УДК: 591. 481. 3.- 019

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CORRECTION BY PEPTIDE BIOREGULATORS OF MICRO-AND ULTRAMICROSCOPIC CHANGES IN THE PINEAL GLAND CAUSED BY STRESS UNDER LIGHT DEPRIVATION

Key words: *pineal gland, chronoperiodical system, melatonin, epithalon, stress, microscopic structure.*

Abstract. *It has been established, that immobilizing stress causes decreasing of light cell's amount in pineal gland, which are the main source of melatonin production like under usual lighting and like under light deprivation. Light deprivation, that stimulates pineal gland function, also provides activation of pre-melatonin biosynthesis that increased production of endogenous melatonin.*

Introduction

An important role of the pineal gland as a synchronizer of biological rhythms in humans and animals is contributed by the fact that the physiology, biochemistry, morphology and ultrastructure of this organ are widely studied in recent decades [1, p. 62-66]. It is known, that the pineal gland is a part of photoperiodic system that is able to perceive changes in lighting of the environment through the impulses to the retinohypothalamic tract - to suprachiasmatic nucleus in particular, and in a lesser extent - to the supraoptic nuclei of the hypothalamus. Changes of lighting, temperature, and humidity of the geomagnetic field will influence the own rhythms of chronoperiodic system.

There were no predominance of any type of pineal cells with maintenance of histological structure of the gland and reduction of general number of the pineolocytes, found during aging [4, p. 52-53]. Authors noticed some changes of the pineal gland cytoarchitectonic in the form of nucleus polarization because of follicle formation, found in 61% cases.

Ultramicroscopic restructuring of pinealocytes under the influence of radiation and hyperillumination was investigated by Logvinov S. The reduction in endoplasmic reticulum and Golgi complex occur at the early stages after hyperillumination of pineolocytes with the normalization of their ultrastructural organization after1-6 months [5, c. 71-75].

Stress response can counteract the negative effects of aging and the effects of moderate sublethal stress may help to live longer. It is shown, that some moderate stressors (radiation, heat and cooling shock, hypergravitation, physical capacity) increase life expectancy, and hypergravitation reduces the degree of behavioral aging. The underlying mechanisms of the stress which increase the life span, have not been studied yet. However, it has been shown, that they can include metabolic regulation and induction of stress proteins [6, c. 95-98].

Objective

In the literature there is not enough information about the impact of the correction of immobilization stress and prolonged darkness on micro- and ultramicroscopic condition of the pineal gland, thereby the objective of our study was to analyze the efficacy of natural and synthetic chronobiotic - melatonin and epithalon to restore post-stressed rearrangements in the studied gland.

Material and methods

Experiments were carried out on 30 old (20-24 months) mongrel male albino rats weighting 280 to 360 g. Animals were kept under standard vivarium conditions, at the controlled temperature and air humidity; free access to water and food was provided. We studied the ability of peptide bioregulators to restore morphofunctional condition of the pineal gland in case of its deprivation and under the influence of 1-hour immobilization stress.

Rats were divided into five groups by six animals each. All stages of the experiments were carried out in accordance with the main requirements of the European Convention for the humane treatment of animals.

Control animals of the 1st group were kept for 7 days under conditions of normal illumination periodicity (12/12 h light/darkness cycle, group LD). Illumination (50 lx in the cages) was provided from 8.00 until 20.00 with luminescent lamps. Rats of group 2 were kept for 7 days under conditions of continuous darkness (group DD, induction of the pineal gland hyperfunction). Animals of the 3rd group were kept during the same period in constant darkness (light deprivation, group DD, induction of the pineal gland hyperfunction) and 1-hour immobilization stress. Rats of group 4 were kept for 7 days under conditions of continuous darkness and immobilization stress with correction of epithalon in dosage 0,5mg/kg, but in the fifth group (group DD+immobilization stress) animals

received melatonin in dosage 2,5 mg/kg.

On the next day after termination of the 7-day-long conditioning period, animals were decapitated under Nembutal anesthesia (40 mg/kg i.p.). The brains were immediately dissected and immersed for 20 h in a 10% formalin solution in phosphate buffer (0.1 M, pH 7.2) at room temperature. After a standard procedure of dehydration and impregnation with chloroform and paraffin, tissue samples were embedded in paraffin.

All surveys were conducted in compliance with EEC Directive № 609 (1986) and the Order of Ukraine № 281 dated 01.11.2000y. "On measures for further improving the organizational norms of using experimental animals". Experiments conducted in accordance with Commission on Bioethics of Bukovinian State Medical University (Minute №3 dated 16.02.2005y.).

Discussion of observed results

The pineal gland of old rats with normal lighting regimen was conical- or drop-shaped. Study of morphofunctional state of pineal gland showed that parenchyma doesn't maintain it shape, with minor signs of age involution in a small number of apoptotic cells, formed as a result of age load against a background of lower melatonin biosynthesis and reduced concentration in the blood.

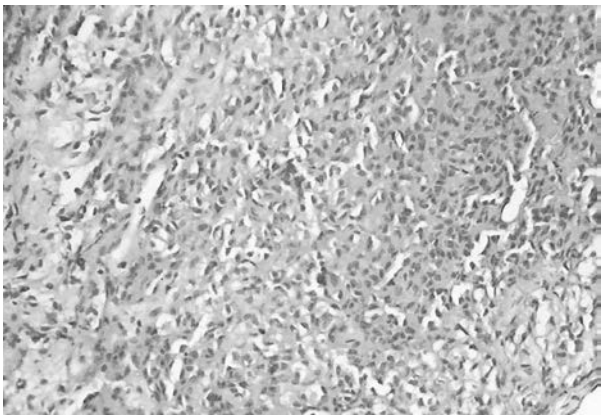

Fig. 1. Morphological condition of the pineal gland of old rat under casual lighting regime. Hematoxylin and eosin. Ob.20x, oc.10x

osmiophilic matrix, ctistae are observed.

Exploring changes in pinealocytes under 7-days constant darkness it has been found, that the ratio of light and dark cells are not significantly different from the averages in animals of the control group and compose of 72± 1,3%:28 ± 1,2% (p>0,05) (Fig. 3). It is even possible to observe the predominance of light-active cells, thereby confirming the inclusion of a protective mechanism in case of complete darkness, stimulating endogenous melatonin as a natural geroprotector (Fig. 4).

Examination of submicroscopic changes of the

Pinealocytes are small, densely located. Pinealocytes which produce indols are intenensively dark, with different shape, nucleoli are not visible. Cytoplasm is low-volumed, looks like a narrow transparent bezel which is surrounding a small irregularly shaped nucleus. Perikaryons of these cells are small, basophilic stained. At histological examination of the pineal gland it has been revealed that the ratio between light and dark pinealocytes amounted to 1,77 ± 0,028 (light pinealocytes: 64 ± 0,9%, dark: 36 ± 0,9%). General morphological picture of intact animals corresponded to literature data on this (Fig. 1) [6, pp. 96-98].

Submicroscopic examination under normal regime of lighting showed that round-oval nuclei with large osmiophilic nuclei are distinctive for the most pinealocytes. In karyoplasm round-oval nuclei containing large nucleolus were found with significant number of ribosomal granules beside them. They are scattered around the nucleus. There is a small lump of heterochromatin. Nuclear membrane is flat and has a relatively uniform perinuclear space, but it is a local thickening, clear nuclear pores.

In cytoplasm various sized, rounded osmiophilic granules of serotonin are found (Fig. 2). Tanks of granular endoplasmic reticulum are expanded, they form vacuole-like structure, large mitochondria with

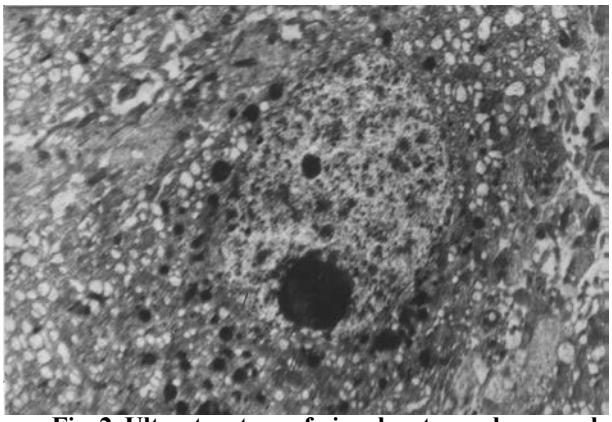

Fig. 2. Ultrastructure of pinealocytes under casual lighting regime. Oval-shaped nucleus (1) with nucleolus. Serotonin granules (2).x 10 000

pineal gland cells revealed euchromatin in karyoplasm, small granules of heterochromatin. Nuclear membrane is rough, forms a single deep invagination under conditions of 24 hours darkness.

The cytoplasm is enriched by narrow tubules of granular endoplasmic reticulum, but somewhere the flake-shaped, electronically low dense fragments are observed, considered to be melatonin granules. Mitochondria have predominantly elongated shape and moderate size. Part of organelles has a partially enlightened matrix (Fig. 5). These ultrastructural changes indicate the overload by melatonin.

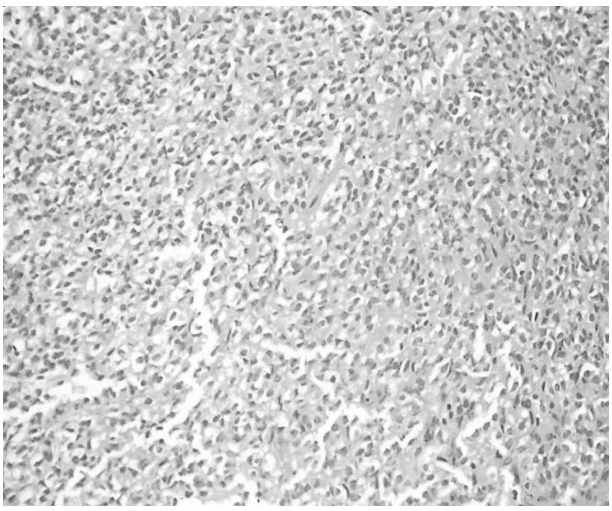

Fig. 3. Morphological condition of the pineal gland of old rat under lighting deprivation. Hematoxylin and eosin. Ob.20x, oc.10x

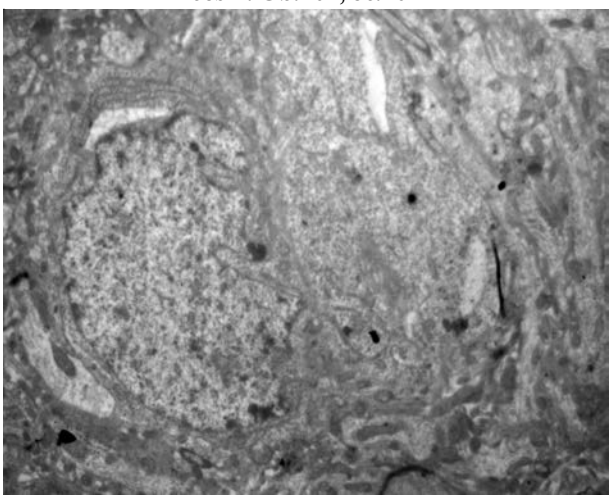

Fig. 5. Ultrastructure of the pineal gland under constant darkness. Nucleus with invagination (1), flake-shape melatonin granules (2) x 9 000

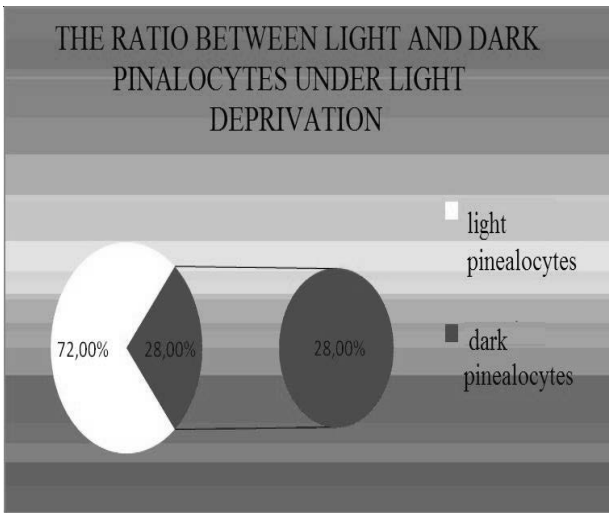

Fig. 4. The ratio between light and dark pinealocytes in old rats under light deprivation

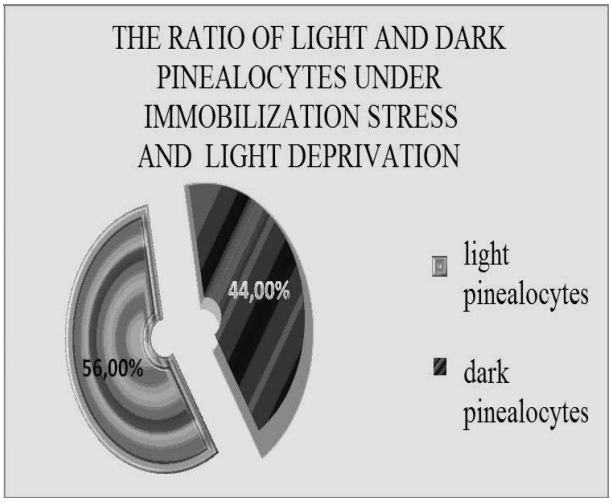

Fig. 6. The ratio of light and dark pinealocytes of old rats s under immobilization stress and light deprivation

Research under immobilization stress and pineal gland hyperfunction showed a manifested reaction of rats' pinealocytes on specified conditions of the experiment. In particular, the ratio between light and dark pinealocytes changed if compared with control values at (56±1,2)% : (44±1,0)% (p=0,0019) (Fig. 6).

The number of light active cells remains in enough amount for reactive capacity to synthesize melatonin as anti-stress factor (Fig. 7). Submicroscopically, pinealocytes have small irregularly shaped nuclei with lots of osmiophilic granules of heterochromatin in karyolemma in the conditions of this experiment.

Perinuclear spaces are not big; amount of nuclear pores is low. Unevenly thickened fragmented tubules of granular endoplasmic reticulum and tanks of Golgi complex are found in the cytoplasm. Oblong or round-oval mitochondria have a few crystals in moderate electronically densed matrix. There are only a few melatonincontaining flaked-shaped structures. Low functional activity of pinealocytes is confirmed by described changes of submicroscopic structure (Fig. 8).

After correction of the changes mentioned above in pinealocytes with epithalon it has been found, that ratio of light and dark pinealocytes had changed toward to control group as (60 ± 1,6)%: (40 ± 1,5)% (p = 0,035) (Fig. 9). A correction with melatonin showed more intensive recovery of light to dark pinealocytes ratio: (67±1,6) % : (32±1,5) % (p=0,036) (Fig. 10). According to ultramicroscopic picture, the obtained received data corresponded to parameters of the control group.

Thus, the results of our numerous studies showed that the pineal gland and its own system of generation and regulation of circadian rhythms in general, play an important role in homeostasis and aging.

Conclusions

Above mentioned findings of the micro-and ultramicroscopic changes in old rats' pineolocytes are indicative of the fact, that light deprivation stimulates

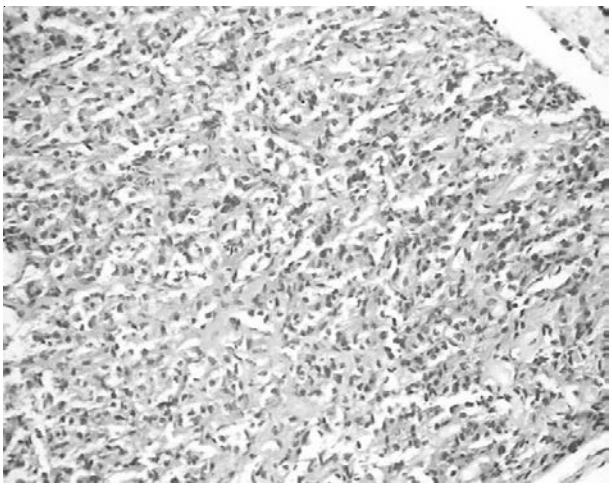

Fig. 7. Light microscopy of the pineal gland of old rat under immobilization sterss and light deprivation. Hematoxylin and eosin. Ob.20x, oc.10x

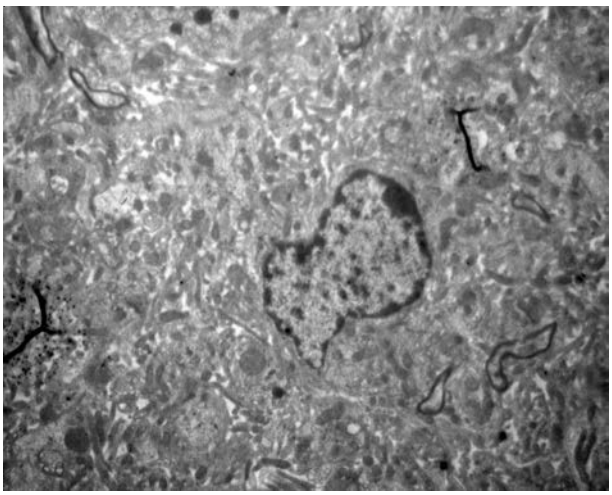

Fig. 8. Ultramicroscopic organization of pinealocytes of old rats under immobilization sterss and light deprivation. Abnormaly shaped nucleus (1), granules of melatonin (2). x 7 000.

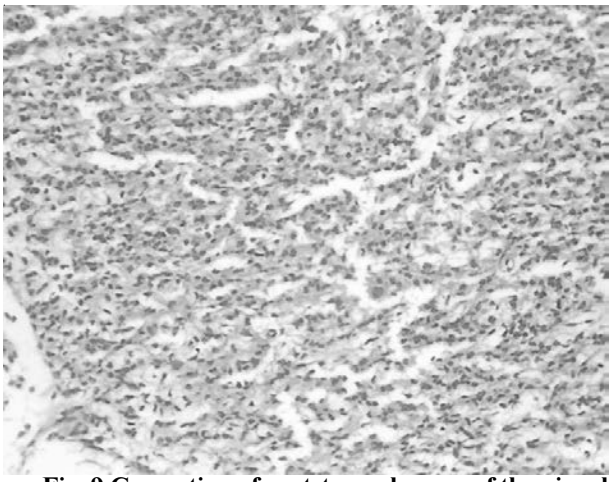

Fig. 9.Correction of poststress changes of the pineal gland with epithalon in old rat under light deprivation and immobilization stress. Hematoxylin and eosin. Ob.20x, oc.10x

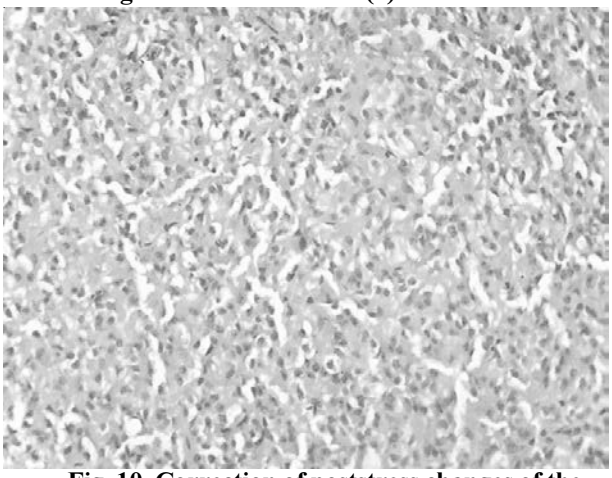

Fig. 10. Correction of poststress changes of the pineal gland with melatonin in old rat under light deprivation and immobilization stress. Hematoxylin and eosin. Ob.20x, oc.10x

the pineal gland function, provides geroprotective effect, actively engaging precursors to melatonin biosynthesis, thereby activating the production of endogenous melatonin (free radical scavenger) in pinealocytes of aging organism. The influence of immobilization stress has negative effects on the morphological characteristics of the pineal gland cells.

Perspectives of the further research

Furthermore, for a deeper understanding of the processes occurring in pinealocytes of old rats under the influence of immobilization stress, we are planning to study micro-and ultramicroscopic changes of these cells under the conditions of light stimulation as well.

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КОРРЕКЦИЯ ПЕПТИДНЫМИ БИОРГЕУЛЯТОРАМИ МИКРО- И УЛЬТРАМИКРОСКОПИЧЕСКИХ ИЗМЕНЕНИЙ ПИНЕАЛЬНОЙ ЖЕЛЕЗЫ ВЫЗВАННЫЕ СТРЕССОМ ПОД ВОЗДЕЙСТВИЕМ СВЕТОВОЙ ДЕПРИВАЦИИ

Ю. В. Ломакина, Р. Е. Булык

Резюме. При изучении морфологических изменений шишковидной железы нами установлено, что иммобилизационный стресс приводит к уменьшению количества

светлых клеток, которые являются основным мелатонинпродуцирующим источником, как при обычном освещении, так и при полной темноте. А световая депривация, что стимулирует функцию эпифиза мозга, обеспечивает активацию биосинтеза предшественников мелатонина, увеличивая тем самым продукцию эндогенного мелатонина.

Ключевые слова: пинеальная железа, хронопериодическая система, мелатонин, эпиталон, микроскопическая структура.

КОРЕКЦІЯ ПЕПТИДНИМИ БІОРГЕУЛЯТОРАМИ МІКРО- ТА УЛЬТРАМІКРОСКОПІЧНИХ ЗМІН ПІНЕАЛЬНОЇ ЗАЛОЗИ ВИКЛИКАНІ СТРЕСОМ ЗА УМОВ СВІТЛОВОЇ ДЕПРИВАЦІЇ

Ю. В. Ломакіна, Р. Є. Булик

Резюме. При вивченні морфологічних змін шишкоподібної залози нами встановлено, що іммобілізаційний стрес призводить до зменшення кількості світлих клітин, які є основним мелатонін-продукувальним джерелом як при звичайному освітленні, так і при повній темряві. А світлова депривація, що стимулює функцію епіфіза мозку, забезпечує активацію біосинтезу попередників мелатоніну, збільшуючи тим самим продукцію ендогенного мелатоніну.

Ключові слова: пінеальна залоза, хроноперіодична система, мелатонін, епіталон, мікроскопічна структура.

Вищий державний навчальний заклад України "Буковинський державний медичний університет", м. Чернівці

Clin. and experim. pathol.- 2015.- Vol.14, №3 (53).-P.85-89.

Надійшла до редакції 2.09.2015
Рецензент доц. О. А. Оленович