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CIRCADIAN DYNAMICS OF OPTIC DENSITY OF MELATONIN RECEPTORS IN THE NEURONS OF HYPOTHALAMIC SUPRAOPTIC NUCLEUS IN RATS UNDER ALTERED PHOTOPERIOD

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Introduction. Melatonin production is considerably suppressed by light and affects the ability to transfer daily rhythm information from the hypothalamus to other neural target sites and thus alters the expression of some biological rhythms. The hormone controls the state of the hypothalamic-pituitary system and endocrine gland activity through melatonin receptors (membrane, cytosolic and nuclear ones). In addition, using a mechanism of the feedback, it interferes with the activity of supraoptic nucleus of the hypothalamus, which regulates water-salt metabolism and responses to stress. Objective: 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 150 – 180 g. The test animals were divided into 3 parts each with 2 groups, kept under the conditions of standard light regime, hyperilluminated and the injection of exogenous melatonin and day-round lighting within 7 days. To perform immunohistochemical methods, we used polyclonal antibodies to melatonin 1A receptors produced by Abcam and streptavidin biotin visualization system LSAB2 (peroxidase mark + diaminobenzidine) produced by Chemicon International Inc. We adhered to protocol standardization of methods for all sections. Additional staining of nuclei was performed with Mayer hematoxylin. Results. The indices of optical density of specific melatonin 1A receptors of supraoptic neurocytes staining obtained in the intact group (at 02.00 AM- 0,488 ± 0,0024, at 02.00 P.M. - 0,464 ± 0,0023, p = 0.002) and in animals subjected to light stress (at 02.00 AM- 0,295 ± 0, 0019, at 02.00 P.M.- 0,286 ± 0,0018, p = 0,012) had a probable value and were characterized by a clear diurnal periodicity. In the group of animals with pineal gland hypofunction modulation (at 02.00 A.M.- 0,216 ± 0,0017, at 02.00 P.M. - 0,214 ± 0,0021, p> 0,05). Conclusions The density of 1A melatonin receptors in rat's hypothalamic supraoptic neurons are normally characterized by an accurate circadian rhythm. The highest density of receptors is observed at 02.00 AM, and at 02.00 PM it is significantly lower (p = 0.002). Immunohistochemical studies revealed that under inhibition of pineal gland activity the circadian rhythm of melatonin receptors density in neurons of supraoptic nuclei of the hypothalamus gets disturbed, which is characterized by an incredible difference of indices in the tested periods of the day.

Key words: supraoptic nucleus, hypothalamus, melatonin receptors, immunohistochemical analysis.

Introduction

Most of the physiological and metabolic body processes are organized in time in response to photoperiod adapting to environmental changes [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 [2, 8].

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 pacemaker of the central biological clock (BC) – suprachiasmatic nucleus (SCN) of the hypothalamus [1].

Production of MT is sharply suppressed by light, which affects the ability to transfer rhyth information from the SCN of the hypothalamus to other neural target sites and thus alters the expression of some biological rhythms [6, 7].

The hormone controls the state of the hypothalamic-pituitary system and endocrine gland activity through melatonin receptors (membrane, cytosolic and nuclear ones) [4]. In addition, using a mechanism of the feedback it interferes with the activity of supraoptic nucleus (SON) of the hypothalamus, which regulates water-salt metabolism and responses to stress reaction [2, 3, 7]. 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

To provide quantitative circadian characteristics of melatonin receptors density in the neurons of the hypothalamic supraoptic nucleus of rats exposed to 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 150 – 180 g. 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, kept under the 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 taking into consideration the cyclical MT production, the rats were decapitated with 12-hour interval (at 02.00 AM and at 02.00PM) on the 8th day. The dates were selected for reasons of different functional activity of the pineal gland and production of leading chronobiotic, MT, in these time periods. Commission on Bioethical Expertise of Higher State Educational Establishment of Ukraine «Bukovinian State Medical University» found all the stages of the experiment were conducted in compliance with the essential requirements of the Helsinki Declaration and the requirements of the European Council 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 5 microns thick sections. 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 assessment of the intensity of staining was conducted as follows. First, we 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); 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 (fig.)

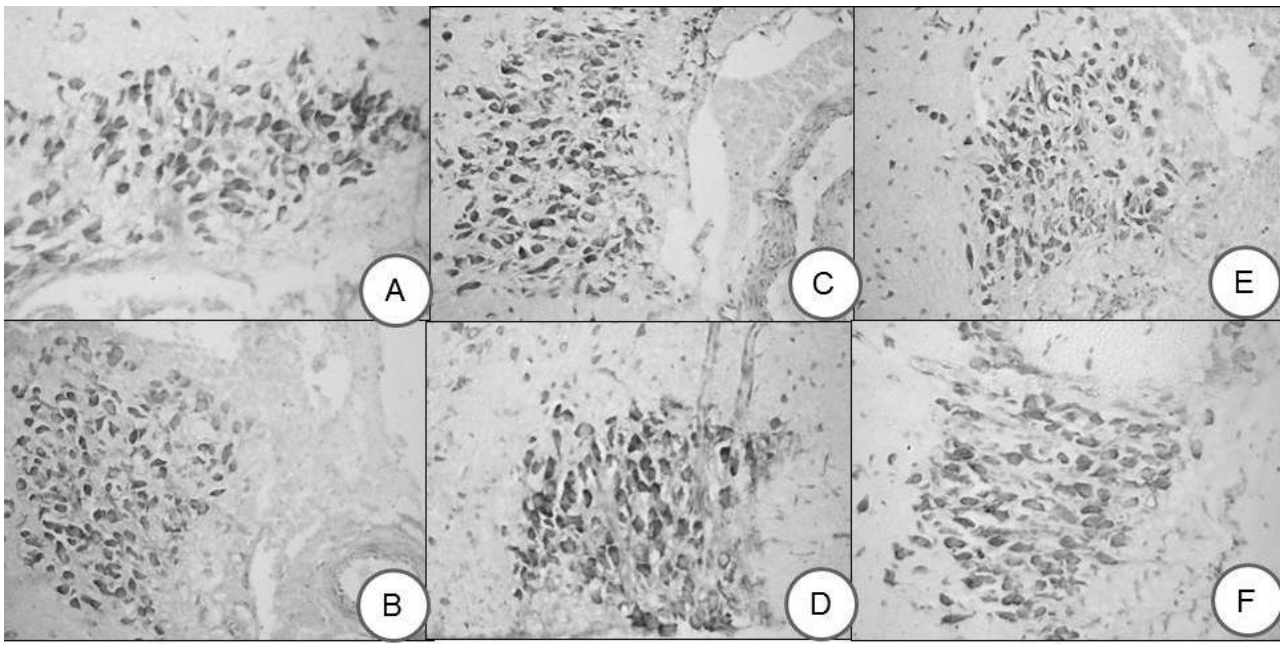

Fig. Melatonin 1 A receptors in the neurons of SON of a rat: under standard illumination at 02.00 AM (A) and at 02.00 PM (B); with hypofunction of the pineal gland at 02.00 AM (C) and at 02.00 PM (D) and after correction with exogenous melatonin at 02.00 AM (E) and 02.00 PM (F).

The highest density of melatonin 1 A receptors in the neurons of rat SON was observed at 02.00 AM compared to 02.00 PM (in sight area of 1600 mcm^2 - p=0.002 by 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.

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

Injections of MT at 02.00 AM under the round-o'clock illumination caused an increase in the density of melatonin receptors 1 A in the rats' hypothalamic neurons of SON compared with the animals that were exposed to constant illumination without injecting the hormon.

Immunohistochemical studies at 02.00 PM when the hormone was injecte, showed likely decrease density in the experimental structures 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 given 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, while at 02.00 PM it is significantly reduced ($p = 0.002$).

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

However, when the pineal gland become activated, the highest value is noted at 02.00 AM, being 0,505 ± 0,0026 units of density. Melatonin injecting for a week against the background of prolonged light exposure is demonstrating the tendency toward the normalization of the density of melatonin receptor 1 A in supraoptic neurons in rats' nuclei that is especially noticeable in the samples selected for the study at 02.00 AM, when the value is within 0,412 ± 0,0025 units of optical density.

Prospects for further research

We are planning to conduct further immunohistochemical analysis with a light deprivation and immobilization stress of rats for possible disturbances in circadian rhythm of density of 1A melatonin receptors in rat's hypothalamic neurons of SON.

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Реферат

ЦИРКАДІАННА ДИНАМІКА ОПТИЧНОЇ ЩІЛЬНОСТІ МЕЛАТОНІНОВИХ РЕЦЕПТОРІВ В НЕЙРОНАХ НАДЗОРОВОГО ЯДРА ГІПОТАЛАМУСА ЗА ЗМІНЕНОГО ФОТОПЕРІОДУ

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Ключові слова: надзорове ядро, гіпоталамус, мелатонінові рецептори, іммуногістохімічний аналіз.

Вступ. Секреція мелатоніну різко пригнічується світлом, що впливає на здатність передавати інформацію про добовий ритм від гіпоталамуса до інших нейронних мішеней і тим самим змінює експресію деяких біологічних ритмів. Гормон контролює стан гіпоталамо-гіпофізарної системи та діяльність ендокринних залоз через рецептори мелатоніну (мембранні, цитозольні та ядерні). Крім того, використовуючи механізм зворотного зв'язку, гормон взаємодіє з надзоровим ядром гіпоталамуса, і регулює водно-сольовий обмін та реагує на стресову реакцію. Мета дослідження. На підставі імуногістохімічної методики поєднано з комп'ютерною мікроденситометрією надати кількісну циркадіанну характеристику щільності мелатонінових рецепторів у нейронах надзорового ядра гіпоталамуса щурів. Матеріал та методи. Експерименти проводились на 60 зрілих самцях щурів масою 150-180 г. Дослідних тварин розділили на 3 серії, по 2 групи, які перебували в умовах стандартного світлового режиму, гіперілюмінізації та цілодобового освітлення з ін'єкціями екзогенного мелатоніну протягом 7 днів. Для виконання імуногістохімічного аналізу використовували поліклональні антитіла до рецепторів мелатоніну 1А, виробника Abcam та стрептавідинбіотинову систему візуалізації LSAB2 (пероксидазна мітка+діамінобензидин) виробника Chemicon International Inc. Додаткове фарбування ядер проводили гематоксиліном Майєра. Висновки. Щільність мелатонінових рецепторів 1А у нейронах надзорового ядра гіпоталамуса щурів у нормі характеризується чітким циркадіанним ритмом. У середньому найвища щільність рецепторів відмічається о 02.00 год доби, а 14.00 год вона суттєво знижується ($p=0,002$).

Імуногістохімічне дослідження дозволило виявити, що за умов пригнічення активності шишкоподібної залози порушується циркадіанний ритм щільності мелатонінових рецепторів у нейронах супраоптичних ядер гіпоталамуса, що характеризується невірогідною різницею показників у досліджувані періоди доби.

Реферат

ЦИРКАДИАННАЯ ДИНАМИКА ОПТИЧЕСКОЙ ПЛОТНОСТИ МЕЛАТОНИНОВИХ РЕЦЕПТОРОВ В НЕЙРОНАХ СУПРАОПТИЧЕСКОГО ЯДРА ГИПОТАЛАМУСА ПРИ ИЗМЕНЕНИЯХ ФОТОПЕРИОДА

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Ключевые слова: супраоптическое ядро, гипоталамус, мелатониновые рецепторы, иммунногистохимический анализ.

Вступление. Секреция мелатонина резко подавляется светом, влияет на способность передавать информацию о суточном ритме от гипоталамуса к другим нейронным мишеням и тем самым изменяет экспрессию некоторых биологических ритмов. Гормон контролирует состояние гипоталамо-гипофизарной системы и деятельность эндокринных желез через рецепторы мелатонина (мембранные, цитозольные и ядерные). Кроме того, используя механизм обратной связи, гормон взаимодействует с супраоптическим ядром гипоталамуса и регулирует водно-солевой обмен, реагируя на стрессовую реакцию. Цель исследования. На основании имуногистохимической методики объединенной с компьютерной микроденситометрией предоставить количественную циркадианную характеристику плотности мелатониновых рецепторов в нейронах супраоптического ядра гипоталамуса крыс. Материал и методы. Эксперименты проводились на 60 зрелых самцах крыс массой 150-180 г. Животных разделили на 3 серии, по 2 группы. Животные поддавались условиям стандартного светового режима, гиперилюминизации и круглосуточного освещения с инъекциями экзогенного мелатонина в течение 7 дней. Для выполнения иммуногистохимического анализа использовали поликлональные антитела к рецепторам мелатонина 1А, производителя Abcam и стрептавидинбиотиновую систему визуализации LSAB2 (пероксидазная метка + диаминобензидин) производителя Chemicon International Inc. Дополнительное окрашивания ядер проводили гематоксилином Майера. Выводы. Плотность мелатониновых рецепторов 1А в нейронах супраоптического ядра гипоталамуса крыс в норме характеризуется четким циркадианный ритмом. В среднем самая высокая плотность рецепторов отмечается в 02.00 ч суток, а 14.00 она существенно снижается ($p = 0,002$). Иммуногистохимическое исследование позволило выявить, что в условиях подавления активности шишковидной железы нарушается циркадианный ритм плотности мелатониновых рецепторов в нейронах супраоптических ядер гипоталамуса, характеризующаяся недостоверной разницей показателей в исследуемые периоды суток.