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ORIGINAL ARTICLE

The study of the circannual relationship between the activity of the epiphysis and gonads in rats of different sex and age

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Abstract

Background: The relationship between the activity of the epiphysis and gonads in rats of different sex and age in different seasons of the year was determined by studying the levels of melatonin and testosterone in the blood plasma. Determination of the levels of melatonin and testosterone in the serum of rats was carried out by enzyme-linked immunosorbent assay. To assess the relationship between the levels of melatonin and testosterone the correlation coefficient was calculated. Based on the study of the levels of melatonin and testosterone in serum the circannual relationship between the activity of the pineal gland and gonads in males of reproductive age has been determined. In females, the relationship between the levels of melatonin and testosterone without the circannual dependence has been determined. The strongest correlation between melatonin and testosterone is present in males at the age of 9 months in autumn, and it corresponds to the human age of 29-30 years.

Keywords: melatonin, epiphysis, gonads, testosterone, age, sex

INTRODUCTION

The phenomenon which is the most significant for wildlife on Earth, is the alternation of day and night, light and darkness. Rotation of our planet around its axis and simultaneously around the sun marks off the day, seasons and years of life. Biological objects are one of the forms of organization of matter, and periodic variations are inherent to them, as well as to inanimate nature. The real progress in the study of biological rhythms was observed in the 30-ies of the XX-th century. There are different classifications of biological rhythms; the most popular one is the classification of F. Halberg who suggested distinguishing circadian (daily), circaseptal (weekly) and circannual (yearly) oscillations.[1] Depending on the level of the biosystem organization the cellular, organ, organismic and population biological rhythms have been described.[2] By frequency they are divided into 5 classes: high frequency rhythms (the period of oscillations from milliseconds up to 30 min), middle frequency rhythms (with the period of oscillation from 30 min to 28 h), mesorhythms (the oscillation period is 28 h – 6 days), macrorhythms with the period from 20 days to 1 year, and megarhythms with oscillations lasting decades and hundreds of years. At present the rhythmic changes of more than 400 physiological functions have been described. Oscillations with periods that are similar to major geophysical cycles are also called adaptive rhythms. These rhythms include daily and seasonal oscillations of physiological parameters; their role is in adaptation of an organism to the periodically changing conditions of the external environment. Diurnal and seasonal periodicity is characteristic of all levels of the biological organization, and it explains the greatest interest of chronophysiologists to them.[3,4] And, if there is a great number of studies concerning circadian rhythms, only some works deal with the problem of the circannual rhythm.

Currently, it is known that rhythms are synchronized by temporal landmarks, such as light and darkness, the tides and the change of seasons. Light falling on the retina through the optic nerves affects suprachiasmatic nuclei (SCN) of the hypothalamus – the main source of oscillations in the body. Despite the fact that SCN exercise control over most functions of the body the projections of neurons of these nuclei mainly fall within the boundaries of the hypothalamus. Through contacts with

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hypothalamic neuroendocrine neurons, which contain releasing hormones, SCN regulate circadian rhythms in secretion of pituitary hormones (level II oscillators). With the help of contacts with autonomic neurons of the hypothalamus these nuclei send their signals to the nerve endings located on the periphery in the organs and endocrine glands, causing rhythmic changes in the hormones synthesized by them – level III oscillators. The pineal gland is the level I oscillator and a unique modulator at the junction of the nervous and endocrine systems, it is capable to integrate various endogenous and exogenous signals, transforming them to the hormonal response.[5] Deprived of the own rhythm specifying abilities in mammals it provides combination, coordination of the different periods of biological rhythms.[6,7] When studying the effect of the pineal gland on the reproductive system primarily the role of hormone melatonin produced by the gland is investigated in this process. Today it is reliably known about the effect of melatonin on sexual development and reproduction, it exhibits the antigonadotropic action. Moreover, decrease in the melatonin content in the blood leads to disinhibition of the cyclic secretion center of gonadotrophin releasing hormones (GnRH) and, consequently, to the increased synthesis by the pituitary gland of luteinizing hormone (LH) and follicle stimulating hormone (FSH).[8] It is known that the function of gonads is regulated by complex neuroendocrine mechanisms. A distinctive feature of the female organism is the presence of sexual cycles, which are lost in the male body organism during the sexual differentiation of hypothalamic nuclei. The preovulatory hypersecretion of GnRH in women is due to the interaction of two external signals in relation to the gonadoliberin neurons – humoral (ovarian) and neuronal (circadian).[9] The mechanism of the humoral signal in women is well studied and is in a sequential alternation of positive and negative feedbacks between the ovaries and the hypothalamus by the hypothalamic-pituitary-ovarian regulation. The positive feedback is characterized by the increased synthesis and secretion of GnRH in the parts of the hypothalamus that are responsible for regulation of the reproductive function in response to the increase of the synthesis of sex hormones by maturing follicles,[10] and it is characteristic only for females. The negative feedback preserved in both sexes is in reducing the level of GnRH and, as a result, gonadotropin rising levels of sex hormones in the blood. As previously stated, the circadian control of the synthesis and secretion of GnRH is also carried out by the neurohumoral way and with involvement of the pineal hormone of SCN of the hypothalamus. It performs the function of a humoral pacemaker of biorhythms, causes rhythmic processes in target organs, including the gonads, via their receptors.

The light conditions of middle and subpolar latitudes where a significant portion of the world's population live are characterized by the presence of oscillations in the length of daylight within the year. The study of cyclic processes of reproduction in women is a subject of many works in modern scientific literature.[11,12] However, we have not come across any works devoted to the study of the relationship between the synthesis of the male sex hormone – testosterone in individuals of different gender and age and the circannual rhythm of melatonin secretion by the pineal gland.

The aim of the work is to study the relationship between the activity of the epiphysis and gonads in rats of different sex and age in different seasons of the year by determining the levels of melatonin and testosterone in the blood plasma.

MATERIALS AND METHODS

The study was carried out on 192 white nonlinear rats divided into groups by gender and age, namely, 3, 9, 15, and 20 months old, and it corresponds to the human age of 14, 29-30, 43-44, 55-56 years, respectively. There were six animals in each group. The studies were conducted at four seasonal points: autumn (October), winter (January), spring (March) and summer (July). The ratio of day/night in different seasons was: autumn – 10:14, winter – 8:16, spring – 12:12, summer – 16:8. During the study animals were kept on a standard diet and the temperature conditions under natural lighting without the influence of artificial light sources. Blood sampling in all experimental seasonal points conducted from 10.00 am to 12.00 am. Time for blood sampling was chosen to avoid the coincidence of circadian rhythms peak secretion of hormones - melatonin for it is from 2.00 to 4.00 of the night, for testosterone – 6.00-8.00 in the morning[6,13], as well as the presence of low amplitude and high amplitude rhythms of melatonin secretion, which is the ratio of the different presented in different age groups – from young high-amplitude rhythms prevail, and the elderly – low amplitude[14]. Determination

TABLE 1: The coefficient of correlation and power connection

The coefficient of correlation	Power connection
1.0	functional relationship
0.9 – 0.99	very strong
0.7 – 0.9	strong
0.5 – 0.7	significant
0.3 – 0.5	moderate
0.1 – 0.3	low
0	no communication

of the levels of melatonin and testosterone in the blood serum of rats was carried out by enzyme-linked immunosorbent assay using a set of Melatonin ELISA ("IBL-International", Germany) and a DRG set of Testosterone ELISA ("DRG", Germany). Euthanasia of animals was performed according to the requirements of the Commission on Bioethics of the National University of Pharmacy (Kharkov, Ukraine) and "General ethical principles of experiments on animals", which are consistent with the provision of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the I-st National Congress on Bioethics (Kyiv, Ukraine, 2001). To assess the relationship between the levels of melatonin and testosterone the correlation coefficient was calculated. The assessment of the relationship was performed according to the Chaddock scale[15] (Table 1).

Statistical significance was assessed using one-way ANOVA test, the difference was considered to be reliable at $p \le 0.05$.[16] The data processing was performed using Statictica 7.0 and Excel software.

RESULTS

In the experiments conducted, it was found that the highest levels of melatonin both in males and in females of all age groups were determined in summer and winter periods, the lowest level was in autumn in Table 2.

However, in the summer period, the highest level of melatonin was determined in males at the age of 15 months, and in females at the age of 3 months, the lowest level was at the age of 20 months in rats of both sexes. In the winter period, the experimental animals of both sexes had the highest level at the age of 3 months, as

TABLE 2: Indicators melatonin levels in rats of different ages and sex in different seasons (M ± m, pmol/L, n = 6)

Season/ age	3 months	9 months	15 months	20 months
		Male		
Autumn	164.12±9.62	127.28±5.11*	156.95±9.72**	127.42±9.16$^{*/***}$
Winter	303.37±7.57$^{\wedge/\&}$	286.81±8.93$^{\wedge/\&}$	292.83±10.29$^{\wedge/\&}$	249.47±13.46$^{*/**/***\wedge/\&}$
Spring	198.66±10.24^	142.33±7.18*	166.84±5.73$^{*/**}$	140.54±8.43$^{*/***}$
Summer	311.9±12.15$^{\wedge/\&}$	302.3±10.3$^{\wedge/\&}$	314.33±14.18$^{\wedge/\&}$	262.37±14.25$^{*/**/***\wedge/\&}$
		Female		
Autumn	159.89±11.95	149.07±11.72	155.80±13.71	125.42±7.86*
Winter	319.28±22.36^	289.53±10.04^	258.57±21.16$^{\wedge/\&}$	183.18±13.08$^{*/**/***\wedge/\&/\#}$
Spring	243.52±16.58$^{\wedge/\#}$	205.25±10.19$^{\wedge/\#}$	156.02±15.75$^{*/**}$	134.09±11.51$^{*/**}$
Summer	310.39±12.91^	302.00±12.22^	261.44±9.28$^{*/**\wedge/\&/\#}$	200.95±13.85$^{*/**/***\wedge/\&/\#}$

Remarks: * $p \le 0.05$ in relation to the rats age 3 months; ** $p \le 0.05$ in relation to the rats age 9 months; *** $p \le 0.05$ in relation to the rats age 15 months; ^ $p \le 0.05$ in relation to the level of autumn; $^{\&}$ $p \le 0.05$ in relation to the level of spring; $^{\#}$ $p \le 0.05$ in relation to males well as with a significant decrease in level by age of 20 months – by 18% in males, and by 42% in females. Indicators of melatonin in spring and autumn were lower in all age groups with the lowest level in animals at the age of 20 months. Moreover, the levels of melatonin in males of the age groups 3, 9, 15 and 20 months in autumn were 47%, 58%, 50% and 51% lower than the levels in summer, and 46%, 56%, 40% and 49% lower than the levels in winter. In females, the levels of melatonin corresponding to the age groups were lower by 49%, 52%, 40%, 38% in autumn than the levels in summer, and by 50%, 49%, 40% and 32% than the levels of melatonin in winter ($p \leq 0.05$). The lowest levels of melatonin in males and females were registered in autumn in the age group of 9 months, being lower in males by 15% than in females. Significant differences between the levels of melatonin in males and females were determined in winter in the age group of 20 months, in spring in animals at the age of 2 and 9 months, in summer in animals at the age of 15 and 20 months.

In parallel with determination of the levels of melatonin in serum the levels of testosterone were studied. Testosterone is the indicator of the male sex gland function and one of the sex hormones that determines functioning of the reproductive system in women (Table 3).

To determine the circannual relationship between the levels of melatonin and testosterone in rats of different sex and age the correlation coefficients were calculated (Figs. 1 and 2).

DISCUSSION

When comparing the levels of melatonin in different age groups the highest levels in all seasons both in males and females occurred at the age of 3 months (corresponding to the human age of 14 years), the lowest levels were found in rats of both sexes aged 20 months (corresponding to the age of 55-56 years) and in males at the age of 9 months (corresponding to the age of 29-30 years). The data obtained are partially consistent with those in the literature where authors noted decrease in the levels of melatonin in the elderly due to age-related involution of the pineal gland.[17] However, in our studies low levels of melatonin were also found in males of the active reproductive age (29-30 years). According to the data obtained in autumn and spring the levels of melatonin were low compared to the values of winter and spring. It coincides with the change of daylight duration and is consistent with the literature data about existence of the physiological desynchronosis in the period of the biological spring and autumn.[18]

The analysis of the results obtained in the determination of testosterone in the plasma shows that circannual oscillations of the testosterone level in females have a significant difference only in the age group of 3 months. There were also reliable differences between the age groups of 3 and 9 months in autumn and winter with oscillations of 7-9%. In comparison with females there is a clear circannual rhythm of the testosterone secretion in males with high levels in autumn and low levels in winter ($p \leq 0.05$),

TABLE 3: Indicators testosterone levels in rats of different ages and sex in different seasons (M ± m, pmol/L, n = 6)

Season/ age	3 month	9 month	15 month	20 month
		Male		
Autumn	5.52±0.27	7.57±0.53*	6.73±0.93	3.88±0.55*/**/***
Winter	3.31±0.29^	3.35±0.36^	3.59±0.94^	3.01±0.28
Spring	3.94±0.65	5.03±1.15	6.68±1.09	3.68±0.56
Summer	3.67±0.57^	5.25±0.68^/&	4.45±0.65	3.27±0.54**
		Female		
Autumn	4.11±0.05#	3.76±0.13*/#	3.68±0.19#	3.53±0.19*
Winter	3.92±0.06^/#	3.63±0.07*	3.59±0.21	3.51±0.29
Spring	3.77±0.09^/#	3.91±0.04&	3.84±0.11#	3.49±0.24
Summer	3.81±0.12^	3.74±0.11#	3.5±0.18	3.39±0.23

Remarks: * $p \leq 0.05$ in relation to the rats age 3 months; ** $p \leq 0.05$ in relation to the rats age 9 months; *** $p \leq 0.05$ in relation to the rats age 15 months; ^ $p \leq 0.05$ in relation to the level of autumn; & $p \leq 0.05$ in relation to the level of spring; # $p \leq 0.05$ in relation to males

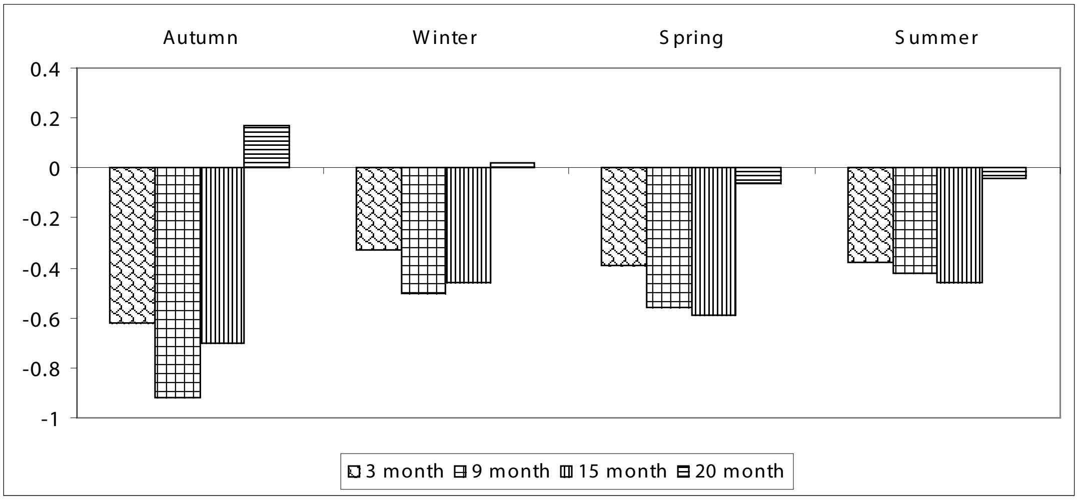

FIG. 1: The correlation relationship between the levels of melatonin and testosterone in male rats of different age in different seasons of the year

and it is partially consistent with the data in the literature.[19] The analysis of oscillations in the seasons in males in different age groups showed that in the age group of 20 months there was the lower level of testosterone without a significant difference in different periods of the year, but with a significant difference relative to males of other ages in autumn. The significant seasonal differences in the age groups 3, 9 and 15 months were also identified with a reliably high level in autumn compared to the indicators of winter by 1.7; 2.3 and 1.9 times, and summer by 1.5; 1.4 and 1.5 times in accordance with the age groups.

Analysis of correlation showed that in the most experimental groups there is a negative correlation of different strength. The correlation strength was significantly different between males and females at the age of 3, 9 and 15 months in different seasons. In males (Fig. 1) it varied at the moderate levels in winter and summer, considerable levels in spring and autumn, and it was strong and very strong in males of 15 and 9 months in autumn, respectively. In females the strength of the correlation relationship in all seasons was weak and did not have an expressed circannual rhythm (Fig. 2). The correlation relationships were bidirectional in experimental animals of both sexes at the age of 20 months during all seasons. The data obtained can be explained by two interrelated mechanisms of regulation – humoral and neuronal (circannual). Firstly, melatonin has an inhibitory action on the

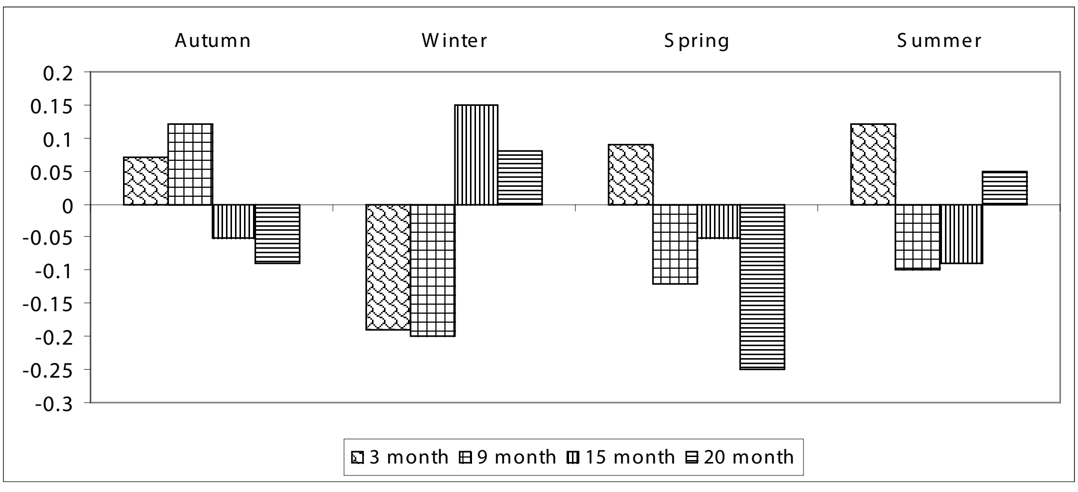

FIG. 2: The correlation relationship between the levels of melatonin and testosterone in female rats of different age in different seasons of the year

system of the hypothalamus-pituitary-androgens due to inhibition of the synthesis of gonadotropin-releasing hormones, and, secondly, the decrease in the synthesis of melatonin leads to increased secretion of FSH and LH, which stimulates the synthesis of testosterone in Leydig cells of the testes in men and the ovaries in women. In turn, a high level of testosterone may lead to a further decrease in the level of melatonin as a result of blocking the translation of mRNA of N-acetyltransferase – the main enzyme of conversion of serotonin to melatonin in the pineal gland.[20] Stronger correlation relationships in males compared to females suggest that the mechanism of the inhibitory action of testosterone on melatonin is evident in men, especially at the age of 29-30 years and 43-44 years, and is not implemented in women. The absence of the similar mechanism in women may be explained by the fact that under normal conditions testosterone in the cells of the mature follicle of the ovaries is metabolized into estrogen, and the level of testosterone in the adrenal glands is not associated with gonadotropic hormones of the pituitary gland. The absence of the relationship between testosterone and melatonin in old age both in males and females indicates changes in the neurohumoral regulation system; they occur with age and are manifested by decrease of the hormonal function in both sexes.[21]

Conclusion

Based on the study of the levels of melatonin and testosterone in the blood serum the circannual relationship between the activity of the pineal gland and gonads in males of the reproductive age has been determined. In females, the relationship between the levels of melatonin and testosterone without the circannual dependence has been determined. The strongest correlation relationship between melatonin and testosterone is present in males at the age of 9 months in autumn, and it corresponds to the human age of 29-30 years.

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