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ULTRASTRUCTURE OF NEURONS OF SUPRAHIASMATIC NUCLEI OF RAT HYPOTHALAMUS UNDER CONDITIONS OF PHOTOPERIOD MODIFICATIONS

Abstract. *Submicroscopic organization of pacemaker cells of the ventrolateral region of the suprachiasmatic nuclei of the anterior hypothalamus in rats was studied. Under the standard LD illumination regime (12.00 – 12.00), the ultrastructure of pacemaker neurons indicates a decrease in their functional activity in the light, and an increase in the darker period of the day. Continuous light stress LL (24.00L:00D) leads to a significant desynchronism of the circadian pacemaker and inhibition of its activity during the observation period. Modeling of the hypothyroidism of the pineal gland causes destructive changes in the components of the studied structures, more pronounced at 2 a.m.*
Key words: *hypothalamic suprachiasmatic nuclei, permanent illumination*

Introduction. Photoperiod is the main timing factor for the synchronization of the rhythms of somatic and visceral functions, as well as the coordination and modulation of the mechanisms of adaptation of the organism to the influence of various factors [2,5]. An oscillator that controls mammals most of the rhythms, in particular, circadian (rituals) rhythms, localized in the pacemaker neurons of the ventrolateral unit of the suprachiosmatic nucleus (SCN) of the hypothalamus [5,8]. Light information is perceived by the retina, is transmitted to the retinogipotalamic tract to the SCN and, later, through the intermediary structures, enters the epiphysis cerebri (pineal gland) [9]. Secretory cells of the gland - pinealocytes synthesized the main neurohormon melatonin [9]. Among the wide range of hormone effects, chrono-regulating is crucial [7]. Physiological control of the function of the pineal gland of mammals is carried out to a large extent by the mode of illumination [4]. In the light melatonin products are suppressed by the gland. Continuous darkness stimulates the secretion of the epiphyseal hormone, and thus causes changes in the activity of the circadian pacemaker, which is reflected on the ultrastructure level [8]. The aim of this work is to elucidate the submicroscopic rearrangements of the neurosecretory cells of the ventrolateral region of the suprachiasmatic nuclei of the hypothalamus in different diurnal periods under constant illumination.

Material and Methods. Experiments were carried out on 24 adult mongrel male albino rats weighing 150 to 180 g. Animals were kept under standard vivarium conditions, at the controlled temperature and air humidity; free access to water and food was provided. The purpose of the study can be considered the pacemaker cells of the ventrolateral unit of the SCN of the hypothalamus.

The animals were divided into two groups; in each of the latter collection of biomaterial was carried out at 2 p.m. and at 2 a.m. The period of the experiment depended on different functional activity of the pineal gland in the specified time of

the day.

Intact animals of the 1st group were kept for 14 days under conditions of normal illumination periodicity (group LD, illumination (50 lx in the cages) was provided from 8.00 until 20.00 with luminescent lamps). Rats of the 2nd group were kept for 14 days under conditions of continuous illumination (group LL, induction of the epiphyseal hypofunction).

On the next day after termination of the 14-day-long conditioning period at 2 p.m. and 8 p.m., animals were decapitated under Ethanal anesthesia (40 mg/kg i.p.).

For the pacemaker neurons of the ventrolateral unit of the SCN of the hypothalamus electron microscopy mapping the samples were collected in conformity with generally accepted rules. The pacemaker neurons were identified in slices of the hypothalamus using electron microscopy. 1-1.5 thick slice of the ventrolateral unit of the SCN of the hypothalamus was dissected. First, the slice was incubated in 2.5% solution of glutaraldehyde in Millonig's buffer (pH 7.2-7.4). Then the fixed material was incubated in buffer solution and washed off for 20-30 min. After this, the post-fixation of the material for 60 min was performed, using 1% solution of osmium tetroxide in Millonig's buffer. After a standard procedure of dehydration in ethanol and acetone and immersion in a mixture of epoxy resins was carried out. A weak red illumination (2 lx) was provided at night, which had little effect on the melatonin biosynthesis with the pineal gland [1] were carried out in accordance with the main requirements of the Decree of The First Regional Meeting of National Bioethics Committees (NBC) "Ethical Principles and Guidelines for Experiments on Animals" (2001), the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of 18 March 1986, the EU Council Directive 86/609/EEC of 24 November 1986 and the Ministry of Health of Ukraine Directive No. 690 of 23 September 2009, No. 944 of 14 December 2009, No. 616 dated 03 August 2012 and the laws of Ukraine.

Results and their discussion. Submicroscopic analysis of the SCN of the hypothalamus of the intact animals at 2 p.m. showed that most of the neurosecretory cells have reduced irregularly shaped nuclei with shallow invasions of the carriel. The carioplasma has lumps of chromatin and a dense osmophilic nucleoi. The neuroplasma takes up small volume densely filled with a small lumen of the granulosa endoplasmic reticulum and Golgi complex cisternae with a few vacuoles and bubbles. The light small matrices and somehow modified mitochondria crystals are observed there. In the neuroplasm of such neuronsecretory cells there is a small amount of hormonal granules. The indicated submicroscopic organization of neurosecretory cells reflects a low functional activity.

The study of the ultrastructural organization of the hypothalamic scintillation in intact animals at 2 a.m. revealed that the neuronsecretory cells frequently have nuclei with significantly uneven nuclear membrane and deep invaginations, therefore increasing the area of aninteraction between the nucleus and the cytoplasm. The euchromatin predominates in the karyoplasma; only small osmophilic lumps of heterochromatin are noticeable. In most nuclei, large nucleoli and many ribosomal granules are visible. The nuclear envelope has numerous nuclear pores.

Most neurosecretory cells of the neuroplasm are occupied with long tubules of granular endoplasmic reticulum with a narrow lumen; and the organelle membranes contain ribosomes. The Golgi bodies (dictyosomes) are characteristically flattened cisternae with perinuclear arrangement having moderate quantity of vacuoles and bubbles filled with osmophilic contents. These are forming neurohormonal granules. The electron microscope revealed a group (pair) arrangement of neurosecretory cells in some fields of view. Their ultrastructure is similar to the one described above. In the axon leaving this cell more intensely osmophilic small hormonal granules are found. Such a pattern indicates the active functional state of the pacemaker cells of the SCN.

The SCN of the hypothalamus ultrastructural organization of the animals kept for 1 week under conditions of light stimulation at 2 p.m. was reflected by the presence of radiant neuronsecretory cells with dense round core. Their karyoplasm usually contains euchromatin and sites of heterochromatin (Fig. 1). In the neuroplasm of the SCN neurons, destructive changes of organelles are identified. Fragmentation and expansion of the tubules of the granular endoplasmic reticulum and Golgi complex cisternae occurs with the vacuoles formation. The mitochondria destruction is accompanied by focal illumination of their matrix. In such neuronsecretory cells the content of hormonal granules is insignificant. There are also dark neuronsecretory cells which usually have pyknotic nuclei with osmophilic karyoplasm and

nuclear membrane invagination. Their electron-density neuroplasm has destructively altered organelles and few horizontal granules (Fig. 1).

For 14 days under conditions of 24 h illumination at 2 a.m. the neurosecretory cells having light nuclei with uneven contours and mild pores were identified submicroscopically. The nuclear membrane contains very few ribosomal granules and occasionally nucleoli. The neuroplasma has a high electron density and ''fuzzy'' membrane organelle. The focal expansion of the granulosa endoplasmic reticulum tubules with the formation of vacuolic structures is found. Part of the mitochondria has a bright matrix and reduced crystals with detached hormone granules (Fig. 2). The ultrastructural state described above indicates a decrease in the functional activity of structures with elements of destruction.

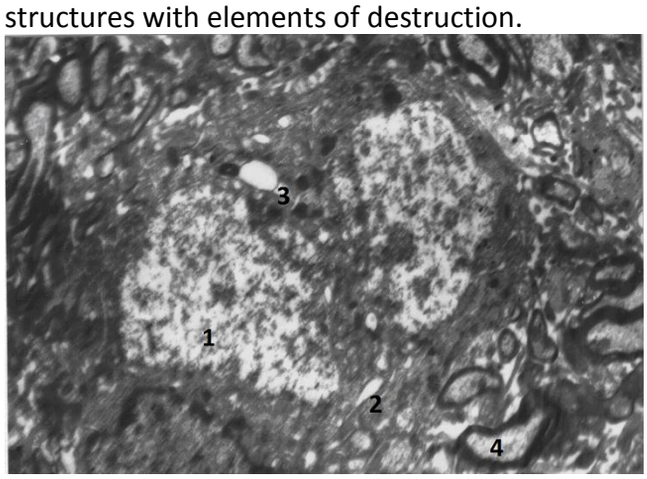

Fig. 1. The submicroscopic organization of the pacemaker cells of the ventrolateral unit of the SCN of the rat anterior hypothalamus at 2 p.m. under constant illumination conditions: 1 - nuclear membrane of dark neurocyte invaginations; 2 - dilated tubules of the granular endoplasmic reticulum; 3 - Golgi complex destruction x 7 000.

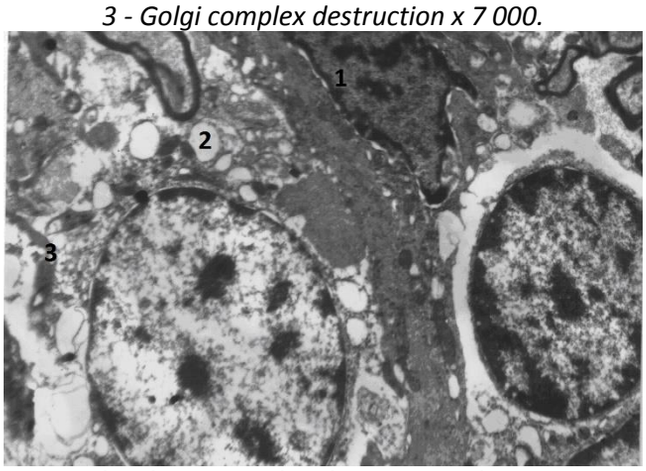

Fig. 2. The changes in the ultrastructural organization of neuronsecretory cells of the ventrolateral unit of the SCN of the rat anterior hypothalamus at 2 a.m. under the light stimulation: 1 - euchromatin nucleus; 2 - destroyed organelles; 3 - neurosecretory granules; 4 - myelinated nerve fibers. x 8 000.

Thus, the ultramicroscopic changes found in the pacemaker cells of the SCN of the hypothalamus can be considered as a reflection of desynchronosis. According to the references, prolonged permanent illumination causes hypofunction of the pineal gland, and, accordingly, a decrease of melatonin production. Inhibition of a synthesis of this natural chronobiotic is the main cause of the functional disorganization of the pacemaker cells of the ventrolateral unit of the SCN of the hypothalamus.

Under the standard illumination regime the submicroscopic organization of pacemaker cells of the ventrolateral unit of the SCN of the rat anterior hypothalamus indicates a decrease in the functional activity of the neurons in the light cycle and its growth in the dark cycle. A long-term light stress leads to significant desynchronosis of circadian pacemaker and inhibition of its activity during the follow-up period. In pineal gland hypofunction modeling the destructive changes in the components of the studied structures are more pronounced at 2 a.m.

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