). Control (*1*, *3*) and Epithalon (*2*, *4*). *$p<0.05$ compared to control old animals at 21.00.

ing melatonin secretion. This is confirmed by a negative correlation between the diurnal dynamics of cortisol and melatonin concentrations in the peripheral blood of young monkeys. Moreover, Epithalon normalized circadian rhythms of melatonin and cortisol.

Normalization of melatonin and cortisol production is of considerable importance, because circadian rhythms of their secretion determine rhythmic activity of various organs, including the nervous, endocrine, cardiovascular, and immune systems [5,12,13].

Thus, Epithalon increases melatonin concentration in the peripheral blood and normalizes circadian rhythms of cortisol production in old female rhesus monkeys. Our findings indicate that Epithalon holds much promise for the correction of age-related hormonal disorders and normalization of functions of various organs and systems.

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