[1,2]О. Тарнопольская, студ.,
[1]А. Котлярова, канд. биол. наук
[1]Институт физиологии имени О. О. Богомольца НАН Украины, Киев, Украина,
[2]Киевский национальный университет имени Тараса Шевченко, Киев, Украина

РЕГИСТРАЦИЯ ИОННЫХ ТОКОВ СКВОЗЬ LCC-КАНАЛЫ ЯДЕРНОЙ МЕМБРАНЫ: ХРОНОБИОЛОГИЧЕСКИЙ АСПЕКТ

На протяжении семи лет исследований транспортных систем ядерных мембран с применением метода patch-clamp наблюдалась определенная закономерность: зимой эффективность работы этим методом значительно снижалась. Поскольку разные сезоны/времена года характеризуются различными световыми и температурными показателями, мы решили обратить внимание на возможное воздействие последних на успешность выполнения исследований. Целью этой работы стала проверка влияния таких сезонных факторов, как изменение продолжительности светового дня, температура, атмосферное давление, количество осадков и облачность на качество patch-clamp-регистраций ионных токов через LCC-каналы ядерной мембраны кардиомиоцитов и нейронов Пуркинье мозжечка. Было выдвинуто предположение, что с уменьшением продолжительности светового дня и понижением температуры уменьшаются качественные и количественные показатели patch-clamp-регистраций. Для проверки этого предположения был применен корреляционный анализ Пирсона с исходными данными о продолжительности светового дня, метеорологических условиях и рассчитанной успеваемостью регистраций (%) за конкретный день. На основе результатов такого анализа было установлено, что существует прямая выраженная линейная зависимость качества и количества регистраций от продолжительности светового дня (r = 0,6) и температуры (r = 0,6), а также слабая обратная зависимость от облачности (r = 0,3). На основе дисперсионного анализа (ANOVA) подтверждено достоверно большую успешность регистраций, выполненных в летний период, по сравнению с зимними того же года. Полученные результаты могут стать основой для оптимизации исследовательской деятельности рабочих групп, изучающих функционирование внутриклеточных транспортных систем электрофизиологическими методами, в частности patch-clamp.

Ключевые слова: биоритмы, хронобиология, метеорологические условия, patch-clamp, ядерная мембрана, ионные каналы, LCC-каналы.

[1,2] O. Tarnopolska, stud.,
[1]A. Kotliarova, PhD
[1]Bogomolets Institute of Physiology NASU, Kyiv, Ukraine,
[2]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

ION CURRENTS REGISTRATION THROUGH LCC-CHANNELS OF THE NUCLEAR MEMBRANES: CHRONOBIOLOGICAL ASPECT

For seven years of researching the transport systems of nuclear membranes using the patch-clamp method, we observed a certain pattern: in winter, this method's efficiency significantly decreased. Since different seasons are characterized by different light and temperature indicators, we decided to pay attention to the latter's possible impact on the success of the research. Therefore, the purpose of this work was to test the influence of seasonal factors such as changes in daylight hours, temperature, atmospheric pressure, precipitation, and cloudiness on the quality of patch-clamp recordings of ion currents through the LCC channels of the nuclear membrane of cardiomyocytes and cerebellar Purkinje neurons. We assumed that with decreasing daylength and decreasing temperature, the patch-clamp registrations' qualitative and quantitative indicators also decrease. We applied Pearson's correlation analysis with initial data on daylight hours, meteorological conditions, and calculated progress of registrations (%) for a specific day to test this assumption. Based on the results of this analysis, we found out that there is a direct pronounced linear dependence of the quality and number of registrations on the length of daylight hours (r = 0.6) and temperature (r = 0.6), as well as a weak inverse dependence on cloudiness (r = 0.3). Analysis of variance (ANOVA) also confirmed a significantly greater success of registrations performed in the summer compared to the winter of the same year. The obtained results can become the basis for optimizing the research activities of working groups studying intracellular transport systems' functioning by electrophysiological methods, in particular, patch-clamp.

Keywords: biorhythms, chronobiology, meteorological conditions, patch-clamp, nuclear membrane, ion channels, LCC channels.

UDK 57.084.1:615.357:616-091.83:591.881
DOI 10.17721/1728_2748.2020.83.17-23

O. Kalmukova, PhD,
M. Dzerzhynsky, Dr. Sci.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

MORPHO-FUNCTIONAL STATE OF RATS PINEAL GLAND AND SUPRACHIASMATIC NUCLEUS OF HYPOTHALAMUS AFTER DIFFERENT REGIMES OF EXOGENOUS MELATONIN ADMINISTRATION

In modern society increase of digitalization associated with grown exceed level of light at night – a new type of pollution. Presence of light at night inhibited endogenous melatonin synthesis by pineal gland, that influence on circadian system work cycles, so organism oftenbroken regime of wake/sleep, meals, physical activity. Also, a lack of melatonin in some certain time of dayand low melatonin concentration both, were shown take some intervention in diseases development through incorrect regulation of clock-depended genes expression. In connect with this, some latest clinical protocol in therapy or clinical trials of many different pathologies (for example, insomnia, metabolic syndrome, cardiovascular diseases, central nervous and immune system trouble, cancer, viral infection, etc.) include exogenous melatonin usage. As melatonin perform his function via endocrine and paracrine ways in variety types of cell, his application take place in wide range of doses and in different time of day (chronotherapeutic approach). Therefore, important to control state of circadian system central elements – pineal gland (main producer of endogenous melatonin) and suprachiasmatic nucleus (SCN) of hypothalamus (central pacemaker of circadian rhythm) in conditionsof exogenous melatonin treatment. Thus, the main goal of our research were analysis of rats pineal gland and hypothalamic SCN morpho-functional state after different time (morning, evening and continuously with drinking water) melatonin daily administration. Melatonin was administered by gavage for 7 weeks in dose 30 mg/kg 1 h before lights-off (M ZT11, evening), or 1 h after lights-on (M ZT01, morning), or continuously with drinking water during day-night period (MW). After melatonin use only in MW group pineal gland demonstrates changes in morphology (pinealocytes nucleus had mild basophilic color) and morphometric (increased cross-sectional area of the pinealocytes nucleus in compare with control group) analysis data. Besides, some similar changes were observed in SCN: the cross-sectional area of the SCN neurons nucleus grown in case of usage each of regime melatonin administration, while morphology characteristic remains without any alteration. In general, it suggesting about having by melatonin non-inhibiting features in context of circadian system feedback loop and supposing wide potential for melatonin use with absent huge side effect on central elements of above mentioned system.

Keywords: melatonin, feedback loop, chronobiology, pinealocytes, neuron, histology, circadian system, side effect.

Introduction. Melatonin – the main signal molecule of circadian system, pineal gland hormone, that has pleiotropic effect on organism insofar as may influence at most different cells in tissue [1]. This action mediated by both: 1) presentation in many cells melatonin's receptors (through membrane receptors 1 MT_1 and 2 types MT_2 [2],

cytosolic receptors 3 MT_3[3]); and 2) orchestration of circadian molecular clock machinery work in each individualcell (through activation RORα1 (retinoid-related orphan receptors), RORα2 and RZRα [4]). Also melatonin and itsderivatives may act directly as antioxidant by bounding active form of oxygen radicals [5]. In addition, perform a huge role in sensing and transform signal to organism about seasonal changing during year; thereby regulatingseasonal reproduction, migration, metabolic rebuilding etc. [6].

Nowadayshad been approved clinical application of melatonin for cure the delayed sleep phase disorder (includingin totally blind or sighted patients), jet-lagin case of circadian system dysfunction (wake-sleep regimes) [7]. Intriguing, melatonin enter in some critical care COVID-19 management protocol as one of necessary component in "cocktail" for prophylaxis and mitigation mildly symptomatic patient [8] (at home and in hospital too, for example, in Eastern Virginia Medical School).

Due to one of the environment features of modern society, characterized by urbanization and infrastructure development; there is the appearance of excessive light at night – the latest type of pollution; most of human had decreased endogenous melatonin level [9]. Also violation of the time regime of meals, wake\sleep, physical activity, circadian rhythms of the body leads to the destruction of melatonin synthesis time-pattern → cyclic expression of temporal genes and, accordingly, genes that are regulated by them – receptors, enzymes, transcription factors, hormones, cytokines [10]. In this connection these factors contribute to the etiology of development many different pathologies. Therefore now, melatonin action study in clinical trials and in experimental models for treat obesity and obesity-related disorders [11], mood deviation (depression, suicide, autism etc.) [12], diabetes [13], cancers [14], cardio-vascular diseases [15], central nervous system pathologies (bipolar disorders, Parkinson disease, Huntington disease, and Alzheimer's disease) [16], pathologies with inflammation process [17].

World FDA organization (Food & Drug Administration) categorized melatonin as dietary supplements, not drugs, since melatonin can be found in many food products (especially plants – phytomelatonin), thou melatonin usage FDA does not regulate [18, 19]. Many researches supported that melatonin had not any side effects in case of usage wide range of dose (0,1 mg/kg – 100 mg/kg) [20, 21]. Moreover, in case of melatonin use, it's necessary totake account of melatonin intervention time, because there aredifferent sensitivity (density, affinity) of melatonin receptors during the day – chrobiological approach an integral part in melatonin therapy [22].

The circadian system for the most partconsists of hypothalamic suprachiasmatic nucleus (SCN) and pineal gland [23]. Pineal gland is the main source of melatonin synthesis, also enterochromaffin cells of intestine contributesin basal melatonin level [24]. The SCN coordinate inputs of environment inoutputs to organism – central pacemaker [25].

So, the main aim of research were analysis of rats pineal gland and hypothalamic SCN morpho-functional state after different time (morning, evening and continuously with drinking water) melatonin daily administration in dose 30 mg/kg during 7 weeks.

Materials and methods. White nonlinear male rats weighing 100 ± 10 g were used in this study. The light cycle was 12-h light and 12-h darkness, with lights-on at 07:00 h (Zeitgeber time, ZT: ZT00) andlights-off at 19:00 h (ZT12). All experiments on animals were carried out in compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (European Convention, Strasburg, 1986), Article 26 of the Law of Ukraine "On the Protection of Animals from Cruelty" (№ 3447-IV, February 21, 2006) as well as all norms of bioethics and biological safety.

All animals received standard rodent chow (15,27 $kJ \cdot g^{-1}$). Food and water were available ad libitum. Animals kept under standard vivarium conditions with constant temperature and humidity. Rats were divided into four groups:

1. Control group without any inductions of melatonin.
2. Group M ZT01 received melatonin in the morning 1 hour after light-on.
3. GroupM ZT11 obtained melatonin administrations1 h before light-off.
4. Group MW (**M**elatonin **W**ater) consumed melatonin solutions continuously with drinking water during all period of experiment.

Thus, the experimental groups are indicated below as: control, M ZT01, M ZT11 and MW.

Melatonin (Alcon Biosciences, USA) was administered daily by single peroral by gavage introductions 2 mL as a solution (group M ZT01 and M ZT11) or constantly with drinking water (the dose required was dissolved in 25–30 mL according to the calculated daily average volume of drinking water consumption per animal [26]) for 7 week (30 mg/kg).

For the treatment of many experimental diseases models [27] and also in case of clinical trials [28], the use of different doses, methods and times of melatonin administration is shown. For the melatonin adverse effects testing was chosen the lowest dose of melatonin at the use of which observed both a simultaneous decrease obese rat's weight gain and the appearance of beige adipocytes, as we are interested in obesity therapy throughbeige adipocyte activation.

On the last day of the experiment, the animals were sacrificedby carbon dioxide asphyxiation and decapitated, and then the pineal gland and hypothalamus was isolated.

Histological examination was performed to characterize the morphology and functional status of pineal gland and suprachiasmatic nucleus of hypothalamus (SCN). Pineal gland were fixed entirely, while whole brain had been collected and previously sectioned in lateral sites throe third ventricles (for rapid fixation process) to obtain fragments in the size of 10 x 10 mm. Tissues were fixed in 4 % of paraformaldehyde in 0.1 M phosphate buffer for 72 hours, after which they were dehydrated and embedded into paraffin according to a standard procedure. From the paraffin blocks, 5 μm sections were performed and stained with Bemer'shematoxylin and eosin (H&E). Five-μm-thick slices were prepared from such blocks; these slices included the SCN, borders of which were identified according to the stereotaxic atlas [29]. Further examination of sections was performed using a light microscope BX41 (Olympus, Japan). Microphotographs were taken using the DP20 (Olympus, Japan) digital camera and the QuickPHOTO MICRO software (Promicra, Czech Republic).

The cross-sectional area of the pinealocytes nucleus were used as criteria for assessing the morphology and functional status of the pineal gland; and the cross-sectional area of the SCN neurons nucleus was used to evaluation the state of the hypothalamus SCN. All parameters were measured using the ImageJ software (National Institutes of Health, USA).

Statistical data analysis was performed using the Statistica 6.0 (StatSoft, USA) and Microsoft Excel 2010 software (Microsoft, USA). The obtained data was presented as mean ± standard error of mean (SEM). The distribution of values was estimated using Shapiro-Wilknormality W-test. Since the deviation of these values distribution of

from the normality was minor, to evaluate the differences between the values was used by one way ANOVA (analysis of variance) and tested by Dunnet's multiple range test with control as post hoc test. The differences with probability of the null hypothesis $p < 0.05$ were considered significant.

Results and discussion. In order to create a complete picture of the exogenous melatonin action at a dose of 30 mg/kg in different regimes of daily administration to important targets onto circadian rhythms regulation systems of body, the morpho-functional state of the pineal gland was studied.

The pineal gland consists of 2 types of cells: pinealocytes (synthesizing melatonin) and neuroglial supporting cells (**Fig. 1**). Pinealocytes differ from neuroglial cells by larger size (**Fig. 1, red arrow**), the presence of long cytoplasmic processes reaching the capillaries (**Fig. 1, green arrow**). Neuroglial cells have a small dark-colored nucleus (**Fig. 1, triangle**), unlike pinealocytes. Pinealocytes are modified neurons that have rounded or ovoid nuclei with a pale cytoplasm that contain granules filled with melatonin. With the melatonin introduction under the conditions of all 3 regimes (M ZT01, M ZT11 and MW) the shape of the nucleus did not change, there was a constant presence of the nucleolus and moderate basophilia (**Fig. 2)**. Cytoplasm conserved brightacidophilic color in M ZT01, M ZT11 and MWgroup that similar to control group. Extracellular accumulations of calcified sediment (brain sand) in the form of conglomerates were not detected in all experimental groups.In some cases, were observed difference in pinealocytes nucleus color between control and MW group: lighter basophilic nucleuses were commonly found in MW group, while in control there had frequently medium shade of color. Both population light and dark pinealocytes had classic morphology and did not demonstrate marked deviation [30].

Morphometric analysis of the cross-sectional area of pinealocytes nucleus showed no effect after melatonin administration in groups M ZT01 and M ZT11, but in group MW this parameter increase by 13,5 % in compare with control group, which indicates about mild stimulating effect of consume melatonin continuously with drinking water (**Fig. 3)**.

Pinealocytes have autocrine mechanisms activation by melatonin, as was shown presentence MT_1 and MT_2 in their own membrane [31]. Weak blocking of morpho-functional activity by exogenous melatonin is connecting with specific regulation of melatonin synthesis, which mediated thought removal of the light blocking action at night [32].

Also, there are no unique data about endogenous melatonin blood concentration after use exogenous melatonin during short or long administration. Ambiguous data presented about melatonin maximal plasma/serum concentration after pharmacokinetics analysis due to different melatonin dose/administration route [33].

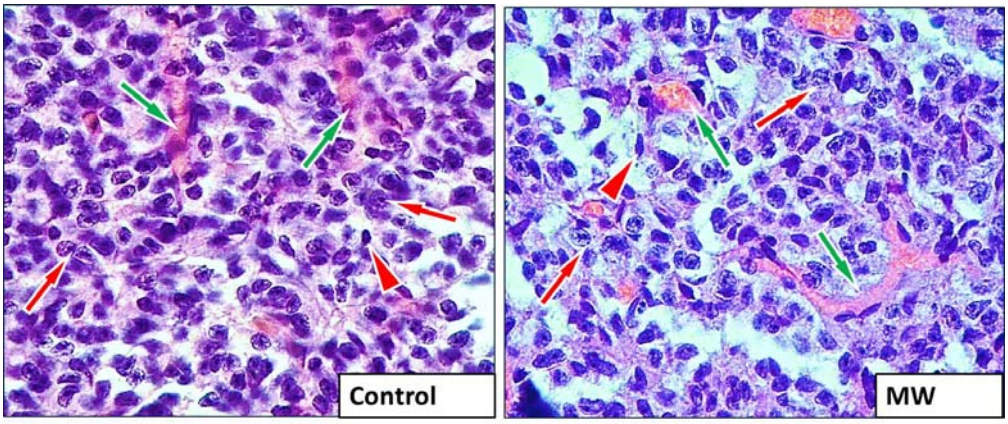

Fig. 1. Microphotographs of rats' pineal gland sections: H&E staining; × 900 magnifications.
Notes: red arrow – pinealocytes nucleus, triangle – nucleus of neuroglial cells, green arrow –blood vessel

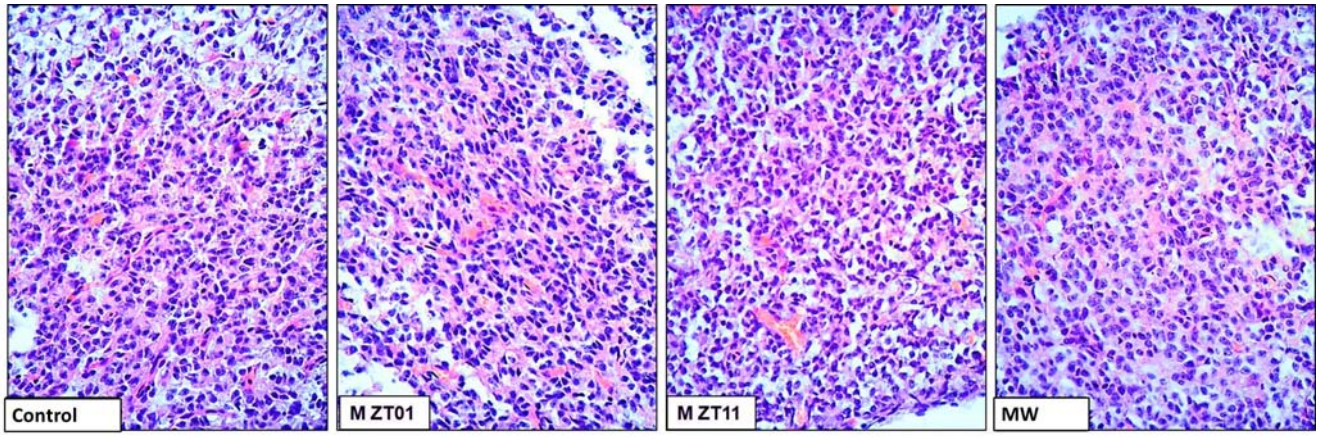

Fig. 2. Microphotographs of rats' pineal gland sections: H&E staining; × 400 magnifications

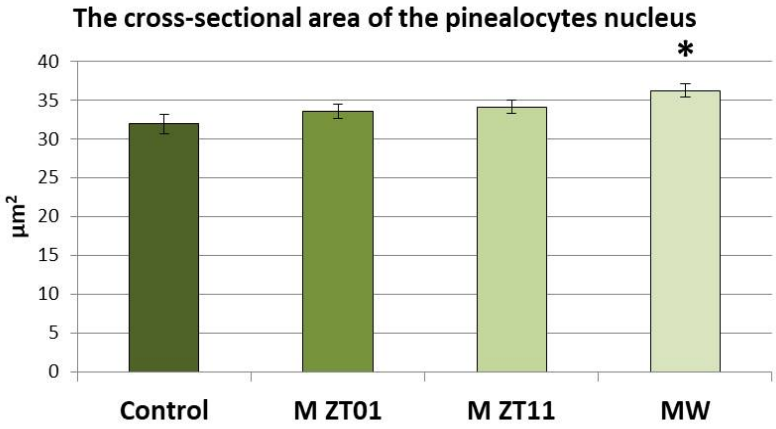

Fig. 3. The morphometric analysis data of the rats' pineal gland.
Notes: * – a significant difference between the control and experimental groups, p ≤ 0.05

The morpho-functional state of SCN neurons of the hypothalamus under the conditions of exogenous melatonin administration was also analyzed (**Fig. 4).** The study of the SCN neurons morphology of the hypothalamus shown that in all groups M ZT01, M ZT11 and MW, the shape of the nuclei remained unchanged – rounded with clear boundaries, the present nucleolus and moderate slightly light basophilia.The perikaryonscytoplasm of neurons in these groups showed weakly acidophilic properties in comparison with the surrounding nerve fibers and glial cells as in control group.

Morphometric calculation of the cross-sectional area of the SCN neurons nucleus (**Fig. 5**) revealed an increase in this parameter after the use of melatonin in M ZT01 by 57 %, in M ZT11 by 21,5 % and in MW by 18,5 % in compare with control group.Also was shown difference between effects after different mode of administration: in compare with MW group the cross-sectional area of the SCN neurons nucleus rise in M ZT01 group by 33 %, while in M ZT11 this parameter did not differ. Taking into account the data of morphological observations and morphometric analysis shows the activating effect of melatonin on the SCN neurons of the hypothalamus.

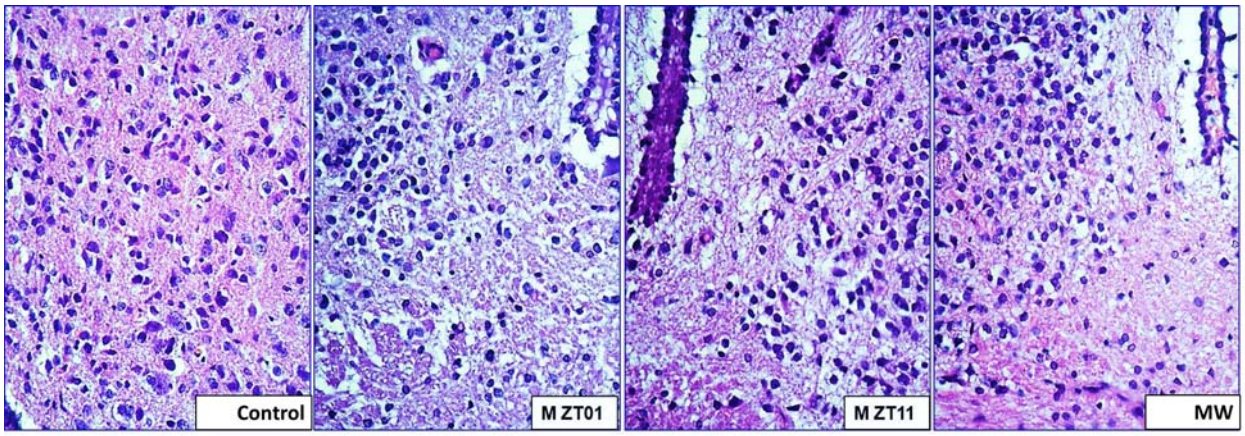

Fig. 4. Microphotographs of rats' hypothalamus SCN sections: H&E staining; ×400 magnifications

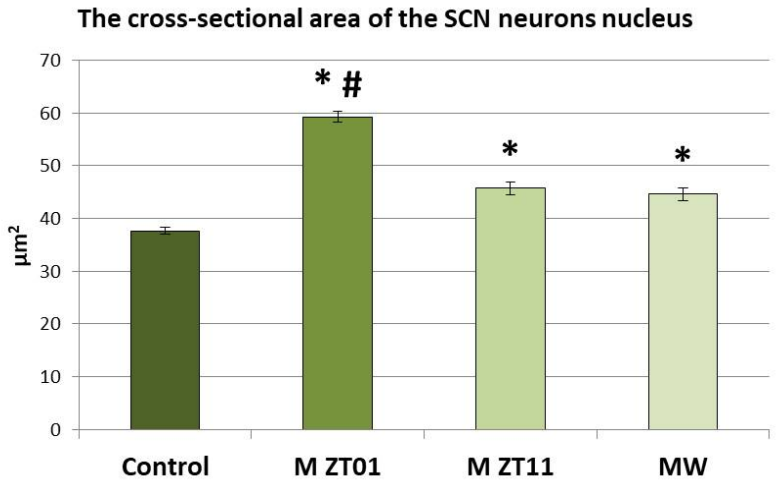

Fig. 5. The morphometric analysis data of the rats' SCN neurons of hypothalamus.
Notes: * – a significant difference between the control and experimental groups, p ≤ 0.05;
– a significant difference between the MW group and M ZT01, M ZT11, p ≤ 0.05

Obtained data are according with SCN-melatonin feedback loop mechanism, as SCN has high concentration of melatonin receptors for entrained and phase-shifting setting own pacemaker working rhythm [34, 35]. Thus, melatonin administrations in dose 30 mg/kg can activate nuclear synthesis, that manifested in grow cross-sectional area of the SCN neurons nucleus. Moreover, in group M ZT11 and MW changes of these parameters did not differ, insofar as rats are nocturnal animals, which consume water mostly at night – MW group – and time of exogenous melatonin peak is approximatelysimilar like in M ZT11 group. While in M ZT01 group striking increaseof cross-sectional area of the SCN neurons nucleuscan be explained by the time of animal's slaughter: they were held in the first half of the day – shortly after the melatonin introduction. Morphology variation during the day of the SCN neuron happen not only with nucleus, also was shown changes in total protrusion and simple dendritic spine density [36]. In the other hand, rise can be provoked by continuation of the night melatoninpeakthrough morning administration [37]. In some cases, out of timechronotherapy can lead to desynchrony and circadian disruption [38], but in human occur a few chronotypes: early and late, taking into account is necessary during treatment [39, 40]. In according of this, morning administration takes place to be in patient with early chronotype.

Conclusions. Daily administration of exogenous melatonin (30 mg/kg, 7 weeks) in different time of day did not inhibitthe activity of such links of the circadian system as pineal gland and suprachiasmatic nucleus of hypothalamus. Furthermore, application melatonin solutions continuously with drinking water during all period of experiment (group MW) lead to mild activation of morpho-functional state of pineal gland (pinealocytes nucleus had lighter basophilic color and cross-sectionalarea of nucleus increase) and of suprachiasmatic nucleus of hypothalamus (cross-sectionalarea of SNC neurons nucleus also grown). In addition, morning (1 hour after light-on, group M ZT01) and evening (1 hour before light-off, group M ZT11) regimes of melatonin administrations conduct in enhance the cross-sectionalarea of SNC neurons nucleus too, but not of pinealocytes nucleus. Thereby, different modes of exogenous melatonin applications rats in dose 30 mg\kg during 7 weeks did not demonstrate negative feedback loop manifestation on morpho-function state of pineal gland and suprachiasmatic nucleus of hypothalamus.

Reference

1. Hardeland R. Melatonin–A pleiotropic, orchestrating regulator molecule / R. Hardeland, DP Cardinali, V. Srinivasan[et al.] //Progress in neurobiology. – 2011. – Vol. 93, №. 3. – P. 350-384.
2. Dubocovich M. L. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors / ML Dubocovich, P Delagrange, DN Krause [et al.] //Pharmacological reviews. – 2010. – Vol. 62, №. 3. – P. 343-380.
3. Nosjean O. Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2 /O. Nosjean, JP. Nicolas, F. Klupsch[et al.] //Biochemical pharmacology. – 2001. – Vol. 61, №. 11. – P. 1369-1379.
4. Jetten A. M. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism / A. M. Jetten//Nuclear receptor signaling. – 2009. – Vol. 7, №. 1. – P. nrs. 07003.
5. Zhang H. M., Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions / H. M. Zhang, Y. Zhang //Journal of pineal research. – 2014. – Vol. 57, №. 2. – P. 131-146.
6. Baekelandt S. Seasonal simulated photoperiods influence melatonin release and immune markers of pike perch Sander lucioperca/ S. Baekelandt, S. Milla, V. Cornet [et al.] //Scientific Reports. – 2020. – Vol. 10, №. 1. – P. 1-10.
7. Cipolla-Neto J., Amaral F. G. Melatonin as a hormone: new physiological and clinical insights / J. Cipolla-Neto, F. G. Amaral//Endocrine Reviews. – 2018. – Vol. 39, №6. – P. 990-1028.
8. Romero A. Coronavirus Disease 2019 (COVID-19) and its neuroinvasive capacity: Is it time for melatonin? / A. Romero, E. Ramos, F. López-Muñoz [et al.] //Cellular and molecular neurobiology. – 2020. – P. 1-12.
9. Russart K. L. G., Nelson R. J. Light at night as an environmental endocrine disruptor / K. L. G.Russart, R. J. Nelson //Physiology & behavior. – 2018. – Vol. 190. – P. 82-89.
10. Tahara Y., Shibata S. Circadian rhythms of liver physiology and disease: experimental and clinical evidence / Y.Tahara, S. Shibata //Nature Reviews Gastroenterology and Hepatology. – 2016. – Vol. 13, №4. – P. 217.
11. Genario R. Melatonin supplementation in the management of obesity and obesity-associated disorders: a review of physiological mechanisms and clinical applications / R. Genario, J. Cipolla-Neto, AA Bueno[et al.] //Pharmacological Research. – 2020. – P. 105254.
12. De Crescenzo F. Melatonin as a treatment for mood disorders: a systematic review / F. De Crescenzo, A. Lennox, JC Gibson [et al.] //ActaPsychiatricaScandinavica. – 2017. – Vol. 136, №6. – P. 549-558.
13. Karamitri A., Jockers R. Melatonin in type 2 diabetes mellitus and obesity / A.Karamitri, R. Jockers//Nature Reviews Endocrinology. – 2019. – Vol. 15, №2. – P. 105-125.
14. Li Y. Melatonin for the prevention and treatment of cancer / Y. Li, S. Li, Y. Zhou [et al.] //Oncotarget. – 2017. – Vol.8, №. 24. – P. 39896.
15. Pourhanifeh M. H. Clinical Application of Melatonin in the Treatment of Cardiovascular Diseases: Current Evidence and New Insights into the Cardioprotective and Cardiotherapeutic Properties / MH Pourhanifeh, E. Dehdashtian, A. Hosseinzadeh[et al.] //Cardiovascular Drugs and Therapy. – 2020. – P. 1-25.
16. Chen D., Zhang T., Lee T. H. Cellular Mechanisms of Melatonin: Insight from Neurodegenerative Diseases / D. Chen, T. Zhang, T. H. Lee //Biomolecules. – 2020. – Vol. 10, №8. – P. 1158.
17. Bondy S. C., Campbell A. Melatonin and Regulation of Immune Function: Impact on Numerous Diseases. – / S. C. Bondy, A. Campbell // Curr Aging Sci. – 2020.
18. Savage RA, Zafar N, Yohannan S, Miller JMM. Melatonin. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; August 21, 2020.
19. Cerezo A. B. Quality control and determination of melatonin in food supplements / A. B. Cerezo, Á. Leal, M. A. Álvarez-Fernández[et al.]//Journal of food composition and Analysis. – 2016. – Vol. 45. – P. 80-86.
20. Soni S. K., Kumar D., Singaravel M. Melatonin-induced phase and dose responses in a diurnal mammal, Funambuluspennantii/ S. K. Soni, D. Kumar, M. Singaravel//Chronobiology International. – 2020. – Vol.37, №5. – P. 641-651.
21. Valizadeh M. Expression levels of two dna repair-related genes under 8 gy ionizing radiation and 100 mg/kg melatonin delivery in rat peripheral blood / M. Valizadeh, A. Shirazi, P. Izadi[et al.]//Journal of biomedical physics & engineering. – 2017. – Vol. 7, №1. – P. 27-36.
22. Pinato L. Day/night expression of MT1 and MT2 receptors in hypothalamic nuclei of the primate Sapajusapella/ L. Pinato, D. Ramos, A. Hataka[et al.]//Journal of chemical neuroanatomy. – 2017. – Vol. 81. – P. 10-17.
23. Dijk D. J., Duffy J. F. Novel approaches for assessing circadian rhythmicity in humans: A review / D. J. Dijk, J. F. Duffy //Journal of Biological Rhythms. – 2020. – Vol. 35, №. 5. – P. 421-438.
24. Tan D. X., Manchester L. C., Reiter R. J. CSF generation by pineal gland results in a robust melatonin circadian rhythm in the third ventricle as an unique light/dark signal / D. X. Tan, L. C. Manchester, R. J. Reiter //Medical hypotheses. – 2016. – Vol. 86. – P. 3-9.
25. Herzog E. D. Regulating the suprachiasmatic nucleus (SCN) circadian clockwork: interplay between cell-autonomous and circuit-level mechanisms / ED Herzog, T. Hermanstyne, NJ Smyllie[et al.]//Cold Spring Harbor perspectives in biology. – 2017. – Vol. 9, №1. – P. a027706.
26. Kalmukova O. Effect of melatonin different time administration on the development of diet-induced obesity in rats / O.Kalmukova, A.Pustovalov, I.Vareniuk[et al.]//Bulletin of Taras Shevchenko National University of Kyiv-Problems of Physiological Functions Regulation. – 2018. – Vol. 23, №. 2. – P. 20-27.
27. Szewczyk-Golec K., Woźniak A., Reiter R. J. Inter-relationships of the chronobiotic, melatonin, with leptin and adiponectin: implications for obesity / K.Szewczyk-Golec, A.Woźniak, R. J.Reiter //Journal of pineal research. – 2015. – Vol.59, №. 3. – P. 277-291.
28. Foley H. M., Steel A. E. Adverse events associated with oral administration of melatonin: A critical systematic review of clinical evidence / H. M.Foley, A. E. Steel //Complementary therapies in medicine. – 2019. – Vol. 42. – P. 65-81.
29. Paxinos G., Watson C. The rat brain in stereotaxic coordinates: hard cover edition. – Elsevier, 2006.
30. Manno F. A. M., Lau C. The pineal gland of the shrew (Blarinabrevicauda and Blarinacarolinensis): a light and electron microscopic study of pinealocytes / F. A. M.Manno, C.Lau //Cell and tissue research. – 2018. – Vol. 374, №3. – P. 595-605.
31. Brunner P. Pineal and cortical melatonin receptors MT1 and MT2 are decreased in Alzheimer's disease /P. Brunner, N. Sözer-Topcular, R. Jockers[et al.]//European Journal of Histochemistry. – 2006. – Vol.50, №4. – P. 311-316.
32. Gorman M. R. Temporal organization of pineal melatonin signaling in mammals / M. R. Gorman //Molecular and Cellular Endocrinology. – 2020. – Vol. 503. – P. 110687.

33. Harpsøe N. G. Clinical pharmacokinetics of melatonin: a systematic review /NGHarpsøe, LPAndersen, IGögenur[et al.]//European journal of clinical pharmacology. – 2015. – Vol. 71, №8. – P. 901-909.

34. Liu C. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock / C. Liu, DR Weaver, X. Jin [et al.] //Neuron. – 1997. – Vol. 19, №1. – P. 91-102.

35. Kandalepas P. C., Mitchell J. W., Gillette M. U. Melatonin signal transduction pathways require E-box-mediated transcription of Per1 and Per2 to reset the SCN clock at dusk / P. C. Kandalepas, J. W. Mitchell, M. U. Gillette //PLoS One. – 2016. – Vol. 11, №6. – P. e0157824.

36. Mendoza-Viveros L. miR-132/212 modulates seasonal adaptation and dendritic morphology of the central circadian clock / L. Mendoza-Viveros, CK Chiang, JLK Ong[et al.]//Cell reports. – 2017. – Vol. 19, №3. – P. 505-520.

37. Reiter R. J., Rosales-Corral S., Sharma R. Circadian disruption, melatonin rhythm perturbations and their contributions to chaotic physiology / R. J. Reiter, S. Rosales-Corral, R. Sharma //Advances in medical sciences. – 2020. – Vol. 65, №2. – P. 394-402.

38. Abbott S. M., Malkani R. G., Zee P. C. Circadian disruption and human health: A bidirectional relationship / S. M. Abbott, R. G. Malkani, P. C. Zee //European Journal of Neuroscience. – 2020. – Vol. 51, №. 1. – P. 567-583.

39. Dallaspezia S. Chronotype influences response to antidepressant chronotherapeutics in bipolar patients /Dallaspezia S, Suzuki M, Clara L [et al.]//Chronobiology International. – 2018. – Vol. 35, №9. – P. 1319-1325.

40. Aulinas A. Physiology of the Pineal Gland and Melatonin //Endotext [Internet]. – MDText. com, Inc., 2019.

Reference (Scopus)

1. Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin--a pleiotropic, orchestrating regulator molecule. ProgNeurobiol. 2011 Mar;93(3):350-84. doi: 10.1016/j.pneurobio.2010.12.004. Epub 2010 Dec 28. PMID: 21193011.

2. Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. Pharmacol Rev. 2010 Sep;62(3):343-80.

3. Nosjean O, Nicolas JP, Klupsch F, Delagrange P, Canet E, Boutin JA. Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2 .BiochemPharmacol. 2001 Jun 1;61(11):1369-79.

4. Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. NuclRecept Signal. 2009;7:e003.

5. Zhang HM, Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. J PinealRes. 2014 Sep;57(2):131-46.

6. Baekelandt S, Milla S, Cornet V, Flamion E, Ledoré Y, Redivo B, Antipine S, Mandiki SNM, Houndji A, El Kertaoui N, Kestemont P. Seasonal simulated photoperiods influence melatonin release and immune markers of pike perch Sander lucioperca. Sci Rep. 2020 Feb 14;10(1):2650. doi: 10.1038/s41598-020-59568-1. PMID: 32060347; PMCID: PMC7021833.

7. Cipolla-Neto J, Amaral FGD. Melatonin as a Hormone: New Physiological and Clinical Insights. Endocr Rev. 2018 Dec 1;39(6):990-1028. doi: 10.1210/er.2018-00084. PMID: 30215696.

8. Romero A, Ramos E, López-Muñoz F, Gil-Martín E, Escames G, Reiter RJ. Coronavirus Disease 2019 (COVID-19) and Its Neuroinvasive Capacity: Is It Time for Melatonin? Cell MolNeurobiol. 2020 Aug 9:1–12. doi: 10.1007/s10571-020-00938-8. Epub ahead of print. PMID: 32772307; PMCID: PMC7415199.

9. Russart KLG, Nelson RJ. Light at night as an environmental endocrine disruptor. PhysiolBehav. 2018 Jun 1;190:82-89. doi: 10.1016/j.physbeh.2017.08.029. Epub 2017 Sep 7. PMID: 28870443; PMCID: PMC5839924.

10. Tahara Y, Shibata S. Circadian rhythms of liver physiology and disease: experimental and clinical evidence. Nat Rev GastroenterolHepatol. 2016 Apr;13(4):217-26. doi: 10.1038/nrgastro.2016.8. Epub 2016 Feb 24. PMID: 26907879.

11. Genario R, Cipolla-Neto J, Bueno AA, Santos HO. Melatonin supplementation in the management of obesity and obesity-associated disorders: A review of physiological mechanisms and clinical applications. Pharmacol Res. 2020 Oct 17:105254. doi: 10.1016/j.phrs.2020.105254. Epub ahead of print. PMID: 33080320.

12. De Crescenzo F, Lennox A, Gibson JC, Cordey JH, Stockton S, Cowen PJ, Quested DJ. Melatonin as a treatment for mood disorders: a systematic review. ActaPsychiatr Scand. 2017 Dec;136(6):549-558. doi: 10.1111/acps.12755. Epub 2017 Jun 14. PMID: 28612993.

13. Karamitri A, Jockers R. Melatonin in type 2 diabetes mellitus and obesity. Nat Rev Endocrinol. 2019 Feb;15(2):105-125. doi: 10.1038/s41574-018-0130-1. PMID: 30531911.

14. Li Y, Li S, Zhou Y, Meng X, Zhang JJ, Xu DP, Li HB. Melatonin for the prevention and treatment of cancer. Oncotarget. 2017 Jun 13;8(24):39896-39921. doi: 10.18632/oncotarget.16379. PMID: 28415828; PMCID: PMC5503661.

15. Pourhanifeh MH, Dehdashtian E, Hosseinzadeh A, Sezavar SH, Mehrzadi S. Clinical Application of Melatonin in the Treatment of Cardiovascular Diseases: Current Evidence and New Insights into the Cardioprotective and Cardiotherapeutic Properties. Cardiovasc Drugs Ther. 2020 Sep 14. doi: 10.1007/s10557-020-07052-3. Epub ahead of print. PMID: 32926271.

16. Chen D, Zhang T, Lee TH. Cellular Mechanisms of Melatonin: Insight from Neurodegenerative Diseases. Biomolecules. 2020 Aug 7;10(8):1158. doi: 10.3390/biom10081158. PMID: 32784556; PMCID: PMC7464852.

17. Bondy SC, Campbell A. Melatonin and Regulation of Immune Function: Impact on Numerous Diseases. Curr Aging Sci. 2020 Jul 11. doi: 10.2174/1874609813666200711153223. Epub ahead of print. PMID: 32651969.

18. Savage RA, Zafar N, Yohannan S, Miller JMM. Melatonin. 2020 Aug 21. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan–. PMID: 30521244.

19. Cerezo, A. B., Leal, Á.,Álvarez-Fernández, M. A., Hornedo-Ortega, R., Troncoso, A. M., &García-Parrilla, M. C. (2016). Quality control and determination of melatonin in food supplements. *Journal of food composition and Analysis*, *45*, 80-86.

20. Soni SK, Kumar D, Singaravel M. Melatonin-induced phase and dose responses in a diurnal mammal, *Funambuluspennantii*. Chronobiol Int. 2020 May;37(5):641-651. doi: 10.1080/07420528.2020.1758127. Epub 2020 Apr 30. PMID: 32349560.

21. Valizadeh M, Shirazi A, Izadi P, TavakkolyBazzaz J, Rezaeejam H. Expression Levels of Two DNA Repair-related Genes under 8 Gy Ionizing Radiation and 100 Mg/Kg Melatonin Delivery In Rat Peripheral Blood. J Biomed Phys Eng. 2017 Mar 1;7(1):27-36. PMID: 28451577; PMCID: PMC5401131.

22. Pinato L, Ramos D, Hataka A, Rossignoli PS, Granado MD Junior, Mazzetto MC, Campos LMG. Day/night expression of MT_1 and MT_2 receptors in hypothalamic nuclei of the primate Sapajusapella. J ChemNeuroanat. 2017 Apr;81:10-17. doi: 10.1016/j.jchemneu.2017.01.005. Epub 2017 Jan 31. PMID: 28159659.

23. Dijk DJ, Duffy JF. Novel Approaches for Assessing Circadian Rhythmicity in Humans: A Review. J Biol Rhythms. 2020 Oct;35(5):421-438. doi: 10.1177/0748730420940483. Epub 2020 Jul 23. PMID: 32700634; PMCID: PMC7543025.

24. Tan DX, Manchester LC, Reiter RJ. CSF generation by pineal gland results in a robust melatonin circadian rhythm in the third ventricle as an unique light/dark signal. Med Hypotheses. 2016 Jan;86:3-9. doi: 10.1016/j.mehy.2015.11.018. Epub 2015 Nov 26. PMID: 26804589.

25. Herzog ED, Hermanstyne T, Smyllie NJ, Hastings MH. Regulating the Suprachiasmatic Nucleus (SCN) Circadian Clockwork: Interplay between Cell-Autonomous and Circuit-Level Mechanisms. Cold Spring HarbPerspect Biol. 2017 Jan 3;9(1):a027706. doi: 10.1101/cshperspect.a027706. PMID: 28049647; PMCID: PMC5204321.

26. Kalmukova, O., Pustovalov, A., Vareniuk, I., and Dzerzhynsky, M., Effect of melatonin different time administration on the development of diet-induced obesity in rats, Bull. Taras Shevchenko Nat. Univ. Kyi – Probl. Physiol. Func. Regul., 2018, vol. 23, no. 2, pp. 20–27.

27. Szewczyk-Golec K, Woźniak A, Reiter RJ. Inter-relationships of the chronobiotic, melatonin, with leptin and adiponectin: implications for obesity. J Pineal Res. 2015 Oct;59(3):277-91. doi: 10.1111/jpi.12257. Epub 2015 Jul 14. PMID: 26103557.

28. Foley HM, Steel AE. Adverse events associated with oral administration of melatonin: A critical systematic review of clinical evidence. Complement Ther Med. 2019 Feb;42:65-81. doi: 10.1016/j.ctim.2018.11.003. Epub 2018 Nov 3. PMID: 30670284.

29. Paxinos, G., & Watson, C. (2006). *The rat brain in stereotaxic coordinates: hard cover edition*. Elsevier.

30. Manno FAM, Lau C. The pineal gland of the shrew (Blarinabrevicauda and Blarinacarolinensis): a light and electron microscopic study of pinealocytes. Cell Tissue Res. 2018 Dec;374(3):595-605. doi: 10.1007/s00441-018-2897-8. Epub 2018 Aug 7. PMID: 30088080.

31. Brunner P, Sözer-Topcular N, Jockers R, Ravid R, Angeloni D, Fraschini F, Eckert A, Müller-Spahn F, Savaskan E. Pineal and cortical melatonin receptors MT1 and MT2 are decreased in Alzheimer's disease. Eur J Histochem. 2006 Oct-Dec;50(4):311-6. PMID: 17213040.

32. Gorman MR. Temporal organization of pineal melatonin signaling in mammals. Mol Cell Endocrinol. 2020 Mar 1;503:110687. doi: 10.1016/j.mce.2019.110687. Epub 2019 Dec 19. PMID: 31866317.

33. Harpsøe NG, Andersen LP, Gögenur I, Rosenberg J. Clinical pharmacokinetics of melatonin: a systematic review. Eur J ClinPharmacol. 2015 Aug;71(8):901-9. doi: 10.1007/s00228-015-1873-4. Epub 2015 May 27. PMID: 26008214.

34. Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. Neuron. 1997 Jul;19(1):91-102. doi: 10.1016/s0896-6273(00)80350-5. PMID: 9247266.

35. Kandalepas PC, Mitchell JW, Gillette MU. Melatonin Signal Transduction Pathways Require E-Box-Mediated Transcription of Per1 and Per2 to Reset the SCN Clock at Dusk. PLoS One. 2016 Jun 30;11(6):e0157824. doi: 10.1371/journal.pone.0157824. PMID: 27362940; PMCID: PMC4928778.

36. Mendoza-Viveros L, Chiang CK, Ong JLK, Hegazi S, Cheng AH, Bouchard-Cannon P, Fana M, Lowden C, Zhang P, Bothorel B, Michniewicz

MG, Magill ST, Holmes MM, Goodman RH, Simonneaux V, Figeys D, Cheng HM. miR-132/212 Modulates Seasonal Adaptation and Dendritic Morphology of the Central Circadian Clock. Cell Rep. 2017 Apr 18;19(3):505-520. doi: 10.1016/j.celrep.2017.03.057. PMID: 28423315; PMCID: PMC5864111.

37. Reiter RJ, Rosales-Corral S, Sharma R. Circadian disruption, melatonin rhythm perturbations and their contributions to chaotic physiology. Adv Med Sci. 2020 Sep;65(2):394-402. doi: 10.1016/j.advms.2020.07.001. Epub 2020 Aug 4. PMID: 32763813.

38. Abbott SM, Malkani RG, Zee PC. Circadian disruption and human health: A bidirectional relationship. Eur J Neurosci. 2020 Jan;51(1):567-583. doi: 10.1111/ejn.14298. Epub 2019 Jan 3. PMID: 30549337; PMCID: PMC7261021.

39. Dallaspezia S, Suzuki M, Clara L, Colombo C, Benedetti F. Chronotype influences response to antidepressant chronotherapeutics in bipolar patients. Chronobiol Int. 2018 Sep;35(9):1319-1325. doi: 10.1080/07420528.2018.1469034. Epub 2018 May 15. PMID: 29764214.

40. Aulinas A. Physiology of the Pineal Gland and Melatonin. 2019 Dec 10. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, Grossman A, Hershman JM, Hofland HJ, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, MorleyJE, New M, Purnell J, Singer F, Stratakis CA, Trence DL, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000–2019. PMID: 31841296.

Надійшла до редколегії 2.10.2020
Отримано виправлений варіант 2.11.2020
Підписано до друку 2.11.2020

Received in the editorial 2.10.2020
Received a revised version on 2.11.2020
Signed in the press on 2.11.2020

О. Калмикова, канд. біол. наук,
М. Дзержинський, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

МОРФО-ФУНКЦІОНАЛЬНИЙ СТАН ЕПІФІЗА ТА СУПРАХІАЗМАТИЧНОГО ЯДРА ГІПОТАЛАМУСА ЩУРІВ ЗА РІЗНИХ РЕЖИМІВ УВЕДЕННЯ ЕКЗОГЕННОГО МЕЛАТОНІНУ

У сучасному суспільстві зростання цифрових технологій пов'язане з перевищенням рівня освітленості в нічну пору – новим типом забруднення. Світло вночі пригнічує синтез ендогенного мелатоніну епіфізом, що впливає на цикли роботи циркадної системи, тому в організмі часто порушуються режими неспання/сну, прийому їжі, фізичної активності. Крім того, показано, що нестача мелатоніну в певний час доби так само, як і його низька концентрація, зумовлює розвиток захворювань шляхом порушення правильної регуляції експресії годинних генів. У зв'язку із цим деякі останні клінічні протоколи або клінічні випробування для терапії багатьох патологій (напр. безсоння, метаболічного синдрому, серцево-судинних захворювань, проблем центральної нервової та імунної систем, раку, вірусних інфекцій) включають використання екзогенного мелатоніну. Оскільки мелатонін виконує свою функцію ендокринними і паракринними шляхами в різних типах клітин, то його застосування відбувається в широкому діапазоні доз і різний час доби (хронотерапевтичний підхід). Тому важливим є контроль стану центральних елементів циркадної системи – епіфіза (основного продуцента ендогенного мелатоніну) і супрахіазматичного ядра (СХЯ) гіпоталамуса (центрального водія ритму циркадної системи) – в умовах лікування екзогенним мелатоніном. Отже, метою нашого дослідження став аналіз морфо-функціонального стану епіфіза і СХЯ гіпоталамуса щурів після щоденного введення мелатоніну в різний час доби (уранці, увечері і безперервно з питною водою). Мелатонін уводили перорально крізь зонд протягом 7 тижнів у дозі 30 мг/кг за 1 год до вимикання світла (M ZT11, вечірнє введення) або 1 год після його увімкнення (M ZT01, ранкове), або безперервно з питною водою протягом дня і ночі (MB). Після застосування мелатоніну тільки у групі MB епіфіз демонстрував зміни морфології (ядра пінеалоцитів мали слабке базофільне забарвлення) і даних морфометричного аналізу (збільшення площі поперечного перерізу ядра пінеалоцитів порівняно з контрольною групою). Крім того, аналогічні зміни спостерігалися й у СХЯ: площа поперечного перерізу ядер нейронів СХЯ зросла при використанні кожного окремого режиму введення мелатоніну, а морфологічна характеристика залишилася без змін. Із цього можна припустити наявність у мелатоніну неінгібуючих властивостей у контексті петлі зворотного зв'язку циркадної системи, що передбачає широкий потенціал його використання за відсутності значного побічного ефекту на центральні елементи вищезгаданої системи.

Ключові слова: мелатонін, петля зворотного зв'язку, хронобіологія, пінеалоцити, нейрон, гістологія, циркадна система, побічна дія.

О. Калмыкова, канд. биол. наук,
Н. Дзержинский, д-р биол. наук
Киевский национальный университет имени Тараса Шевченко, Киев, Украина

МОРФО-ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ЭПИФИЗА И СУПРАХИАЗМАТИЧЕСКОГО ЯДРА ГИПОТАЛАМУСА КРЫС ПОСЛЕ РАЗНЫХ РЕЖИМОВ ВВЕДЕНИЯ ЭКЗОГЕННОГО МЕЛАТОНИНА

В современном обществе рост цифровых технологий связан с превышением уровня освещенности в ночное время – новым типом загрязнения. Свет ночью подавляет синтез эндогенного мелатонина эпифизом, что влияет на циклы работы циркадной системы, поэтому в организме часто нарушаются режимы бодрствования/сна, приема пищи, физической активности. Недостаток мелатонина в определенное время суток, равно как и низкая его концентрация, влияют на развитие заболеваний путем неправильной регуляции экспрессии часовых генов. В связи с этим некоторые последние клинические протоколы или клинические испытания для терапии различных патологий (напр. бессонницы, метаболического синдрома, сердечно-сосудистых заболеваний, проблем центральной нервной и иммунной систем, рака, вирусных инфекций) включают использование экзогенного мелатонина. Поскольку мелатонин выполняет свою функцию эндокринным и паракринным путями в различных типах клеток, его применяют в широком диапазоне доз и в разное время суток (хронотерапевтический подход). Поэтому важен контроль состояния центральных элементов циркадной системы – эпифиза (основного продуцента эндогенного мелатонина) и супрахиазматического ядра (СХЯ) гипоталамуса (центрального водителя ритма циркадной системы) в условиях лечения экзогенным мелатонином. Исходя из вышесказанного, основной целью нашего исследования стал анализ морфо-функционального состояния эпифиза и СХЯ гипоталамуса крыс после ежедневного введения мелатонина в разное время (утром, вечером и непрерывно с питьевой водой). Мелатонин вводили перорально через зонд в течение 7 недель в дозе 30 мг/кг за 1 час до выключения света (M ZT11, вечер) или через 1 час после его включения (M ZT01, утро), или непрерывно с питьевой водой в течение дня и ночи (MB). После применения мелатонина только в группе MB эпифиз демонстрировал изменения морфологии (ядро пинеалоцитов имело слабую базофильную окраску) и данных морфометрического анализа (увеличение площади поперечного сечения ядра пинеалоцитов по сравнению с контрольной группой). Кроме того, аналогичные изменения наблюдались и в СХЯ: площадь поперечного сечения ядра нейронов СХЯ увеличилась при использовании каждого отдельного режима введения мелатонина, а морфологическая характеристика осталась без изменений. В целом это предполагает наличие у мелатонина неингибирующих свойств в контексте петли обратной связи циркадной системы и широкий потенциал его использования при отсутствии значительного побочного эффекта на центральные элементы вышеупомянутой системы.

Ключевые слова: мелатонин, петля обратной связи, хронобиология, пинеалоциты, нейрон, гистология, циркадная система, побочный эффект.