ANTISTRESOOR MEDICINE MELATONIN FOR RECOVERMENT OF MICROSCOPIC CHANGES IN THE PINEAL GLAND CAUSED BY LIGHT DEPRIVATION

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Key words: *pineal gland, chronoperiodical system, melatonin, ultramicroscopic structure.*

Abstract. *It has been established, that light alteration causes changes 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.*

Резюме. *Изучено, что изменения освещенности вызывают перестройку количества светлых пинеалоцитов, которые являются основным источником продукции мелатонина как при условии обычного освещения, так и при световой депривации. Световая депривация стимулирует функциоанльную активность пинеальной железы и, обеспечивает активацию биосинтеза предшественников мелатонина, увеличивая тем самым продукцию эндогенного мелатонина.*

Topicality. 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].

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

Material and methods. Experiments were carried out on 30 old (20-24months) 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 bioregulator to restore morphofunctional condition of the pineal gland in case of its deprivation.

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). Rats of group 3 were kept for 7 days under conditions of continuous darkness with correction of melatonin in dosage 2,5 mg/kg (group DD+melatonin).

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.

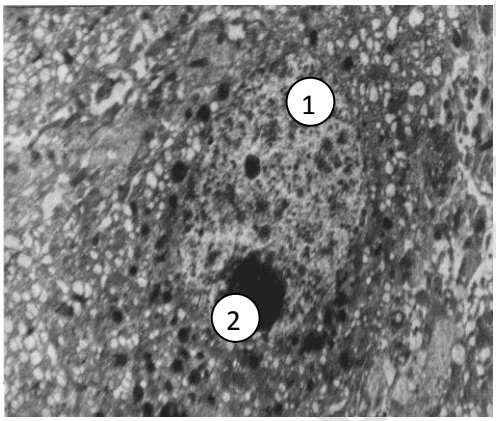
All surveys were conducted in compliance with EEC Directive № 609 (1986) and the Order of Ukraine № 281 dated 01.11.2000y. "On measures for further improving the organizational norms of using experimental animals". Experiments conducted in accordance with Commission on Bioethics of HSEE “Bukovinian State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

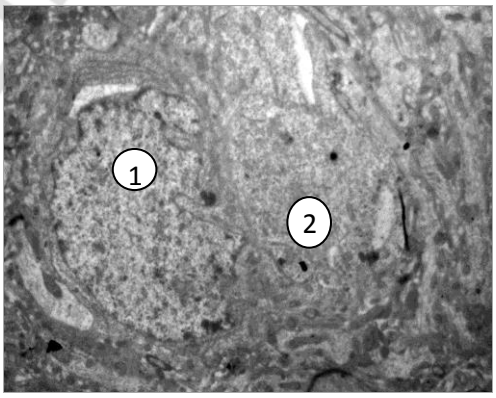
Submicroscopic examination under normal regime of lighting showed that round-oval nuclei with large osmiophilic nuclei are distinctive for the most pinealocytes. In karyoplasm round-oval nuclei containing large nucleolus were found with significant

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. 1). Tanks of granular endoplasmic reticulum are expanded, they form vacuole-like structure, large mitochondria with osmiophilic matrix, ctistae are observed.



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



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

Exploring changes in pinealocytes under 7-days constant darkness it has been found, that the 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 being melatonin granules. Mitochondria have predominantly elongated shape and moderate size. Part of organelles has a partially enlightened matrix (Fig. 2). These ultrastructural changes indicate the overload by melatonin.

After correction of the changes mentioned above in pinealocytes with melatonin showed 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 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.

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