Ultrastructural Changes in Neurons of Medullary Giant-Cell Nucleus in Growing Rat Exposed to Acute Stress

V. B. Pisarev, A. V. Smirnov, P. A. Khloponin, and V. P. Tumanov

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Ultrastructural transformations in neurons of the medullary giant-cell reticular nucleus of young rats during exposure to acute emotional and painful stress attest to activation of the protein synthesis system (nucleolar hypertrophy, well-developed elements of the granular endoplasmic reticulum, increased content of free ribosomes and polysomes) in the majority of cells and the appearance of organelle injuries in some these cells.

Key Words: *ultrastructure; neuron; medulla oblongata; young rats; emotional and painful stress*

Exposure to stress factors sharply increases the load to vital systems of growing organisms [1,7]. Activation of the sympathoadrenal system observed during exposure is mediated by structures of the brainstem reticular formation [2,11]. Giant-cell reticular nuclei (GRN) provide modulation of the respiratory rhythm, integration of nociceptive information, and contribute to coordination of the motor and autonomic responses [6,8,9].

We studied ultrastructural changes in GRN regions of young rats during the early periods of emotional and painful stress.

MATERIALS AND METHODS

The study was carried out on 40 young albino rats. Acute emotional and painful stress (EPS) was induced by the method of group fixation of 30-day-old rats by the withers [4]. Experimental animals of groups 1 (n=10) and 2 (n=10) were daily exposed to 3-h EPS procedure for 3 and 7 days, respectively. Groups 3 and 4 were control animals aged 33 (n=10) and 37 (n=10) days, respectively; they were kept under standard vivarium conditions. The animals were sacrificed under ether narcosis in accordance with the Regulations for Handling Experimental Animals.

Volgograd Research Center of Russian Academy of Medical Sciences. ***Address for correspondence:*** alexey-smirnov@rambler.ru. A. V. Smirnov

Fragments of the medulla oblongata (1 mm^3) for electron microscopy were fixed in 4% paraform in 0.1 M cacodylate buffer for 12 h and postfixed in 1% OsO_4 in 0.1 M cacodilate buffer (pH 7.4) at 4°C for 2 h [5]. After repeated washout in cacodylate buffer the material was dehydrated in ascending alcohols and embedded in epon-araldite mixture.

Ultrathin sections (50-90 nm) were made on an LKB-8800 ultramicrotome. Semithin epon-araldite sections (1 μ) were stained with methylene blue. Ultrathin sections were mounted on copper lattices. After contrasting with 2.5% uranyl acetate in 50° ethanol for 40 min and 0.3% lead citrate for 20 min the sections were examined under JEM-100S and Tesla BS-540 electron microscopes at 60 kV accelerating voltage. The preparations were photographed using plates for nuclear studies. Electron microphotos were printed on Fujifilm photopaper.

RESULTS

Ultrastructural changes in neurocytes of different degree and heteromorphism were detected in young rats exposed to 3-day EPS. Changes in compartmentalization of organelle location in neurons were noted. Well-developed granular endoplasmic reticulum was seen in the perinuclear and peripheral regions of the perikaryon. The size and shape of mitochondria varied from small round to large dumbbell-like. The majority

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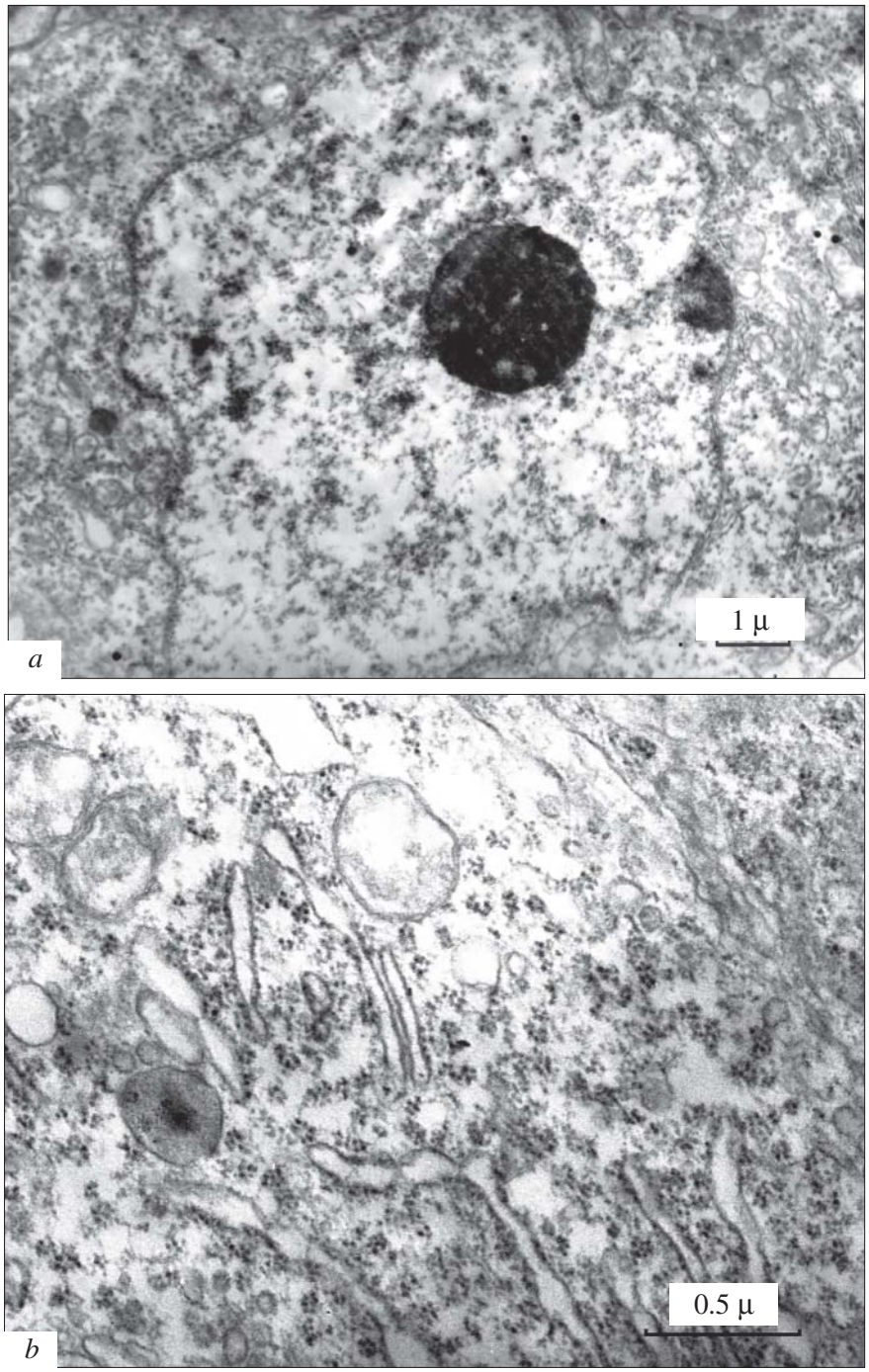

Fig. 1. Ultrastructure of a neuron in the giant cell reticular nucleus of the medulla oblongata in a 30-day-old rat exposed to emotional and painful stress for 3 (*a*) and 7 (*b*) days.

of the mitochondria were seen in the intermediate compartment of the perikaryon. The cristae were numerous, with lamellar structure. The matrix had moderate electron density. The cytoskeleton in the neuronal perikarya was well-developed. More pronounced ultrastructural differences between "dark" and light neurons were seen. The cytoplasm in light neurons was characterized by low electron density of the cytosol and numerous free ribosomes and solitary multivesicular bodies. Euchromatin predominated in the nuclei of the majority of studied neurons. Small islets of heterochromatin in the karyoplasm and chromatin associated with the nucleolus were seen. The nucleoli in light neurons were round, large, sharply hypertrophic, eccentrical, looking compact at the expense of *pars granulosa* (Fig. 1, *a*). The nucleolus in the dark neurons was medium-sized, sometimes was not visualized. The nuclear membrane of the majority of neurons had uneven contour.

On day 7 of EPS well-developed elements of the granular endoplasmic reticulum were seen in the perinuclear and peripheral areas of perikarya. Focal dilata-

tion of tubules developing into pronounced edema with the formation of vacuoles with electron-light contents was seen (Fig. 1, *b*). The content of free ribosomes and polysomes increased. Focal clarification of the matrix with signs of edema and extension of the intermembrane space were seen in some mitochondria. Peroxisomes with compact core and focal impairment of the membrane integrity were seen in the cytoplasm. Euchromatin predomination, hypertrophic and ectopic nucleoli were seen in the nuclei of the majority of neurons. Small accumulations of heterochromatin under the nuclear membrane were seen in solitary neurons. The karyolemma in the majority of neurons of experimental animals had uneven contour and formed invagination. Areas with extended perinuclear space were seen. The plasma membrane participated in the formation of numerous axosomatic synapses. The degree of ultrastructural changes varied significantly in different regions of GRN.

These findings suggest that neurons of the giant-cell reticular nuclei in a growing organism retain their viability and demonstrate ultrastructural signs of activation of the protein synthesis system during the first week of EPS exposure. These changes can be regarded as a manifestation of complex adaptation reaction of giant-cell nucleus neurons of the reticular formation, during which in adult rats hyperexpression of Fos protein and increase in the counts of Fos-positive neurons were observed [12]. Ultrastructural findings are in line with our previous results of immunohistochemical study [3] demonstrating increased level of NF-200 protein in axons of the reticular formation neurons in the brain stem of growing rats exposed to acute stress indicating active restructuring of the cytoskeleton elements.

More pronounced heteromorphism of neurons in the medullary nucleus detected under conditions of EPS is a manifestation of changed rate of normal histogenesis in the presence of neuronal resistance to apoptogenic stimuli. This resistance in neurons of young rats is higher than in adult animals [10]. However, the appearance of the initial ultrastructural signs of injury in some organelles seems to be associated also with changes in the microenvironment [3] and can precede irreversible processes leading to disorders in the neuronal growth and differentiation under conditions of longer stress exposure.

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