Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Synthetic tetrapeptide epitalon restores disturbed neuroendocrine regulation in senescent monkeys

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Submitted: *June 22, 2001*
Accepted: *June 28, 2001*

Key words: **Epitalon; melatonin; cortisol; circadian rhythms; monkeys**

*Neuroendocrinology Letters 2001; **22**:251–254 pii: NEL220401A03 Copyright © Neuroendocrinology Letters 2001*

Abstract

The **OBJECTIVE** of this research was to investigate the regulatory effect of Epitalon on the production of melatonin and cortisol in senescent monkeys.

MATERIAL AND METHODS: We investigated the character of melatonin and cortisol secretion by immunoferment assay in Epitalon-administered female *Macaca mulatta* in different age periods.

RESULTS: Epitalon was proven to significantly stimulate melatonin synthesis in senescent monkeys in the evening, thereby normalising the circadian rhythm of cortisol secretion.

Introduction

Results of a series of investigations demonstrate a pronounced decrease in the content of melatonin in the blood of aging humans and animals [11–13]. This hormone has been proven to play the key role in biologic rhythm control and exert diverse effects upon the functioning of the endocrine, nervous and immune systems [12, 13]. Reduced melatonin production is considered to entail age-related neurodegenerative changes and certain diseases [12, 14]. Introduction of melatonin produces a geroprotective influence [2, 5, 9, 12, 13]. However, this could in some cases provoke considerable side effects, such as neoplastic growth etc. [6]. These circumstances necessitate the search for effective stimulants of endogenous melatonin secretion. Epithalamin and Epitalon – physiologically active preparations of the pineal gland – appear to be among the most promising medications of their kind [1, 6].

The Pharmacopoeic drug Epithalamin is a complex of peptides extracted from the pineal gland. Tetrapeptide Epitalon (Ala-Glu-Asp-Gly) has been designed on the basis of Epithalamin amino acid analysis and synthesized at the Laboratory of Peptide Chemistry (headed by Dr. Grigoriev, Ph.D.) of the St. Petersburg Institute of Bioregulation and Gerontology. The expressed regulatory effect of these drugs on the functions of different organs and tissues has been demonstrated in previous research [1, 6].

In this work we would like to present the results of studying the effect of Epitalon on melatonin and cortisol secretion in senescent monkeys in different age periods.

Material and methods

The experiments were carried out on 6 young puberal (aged 6–8 years, average age 7.0±0.3) and 6 senescent (aged 20–26 years, average age 22.8±1) female *Macaca mulatta* from the Adler Primatological Center (Adler, Russia). Young monkeys weighed 5.1±0.9 kg and senescent ones 4.8±0.2 kg. All the young animals revealed normal ovarian menstrual cycles, while the old individuals had various reproductive disorders (from a relatively short amenorrhea to complete absence of menstrual cycles).

The monkeys were kept in individual metabolic cages in a separate room. Regular night/day cycle was maintained, lighting provided from 8 a.m. to 19 p.m. in the summertime (June–July). The animals received a well-balanced diet and unrestricted access to water.

After a 3-week adaptation to the experimental conditions and the procedure of blood taking, the animals were administered with Epitalon (intramuscularly, 10 μg in 1 ml per animal) or with natural saline solution as placebo (intramuscularly, 10 ml per animal). The monkeys were divided into 2 control (placebo) and 2 experimental (Epitalon) groups, three individuals in each, with respect to their age. Epitalon and natural saline solution were injected at 9 a.m. for 10 days. Blood samples were taken from the ulnar and femoral veins twice a day (at 9 a.m. and 9 p.m.) on the 10th day of injections with heparin applied as anticoagulant. The blood was centrifuged for 15 min. at *2000g*, plasma was separated and stored at –50°C till analysis for hormones was to be done.

Immunoferment assay was made in the blood plasma samples to define the content of cortisol ("Alcor Bio", St. Petersburg, Russia), dehydroepiandrosterone (DHEA) ("Diagnostic System Laboratories, Inc.", Texas, USA), dehydroepiandrosterone sulphate (DHEAS) ("Diagnostic System Laboratories, Inc."), total thyroxin ("Alcor Bio"), estradiol ("Diagnostic System Laboratories, Inc."), progesterone ("Diagnostic System Laboratories, Inc.") and melatonin ("Immuno Biological Laboratories", Hamburg, Germany).

Correlative coefficients for the levels of all the investigated hormones did not exceed 12 % in one reaction and 15 % in different reactions. The investigation results were statistically processed by Student's t-criterion.

Results and discussion

Melatonin content in the senescent control animals was twice less ($p<0.01$) than that in the young controls, especially in the evening (Figure 1). This corresponds to the published data on decreased melatonin content in human and animal blood in aging [11–13].

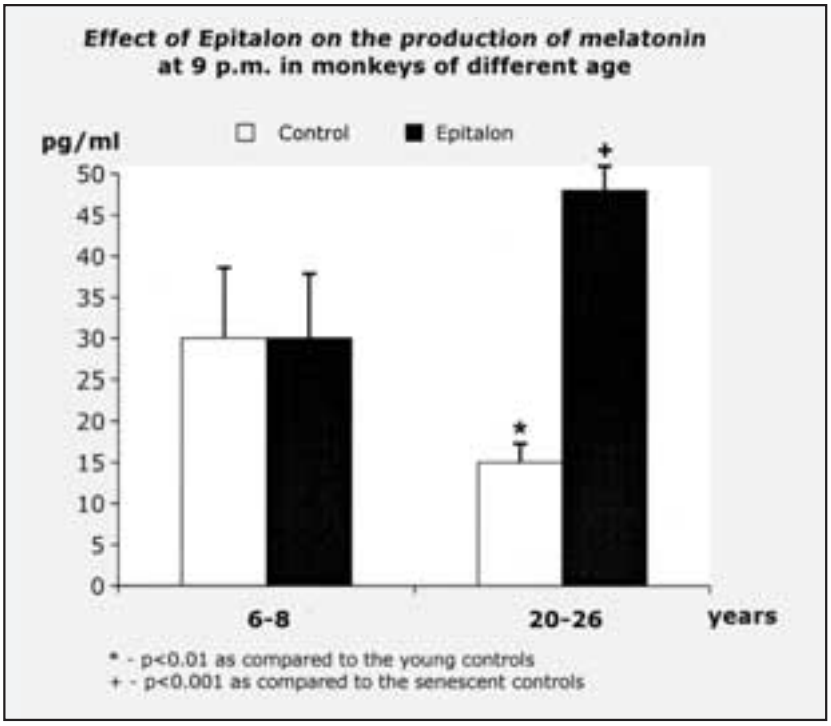
It is important that the melatonin concentration both in the young and senescent control monkeys at 9 p.m. exceeded that at 9 a.m. (30±9 and 17±7 pg/ml in the young females; 15±3 and 10±2 pg/ml in the old ones, respectively). This also conforms to the evidence of increased melatonin secretion in the evening [11–13].

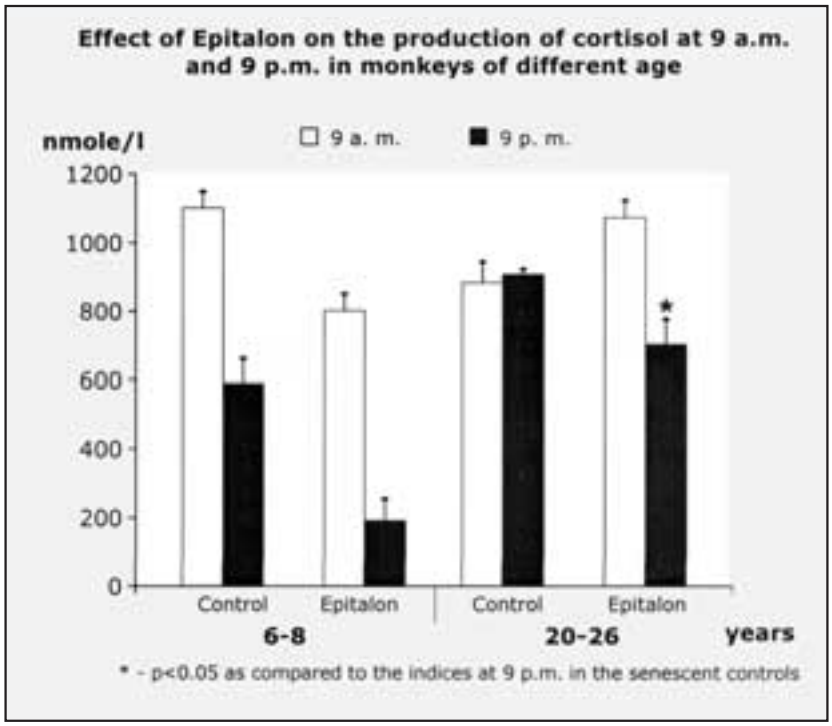
We registered a significant increase in the evening-time melatonin concentration in the Epitalon-administered animals (Figure 1). For instance, it exceeded the corresponding control value in 20–26 year old Epitalon-affected monkeys ($p<0.001$) more than 3 times, while in the young animals Epitalon introduction did not exert any significant effect upon melatonin content in the blood.

Epitalon did not significantly influence the basal production of adrenal androgens (DHEA, DHEAS), sex steroid hormones (estradiol and progesterone) and thyroxin.

In the analysis of the obtained data it is important to consider the age-related differences in basal melatonin production and the changes in melatonin biosynthesis in case of Epitalon administration. The selective stimulating action of Epitalon on melatonin production in senescent animals could presumably be explained by its modulating effect upon the sensitivity of melatonin-synthesizing system to the degree of outer illuminance, which usually decreases with age. This fact is confirmed by both lower basal levels of evening melatonin in senescent monkeys, as well as daytime- and, consequently, luminance-depending stimulating effect of Epitalon (48±5 pg/ml of melatonin at 9 p.m. and 10±2 pg/ml at 9 a.m., $p<0.001$). This presumption is supported by the data on the inhibitory effect of light on melatonin synthesis and secretion, as well as by the results of investigations demonstrating the lack of any age-related lesions both in the structure of pinealocytes and in the activity of the key ferment systems of pineal melatonin synthesis in humans [7, 8, 10, 12].

Adrenergic innervation of the pineal gland is known to be primarily important in the regulation of melatonin synthesis [12, 13]. Consequently, if we take





into account the age-related decrease in the sensitivity of pineal β-photoreceptors in rodents [4], we shall – with all probability – suggest the stimulating effect of Epitalon on the amount and/or affinity of β-adrenergic receptors to noradrenalin on the pinealocytic membranes.

Epitalon administration to senescent animals ceased not only melatonin secretion, but also the circadian rhythms of cortisol content in the peripheral blood (Figure 2). In aging, there is no expressed decrease in the level of evening cortisol (at 9 p.m.), which evidences reduced amplitude of cortisol circadian rhythm. A similar picture has been previously registered in humans as well [12].

Since close correlation between the epiphysis and adrenal glands has been proven [7, 12], we may presume that Epitalon restoring influence on cortisol circadian rhythms in old animals is mediated through the recovery of melatonin secretion level. This is confirmed, first, by the negative correlation between daily dynamics of cortisol and melatonin levels in the peripheral blood of young monkeys and, secondly, by the normalization of melatonin and cortisol circadian rhythms in the event of Epitalon administration.

Normalized production of melatonin and cortisol is essential for the organism, since it is the circadian rhythms of these very hormones that determine the daily rhythmic activity of various organs and, above all, of the nervous, endocrine, cardiovascular and immune systems [5, 12, 13].

For the first time the stimulating effect of peptide Epitalon on melatonin level in the peripheral blood accompanied by normalization of cortisol circadian rhythms in senescent female *Macaca mulatta* in the performed investigation was stated. These results point to the prospects of applying Epitalon in the correction of age-related hormonal imbalance and functional normalization of the vitally important organs and systems.

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