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Ultrastructure of Neurons in the Hypothalamic Suprachiasmatic Nucleus of Rats with Different Stress Reactivity

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The rats were divided into groups demonstrating extremely high and low stress reactivity depending on the results of testing for the nociceptive threshold and thermolability in response to bacterial lipopolysaccharide administration. Specific structural features of the nucleus and mitochondria were revealed in neurons of the hypothalamic suprachiasmatic nucleus in rats with constitutionally high reactivity, which reflects high functional activity and stress-induced lability of these structures. Ultramicroscopic study revealed phenotypic differences in one of the key hypothalamic nucleus, which enables potent and rapid neurogenic response of the stress system in animals with high stress reactivity.

Key Words: *hypothalamus; suprachiasmatic nucleus; stress reactivity*

The adaptive response to stress factors (particularly psychoemotional agents) and risk for the development of diseases are determined by not only strength and duration of exposure, but also stress reactivity of the organism [5,7,8,10]. Our previous studies revealed specific features of hypothalamic, striopallidal, and limbic structures and cerebral cortex for various constitutional phenotypes that are associated with high risk of stress-induced disorders [1,3,4].

A theoretically and practically important problem of the existence of morphological specific features in hypothalamic structures of animals with documented high (HSR) or low stress reactivity (LSR) was not solved. This hypothesis was tested by ultrastructural study of neurons in the hypothalamic suprachiasmatic nucleus (SCN), which plays a key role in the progressive development of the stress response.

Here we studied intracellular structural characteristics of SCN neurons in intact rats with different constitutional stress reactivity.

MATERIALS AND METHODS

Experiments were performed on adult male outbred rats weighing 220-280 g. Using practically harmless tests estimating vocalization threshold during stimulation with low-voltage direct current and gradient of temperature rise after administration of bacterial lipopolysaccharide (LPS) in ultralow doses [2] we selected 8 of 120 animals with hypothetically LSR and 8 rats with HSR. Half of animals in each group were subjected to 24-h immobilization stress [5] 14 days after testing. Histopathological features of tissues in the stomach, thymus, and adrenal glands confirmed differences between LSR and HSR rats. Four animals of each group were maintained in a

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vivarium for 2 weeks under conventional conditions. The animals were killed by intravenous infusion of 4% paraform in 0.1 M cacodylate buffer (supravital fixation) under nembutal anesthesia according to the rules of studies with experimental animals. The brain was cut into 3 frontal sections immediately after removal from the cranium. SCN was identified dorsal to the optic chiasm (AP_0 sections, Horsley—Clark coordinates) [9]. Ultrathin sections (50-90 μ) were prepared on a LKB-8800 ultramicrotome and mounted on copper grids. These sections were contrasted with 2.5% uranyl acetate in 50% ethanol (40 min) and 0.3% lead citrate (20 min) and examined under a Tesla BS-540 electron microscope (accelerating voltage 60 kV). Scanned ultramicrophotographs were analyzed by means of AM Lab Hesperus v3.0 beta software. Structural study included measurement of the volume ratio of mitochondria in perikaryon and calculation of the brightness ratio for euchromatin/heterochromatin, euchromatin/cytoplasmic matrix, mitochondrial matrix/cytoplasmic matrix, and cytoplasmic matrix/synaptosomal contents in RGB three-dimensional coordinates. We also analyzed the shape of the nuclear membrane and outer mitochondrial membrane and index of mitochondrial cristae.

RESULTS

Cells with polygonal perikarya were found in SCN neurons of HSR rats. The nuclei had regular round shape without invaginations. Chromatin was presented by small clumps of highly condensed material. The amount of condensed chromatin was maximum on the inner surface of the nuclear membrane. Electron density of the karyoplasm was very low. The karyolemma consisted of 2 leaflets with low osmiophilic intermembrane content. The perikaryon was of small width. The nucleus occupied more than 50% space of the perikaryon. The hyaloplasm had high electron density (Fig. 1, *a*).

The hyaloplasm included a considerable number of compartments of the endoplasmic reticulum with abundant ribosomes. The hyaloplasm also contained considerable number of freely floating polysomes. The Golgi complex was presented by widened dictyosomes with electron-transparent contents. The surface of dictyosomes was relatively smooth with a low number of convexities and concavities (which were in a certain equilibrium).

Mitochondria appeared as relatively large organelles whose shape varied from round and spindle-like to irregular polygonal. The mitochondrial contents had relatively low electron density (Fig. 1, *b*). The outer membrane was smooth. The inner membrane formed small cristae that were chaotically oriented relative to the outer surface. There were a small number of symmetric axosomatic contacts. Synaptic vesicles contained electron-transparent substance.

SCN neurons in LSR rats had perikaryon of polygonal shape and relatively regular nucleus, the nucleoplasm was characterized by low electron density. Euchromatin was diffusely distributed over the volume of the nucleus. Heterochromatin was presented by large osmiophilic clumps, which occupied nearly all space near the inner surface of the nuclear membrane. The nucleolus did not necessarily appear on section. The nucleolus looked like a round structure with predominance of the granular compartment. The nuclear membrane had normal ultrastructure and formed large invaginations (up to 1/3 of the nuclear radius; Fig. 2, *a*). The width of the perikaryon varied and, therefore, the nucleus was shifted toward the peripheral region. The hyaloplasm had moderate electron density. The cytoplasm had a small number of elements of the endo-

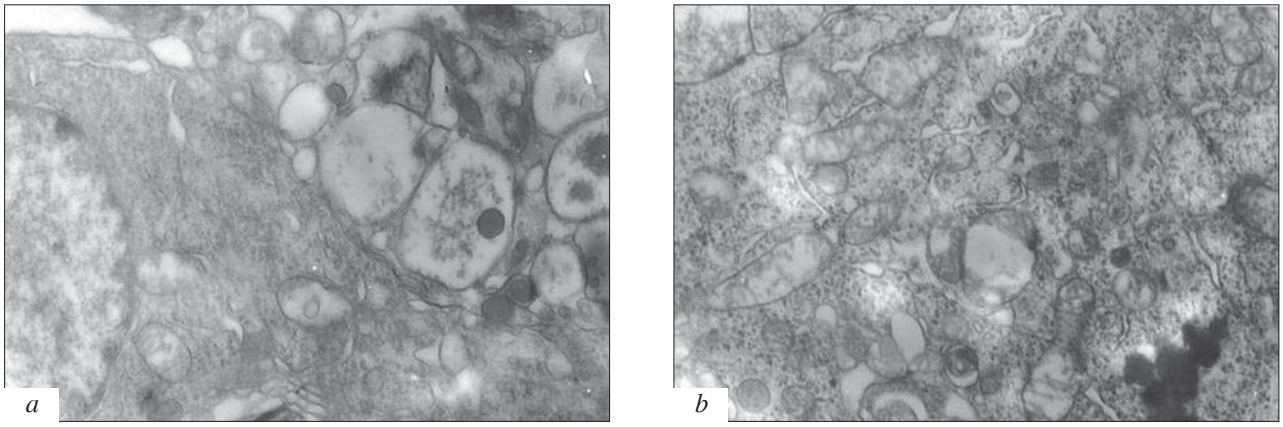

Fig. 1. SCN neurons in HSR rats, ×10,000. Absence of nuclear invaginations, highly osmiophilic cytoplasm of neurons (*a*); reduced electron density of mitochondria, considerable number of cisternae and membranes of the endoplasmic reticulum (*b*).

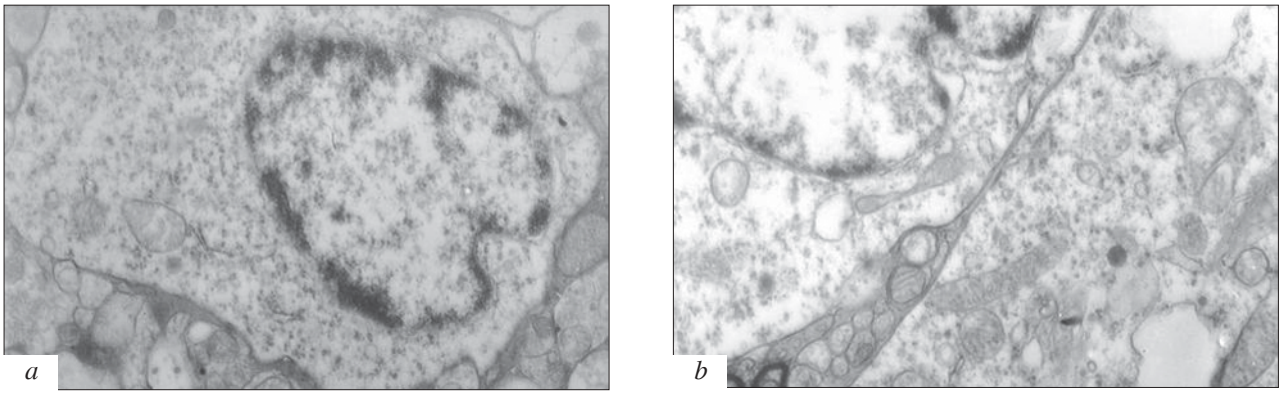

Fig. 2. SCN neurons in LSR rats, ×10,000. Large invaginations of nuclei (*a*); small number of electronically transparent synaptic vesicles in axosomatic contacts (*b*).

TABLE 1. Morphometric Parameters of SCN Neurons in Intact Rats with Different Stress Reactivity (*M*±*m*)

Parameter	HSR rats	LSR rats
Euchromatin/heterochromatin RGB brightness ratio	0.42±0.01	0.75±0.02*
Volume ratio of mitochondria, μ^3/μ^3	0.25±0.01	0.17±0.01*
Index of mitochondrial cristae	7.14±0.20	3.25±0.14*
Cytoplasmic matrix/synaptosome RGB brightness ratio	1.41±0.05	1.25±0.04*

Note. *$p \leq 0.05$ compared to HSR rats.

plasmic reticulum with a moderate amount of ribosomes. The Golgi complex was presented by a small number of dictyosomes. They were localized perinuclearly and mainly had well-developed concave surface. Mitochondria appeared as organelles with normal ultrastructure. The matrix had moderate electron density. The outer membrane was smooth. The inner membrane formed cristae that were oriented perpendicularly to the outer membrane (Fig. 2, *b*).

Morphometry showed that among quantitative ultrastructural characteristics of the nucleus only the euchromatin/heterochromatin brightness ratio is significantly lower in HSR animals (Table 1).

These differences confirmed a specific quantitative feature of the neuronal nucleus in HSR rats (relatively heterogeneous structure of the nucleus and considerable condensation of chromatin). The results of previous studies [4] suggest that this structure of the nucleus in SCN neurons provides greater functional activity of the stress system.

The volume ratio of mitochondria in perikarya of SCN neurons in HSR rats was 38.8% higher than in LSR animals ($p<0.01$). No inter-group differences were found in the shape of the outer mitochondrial membrane and mitochondrial matrix/cytoplasmic matrix brightness ratio. However, the shape of cristae that characterizes functional activity was 2.2-fold higher in HSR rats compared to LSR animals ($p<0.001$).

Significant differences were revealed in the cytoplasmic matrix/synaptosome brightness ratio. This parameter in SCN neurons of HSR rats was 1.12-fold higher compared to LSR animals ($p \leq 0.05$). These differences reflected greater lability of synaptic processes in SCN of HSR rats [7].

A comparative ultrastructural study of SCN neurons demonstrated significant intracellular differences in the nucleus, mitochondria, and other cytoplasmic organelles. They can be considered as a substrate for high functional activity and lability of SCN in animals with constitutionally high stress reactivity.

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