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EFFECT OF MELATONIN ON DAILY OSCILLATIONS OF THE DENSITY OF MELATONIN RECEPTORS 1A IN NEURONS OF THE LATERAL PREOPTIC NUCLEUS OF THE HYPOTHALAMUS IN RATS UNDER DIFFERENT LIGHT MODE

Abstract. *The analysis and generalization of results of own immunohistochemical research of density of receptors to melatonin of type 1A in neurons of a lateral preoptic nucleus of the hypothalamus of mature rats are given in the article. Representative immunohistochemical staining of melatonin type 1A receptors was observed in all neurons of the lateral preoptic nucleus of the hypothalamus, regardless of their location on the nucleus. The staining was mostly fine-grained or almost diffuse in nature and covered the membrane of neurons and their cytoplasm. It is proved that the daily fluctuations of the optical density of the studied structures are clearly subordinated to circadian rhythms, with the highest values at 2 a.m., while at 2 p.m. it decreases ($p<0.001$). Light stimulation (24.00L and 00.00D) leads to desynchrony and decreased density of melatonin receptors type 1A in neurons of the lateral preoptic nucleus of the hypothalamus of rats. With the injection of exogenous melatonin at a dose of 0.5 mg/kg on the background of constant illumination, a probable ($p<0.001$) increase in the average values of receptor density to melatonin type 1A in neurons of the lateral preoptic nucleus of the hypothalamus was observed.*

Key words: *sleep-wake cycle, lateral preoptic nucleus of the hypothalamus, circadian rhythms, photoperiod, melatonin.*

One of the fundamental properties of all living organisms is rhythmic oscillations [1], which ensure the adaptation of living organisms to the constantly changing environment [2].

The sleep-wake cycle is the most obvious circadian rhythm observed in humans and many animals [3]. People spend a third of their lives asleep, and its quality determines the overall level of health [4]. Sleep is an extremely complex physiological process [5], which is regulated by homeostatic and circadian processes involving various neural structures [6]. In the human body, there is a cerebral system of regulation of the sleep-wake cycle [7], where the key role is played by the lateral preoptic nucleus of the hypothalamus [8]. Neurons of this nucleus provide gamma-aminobutyric innervation of key monoamine systems of the brain, which are responsible for the period of wakefulness [9], furthermore, damage to this nucleus can cause insomnia [10].

The hormone synthesized by the pineal gland, melatonin, and to a lesser extent human and social activity are important factors in synchronizing the circadian rhythm with the 24-hour day. Melatonin secretion is subject to a circadian rhythm, with an increase in its concentration at night [11] and a decrease during the day [12]. The presence of a circadian rhythm of melatonin production is a marker of the normal operation of the circadian system of biorhythms and their synchronization with the change of day and night [13] and the regulation of the sleep-wake cycle [4].

Excessive light stimulation and night activity are the most common causes of sleep-wake disorders [14], leading to an increased risk of cancer, metabolic, cardiovascular disease [15], and premature death [16].

The objective of the research was to investigate the effect of melatonin on the daily fluctuations in the density of melatonin receptors

type 1A in the neurons of the lateral preoptic nucleus of the hypothalamus of rats, which were kept under conditions of light stimulation.

Material and Methods. The experiments were performed on 36 mature white male rats. Animals of the first group were in the standard light regime for seven days (lighting from 8 a.m. to 8 p.m. with fluorescent lamps, the level of illumination at the level of a cage - 500 Lk). The rats of the second group were kept for seven days in the conditions of light stimulation (round-the-clock lighting, the level is similar to the rats of the first group). The third group - rats, which were for seven days in the background of round-the-clock lighting and received intraperitoneal injection of exogenous melatonin (Sigma, USA) at a dose of 0.5 mg/kg body weight of rats.

Given the daily rhythm of melatonin synthesis, the material was taken at 12-hour intervals (at 2 p.m. and at 2 a.m.), performing a one-time decapitation under etaminal anesthesia (40.0 mg/kg, intraperitoneally). All stages of the experiment were carried out in compliance with the basic requirements of the European Convention for the Humane Treatment of Animals.

The removed rat brain was fixed for 22-24 hours in neutral buffered 10% formalin solution. After that, plates about one millimeter thick were cut: the first section passed through the anterior part of the optic chiasm, and the second section passed through the posterior part of the optic chiasm. Next, dehydration of the excised plates of brain tissue was performed in an ascending battery of alcohols. At 58^0C was poured into the paraffin and using a sled microtome made serial histological sections with a thickness of 5µm. According to the protocols provided by the manufacturer immunohistochemical techniques using primary antibodies against receptors for melatonin type 1A (Abcam) were performed on the sections. A Delta Optical Evolution 100 microscope and an Olympus SP550UZ digital camera were used to visualize the primary antibodies. Digital copies of images using a licensed copy of ImageJ v1.48 were evaluated for color intensity by computer microdensitometry, ranging from 0 (absolute transparency) to 1 (absolute opacity).

Statistical processing of the obtained results was performed using a licensed copy of the computer program PAST. First, using the Wilki-Khan-Shapiro test, the normality of the distribution was checked. Then parametric methods of statistical analysis were used: calculation of arithmetic mean and its error (M ± m), odd two-sided Student's criterion. At the same time, the Mann-Whitney test was additionally used to increase the reliability of the results of the test of differences between the study groups.

Results and their discussion. Representative immunohistochemical staining of melatonin type 1A receptors was observed in all neurons of the lateral preoptic nucleus of the hypothalamus, regardless of their location on the nucleus. The staining was mostly fine-grained or almost diffuse in nature and covered the envelope of neurons and their cytoplasm. Observing the intensity of the optical density of the immunohistochemical staining of neurons, it can be stated that it had different strengths with respect to both the membrane and the cytoplasm. In particular, there were neurons with strong staining and moderate or even weak staining, so all measurements of the optical density of immunohistochemical staining for melatonin receptors type 1A in neurons of the lateral preoptic nucleus of the hypothalamus were performed on the cytoplasm of cells with stable staining. The average optical density of staining for melatonin receptors type 1A neurons of the lateral preoptic nucleus of the hypothalamus at 2 p.m. and 2 a.m. under different lighting modes are listed in table.

From the data shown in table 1, it is seen that the optical density to melatonin receptors type 1A in the neurons of the lateral preoptic nucleus of the hypothalamus of rats at 2 a.m. is on average higher than at 2 p.m.

Under the conditions of light stimulation (24.00L and 00.00D), the intensity of the optical density of a specific color for melatonin receptors 1A in the neurons of the lateral preoptic nucleus of the hypothalamus decreased sharply. It should be noted that under constant illumination we did not register differences in the average trends between the optical density of a specific color in the neurons of the lateral preoptic nucleus of the hypothalamus between the study periods at 2 a.m. and 2 p.m.

Injection of exogenous melatonin (Sigma, USA)

Table

Optical density of specific staining for melatonin receptors type 1A in neurons of lateral preoptic nucleus of the hypothalamus of rats under different lighting conditions (M ± m)

Time of the day	Optical density of immunohistochemical staining on melatonin 1A receptors (in unit of optical density)		
	Standard lighting	Light stimulation	Light stimulation + melatonin
2 p.m.	0,248±0,0018	0,180±0,0018	0,249±0,0017
2 a.m.	0,264±0,0016 (p<0,001)	0,182±0,0017	0,275±0,0015 (p<0,001)

Note: p - probability of difference compared to the previous time interval within one series.

to light-stimulated rats resulted in an increase in the average value (p<0.001) of optical density of specific staining for melatonin receptor type 1A in neurons of the lateral preoptic nucleus of the hypothalamus at 2 a.m. and at 2 p.m. In particular, the optical density increased by 2 p.m. - to the level of rats kept under standard lighting, and at 2 a.m. - even higher than rats kept under standard lighting.

Conclusions. 1. The density of melatonin receptors type 1A in neurons of the lateral preoptic nucleus of the hypothalamus is obeyed by a clear circadian rhythm, with the highest values at 2 a.m., while at 2 p.m. it decreases (p<0.001).

2. Light stimulation leads to a violation of the circadian rhythm and a decrease in the density of melatonin 1A receptors in the neurons of the lateral preoptic nucleus of the hypothalamus.

3. Injection of exogenous melatonin at a dose of 0.5 mg/kg leads to a probable (p<0,001) increase in the average density of receptor density for melatonin type 1A in the neurons of the lateral preoptic nucleus of the hypothalamus.

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