INFLUENCE OF MELATONIN ON THE DENSITY OF MELATONIN RECEPTORS IN THE NEURONS OF HYPOTHALAMic SUPRAOPTIC NUCLEUS UNDER THE CONDITION OF ALTERED PHOTOPERIOD

Vlasova K.V., Bulyk R.Ye.

Higher State Educational Institution of Ukraine
«Bukovinian State Medical University»
Department of medical biology and genetics, Chernivtsi

Ключевые слова: *супраоптические ядра, нейроны, мелатониновые рецепторы, иммунно-гистохимический анализ.*

Резюме. *Путем иммуногистохимического анализа выявлено корректирующее воздействие мелатонина на нарушение циркадианного ритма экспрессии мелатониновых рецепторов 1 А типа в нейронах супраоптического ядра гипоталамуса в условиях подавления активности шишковидной железы .*

Abstract. *Using immunohistochemical analysis we revealed adjustment effect of melatonin on the disturbance in the circadian rhythm of expression of type 1 A melatonin receptors in the neurons of the hypothalamic supraoptic nucleus under the conditions of pineal gland suppression.*

Topicality. Most of the physiological and metabolic processes of the body are organized in time in response to photoperiod, adapting to environmental changes [1, 7].

Chemical signal of darkness for living organisms is the hormone melatonin (MT) produced by photoreceptors of the retina, by pinealocytes of the pineal gland, and by the peripheral organs (liver cells, kidney, adrenal gland, gall bladder, ovary, endometrium, placenta, endothelium, thymus, blood (leukocytes, platelets),intestinal vermiform appendix and other parts of the gastrointestinal tract [1, 6].

It is known that in night and daytime animals, melatonin is produced in the dark period of the day and encods the information signal of the time and duration of the day, coming to the rhythm driver of the central biological clock (BC) – suprachiasmatic nucleus (SCN) of the hypothalamus [9, 10].

Production of MT is sharply suppressed by light, which affects the ability to transfer rhythmic information from the SCN of the hypothalamus to other neural target sites and thus alters the expression of some biological rhythms [3].

The hormone controls the state of the hypothalamic-pituitary system and endocrine glands activity through melatonin receptors (membrane, cytosolic and nuclear ones) [4, 5]. In addition, using a mechanism of the feedback it interferes with the activity of supraoptic nucleus (SON) of the hypothalamus, which regulates water-salt exchange and responses to stress reaction [1, 2, 8]. However,there are practically no data on characteristics of melatonin receptors in the hypothalamic neurons of SON in the rat's brain under the condition of the altered photoperiod.

Objective: Based on immunohistochemical techniques combined with computer microdensitometry, to provide quantitative circadian characteristics of melatonin receptors

density in the neurons of the hypothalamic supraoptic nucleus of rats being under light stimulation as well as the correction of changes after injecting exogenous melatonin.

Material and methods. The experiments were conducted on 60 white mongrel mature male rats weighing 0,15-0,18 kg. The animals were kept in cages at a constant temperature, humidity and free access to water and food. Experimental animals were divided into 3 parts each with 2 groups (10 animals in each), which stayed under conditions of standard light regime – 12.00L: 12.00D (light from 08.00 AM to 08.00 PM was provided by means of a fluorescent lamp LB-40, illuminance of the room near the animals was 200 luxes), hyperilluminated (day-round light (24.00L: 00D) by fluorescent lamps LB-40, illuminance of the room near the animals was 500 luxes), the injection of exogenous MT and day-round lighting (24.00L: 00D + MT) within 7 days. In order to detect circadian differences in melatonin receptors and talking into consideration the cyclical production of MT, euthanasia of rats was performed with 12-hour interval (at 02.00 AM and at 02.00PM) by their decapitation on the 8^{th} day. The selected dates for the experiment were caused by different functional activity of the pineal gland and production of leading chronobiotic – MT in these time periods. Commission on bioethical expertise of Bukovinian State Medical University found that all stages of the experiment were conducted in compliance with the essential requirements of the Helsinki Declaration and the requirements of the Council of Europe on Human Rights and Biomedicine (1977), the provisions of the WHO, International Code of Medical Ethics (1983) and the laws of Ukraine (protocol number 22 of November 28, 2007.).

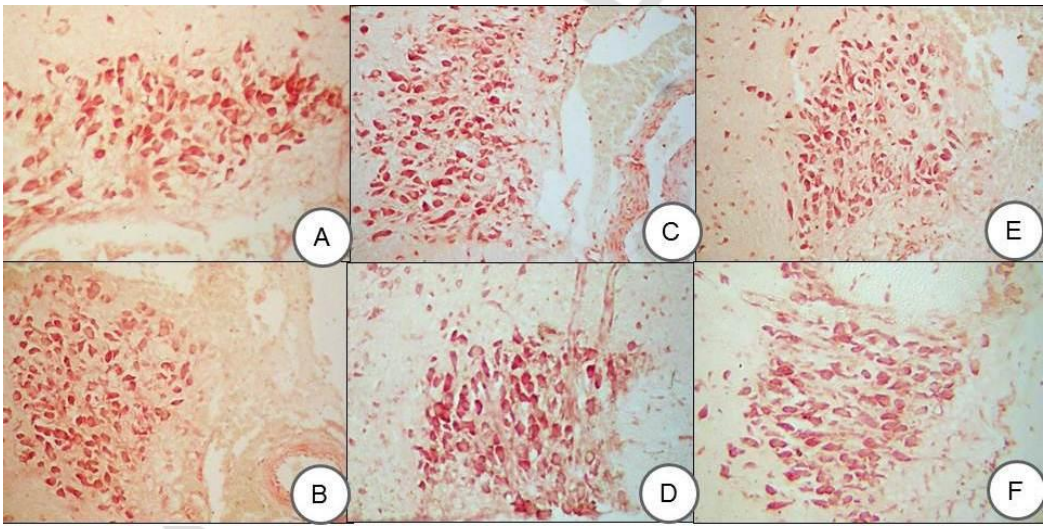
For immunohistochemical study fragments of the cerebral hemispheres with the area of the supraoptic nucleus of the hypothalamus were fixed in 10% solution of neutral buffered formalin for 22 hours. Then we performed accelerated dehydration in ascending alcohol concentrations, embedded in paraffin at 58^0C, with further obtaining histological sections 5 microns thick.

In order to perform immunohistochemical methods we used polyclonal antibodies to melatonin 1A receptors produced by Abcam (UK) and streptavidin biotin visualization system LSAB2 (peroxidase mark + diaminobenzidine) produced by Chemicon International Inc. (USA). We adhered to protocol standardization of methods for all sections. Additional staining of nuclei was performed with Mayer hematoxylin.

Quantitative research of the intensity of staining was conducted as follows. First, the used a microscope lens x 40 and received digital copies of optical images, which were then analyzed by a licensed copy of the computer program "VideoTest – Size 5.0" (Videotest company, Russia) – namely we performed computer microdensitometry. The analysis was performed on the basis of measurements by means of microprobe technique in the fields of positive coloring in terms of "optical density" (in relative units within a range of 0-1, with "0" corresponding to absolute transparency in the optical microprobe, and "1" is an absolute optical opacity). The intensity of a specific staining (term "Optical density") was identified with the degree of density of melatonin receptors.

Given the need to perform multiple statistical comparisons of averages in statistical sampling to determine the differences between the aggregations, we used the Newman-Keuls test.

Results. Melatonin 1A receptors in the form of granules of different sizes and optical density with transmembrane location were in the neurons of the hypothalamic supraoptic nucleus (figure 1)



Pic. 1 - Melatonin 1 A receptors in the neurons of SON of a rat:
under standard illumination at 02.00 AM (A) and at 02.00 PM (B); with hypofunction of the pineal gland at 02.00 AM (C) and at 02.00 PM (D) and after correction with exogenous melatonin at 02.00 AM (E) and 02.00 PM (F)

Notice. We showed performance increase – Ob.40^x, Oc.10^x. Immunohistochemical method of polyclonal antibodies to melatonin receptor 1 A and streptavidin biotin visualization system LSAB2 (peroxidase mark + diaminobenzidin). Additional staining of cellular nuclei with Mayer's hematoxylin.

The results of measurement of optical density of a specific staining to melatonin receptors 1A under standard light regime are shown in the table.

Table 1. Circadian dynamics of the optic density of immunohistochemical staining on 1 A melatonin receptors in the neurons of the rat's hypothalamic supraoptic nucleus (x±Sx)

Time	Optic thickness of the specific staining (n=10)	Value of likelihood (p) of differences between experimental groups according to Newman-Keuls test
02.00 AM	0,488±0,0024	p=0,002*
02.00 PM	0,464±0,0023	

Notice. n – number of animals, * – likelihood of differences compared to the previous time interval.

As we can see from the table, the highest density of melatonin 1 A receptors in the neurons of rats' SON was observed at 02.00 AM compared to 02.00 PM (in sight area of 1600 mcm^2 - p=0.002 for the Newman-Keuls test).

Under the day-round lighting the number of positively stained for melatonin 1 A receptors in neurons of SON in the sight area 1600 mcm^2 amounted to about 0,216 ± 0,0017 at 02.00 AM and 0,214 ± 0,0021 at 02.00 PM in units of optical density. Differences after the Newman-Keuls test between these experimental groups are not true (p>0,05).

However, there is a substantial reduction of this value in the study periods (p<0,001) compared with animals that are kept in conditions of standard light regime.

It was melatonin (Sigma, USA) in the dose 0,5 mcg/kg of the animal's body weight that was used for correction of changes caused by prolonged exposition to constant illumination while determining the density of 1 A receptors in the neurons of the hypothalamic supraoptic nucleus (fig.).

Injections of MT under conditions of constant illumination caused at 02.00 AM an increase of the density of melatonin receptors 1 A in the rats' hypothalamic neurons of SON compared with animals that were in conditions of constant illumination and without injecting the drug.

Immunohistochemical studies at 02.00 PM when injecting the drug, showed probable decrease of the experimental structures density to 0,324 ± 0,0027 units compared with that at 02.00 AM (p <0,001 by the Newman-Keuls test). In particular, the number of positively stained melatonin receptors 1 A in SON neurons was higher in the study period compared with the animals which were not administered melatonin against the background of light stress.

Conclusions: The density of melatonin receptors 1 A in rats' hypothalamic neurons of SON is normally characterized by a distinct circadian rhythm. The highest density of receptors is observed at 02.00 AM and at 02.00 PM it is significantly reduced (p = 0.002).

Immunohistochemical studies revealed that under inhibition of the pineal gland the circadian rhythm of melatonin receptors density in neurons in supraoptic nuclei of the hypothalamus is disturbed, which is characterized by a false difference in the periods of the experiment.

However, when you activate the pineal gland, the highest value is noted at 02.00 AM, being 0,505 ± 0,0026 units of density. Injecting melatonin for a week against the background of prolonged lighting manifests tendency to normalizing density of melatonin receptor 1 A in supraoptic neurons in rats' nuclei, which is especially noticeable in the samples selected for the study at 02.00 AM, when the value was within 0,412 ± 0,0025 units of optical density.

Reference

1. Пішак В. П. Молекулярно-генетичні механізми часової організації фізіологічних функцій у ссавців (огляд літератури та власні дані) [Текст] / В. П. Пішак, Р. Є. Булик, К. В. Власова // Бук. мед. вісник. – 2014. – Т. 18, №1. – С. 172–177.
2. Хильдебрандт, Г. Хронобиология и хрономедицина [Текст] / Г. Хильдебрандт, М. Мозер, М. Лехофер. – М.: Арнебия, 2006. – 144 с.

3. Чибисов, С. М. Биоритмы и космос: мониторинг космобиосферных связей [Текст] / С. М. Чибисов, Г. С. Катинас, М. В. Разгульская. – М.: Монография, 2013. – 442 с.
4. Chan, K. H. A molecular and chemical perspective in defining melatonin receptor subtype selectivity [Text] / K. H. Chan, Y. H. Wong // Int. J. Mol. Sci. – 2013. – Vol. 14 (9). – P. 18385–18406.
5. Distribution of MT1 melatonin receptor immunoreactivity in the human hypothalamus and pituitary gland : colocalization of MT1 with vasopressin, oxytocin, and corticotropin-releasing hormone [Text] / Y. H. Wu, J. N. Zhou, R. Balesar, [et al.] // J. Comp. Neurol. – 2006. – Vol. 499 (6) – P. 897–910.
6. Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology [Text] / R. J. Reiter, D. X. Tan, A. Korkmaz, S. A. Rosales-Corral // Hum. Reprod. Update. – 2014. – Vol. 20 (2). – P.293–307.
7. Phase relationships between core body temperature, melatonin, and sleep are associated with depression severity: Further evidence for circadian misalignment in non-seasonal depression [Text] / B. P Hasler, D. J Buysse, D. J. Kupfer, A. Germain // Psychiatry Research. – 2010. – Vol. 178. – P. 205–207.
8. The influence melatonin receptors antagonists, luzindole and 4-phenyl-2-propionamidotetralin (4-P-PDOT), on melatonin-dependent vasopressin and adrenocorticotropic hormone (ACTH) release from the rat hypothalamo-hypophysial system. In vitro and in vivo studies [Text] / M. Juszczak, M. Roszczyk, E. Kowalska, B. Stempniak // J. Physiol. Pharmacol. – 2014. – Vol. 65 (6). – P. 777–784.
9. Therapeutic uses of melatonin and melatonin derivatives: a patent review (2012 - 2014) [Text] / S. Rivara, D. Pala, A. Bedini, G. Spadoni // Expert Opin. Ther. Pat. – 2015. – “in press”
10. Trivedi, A. K. Melatonin: an internal signal for daily and seasonal timing [Text] / A. K. Trivedi, V. Kumar // Indian J. Exp. Biol. – 2014. – Vol. 52 (5). – P. 425–437.