MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructural Changes in the Lateral Reticular Nucleus of the Medulla Oblongata in Prepubertal Rats under the Effect of Long-Term Stress Exposure

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Ultrastructural changes in the lateral reticular nuclei of the medulla oblongata of rat pups developing under the effect of chronic mental and pain stress indicate impaired histogenesis of structures of the medulla oblongata reticular formation and appearance of pronounced morphofunctional differences between the neurons.

Key Words: *mental and pain stress; medulla oblongata; prepubertal rats; ultrastructure*

The growth and formation of the nervous system is completed during the prepubertal period [5,10]. One-week exposure of rat pups to stress factors during the 2nd month of postnatal development leads to structural changes in neurons in some brain stem nuclei [2,3]. It is assumed that structures of the brain stem reticular formation mediate activation of the sympathoadrenal system during stress [1,4]. Ventrolateral nuclei of the reticular formation of the medulla oblongata contain tonically active neurons inducing generalized vasoconstriction and adrenergic vasoconstrictor effects [9].

We studied ultrastructural changes in neurons of the lateral reticular nucleus (LRN) in rat pups exposed to long-term mental and pain stress (MPS).

MATERIALS AND METHODS

The study was carried out on 40 outbred prepubertal albino rats (aged 30 days at the start of the experiment). Long-term MPS was induced by group fixation of animals by the withers [2-4]. The animals were daily exposed to 3-h MPS for 15 (group 1; n=10) or 30 days (group 2; n=10). Groups 3 and 4 consisted of 10 controls each, aging 45 and 60 days, respectively. The animals were sacrificed under ether narcosis in accordance with "Regulations for Handling Experimental Animals".

Fragments of the medulla oblongata (1 mm^3) for electron microscopy were fixed for 12 h in 4% paraform solution in 0.1 M cacodylate buffer, postfixed (2 h) in 1% OsO_4 in 0.1 M cacodylate buffer at 4°C [6], washed in several portions of cacodylate buffer, dehydrated in ascending alcohols, and embedded in epon-araldite.

Semithin sections (1 μ) were stained with methylene blue. Ultrathin sections (50-90 nm) were prepared on an LKB-8800 ultramicrotome and mounted on copper grids. After contrasting with 2.5% uranyl acetate in 50% ethanol (40 min) and 0.3% lead citrate (20 min) the sections were examined under a Tesla BS-500 electron microscope (accelerating voltage 60 kV). Photographs were made on photographic plates for nuclear studies.

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RESULTS

Pronounced ultrastructural changes were detected in the LRN. Euchromatin unevenly distributed in the karyoplasm predominated in the nuclear ultrastructure of the majority of medium-sized and small neurons. Significant accumulations of marginally located chromatin with uneven contours appeared. These ultrastructural changes became more pronounced by day 30 of MPS.

By day 15 of MPS, a narrow area formed around the nucleus in the perikaryon cytoplasm in the majority of medium-sized and small neurons; this area appeared as a result of slower neuron growth, which manifested in higher values of the nucleus/cytoplasm ratio. Large mitochondria with clarified matrix and focal lysis of cristae and sharply dilated cisterns of the rough endoplasmic reticulum were seen in the perikaryon cytoplasm (Fig. 1). The relative density of free and bound ribosomes decreased. These ultrastructural changes in the mitochondria persisted on day 30 of MPS and were paralleled by a pronounced pericellular edema.

In contrast to the majority of medium and small neurons, in which MPS decreased the number of ultrastructures involved in protein synthesis, the perikaryons of large neurons contained well-developed rough endoplasmic reticulum cisterns and numerous mitochondria with intact cristae.

On day 15 of MPS, numerous distorted of cross-section profiles of myelin nerve fibers of different diameters were seen in the neuropile. The thickness of the myelin sheath varied. Large nerve fibers with thick myelin sheath looked less damaged. In some cases, edema involved the axial cylinder. In many neurons, foci of myelin sheath loosening were combined with areas of its destruction, appearance of numerous small vesicles of low electron density. These changes persisted on day 30 of MPS; "foamy" axoplasm sites appeared (Fig. 1). Large irregularly shaped vacuoles formed between the axolemma and inner coils of the mesaxon and between the lamellae, which led to loss of fibers in the myelin membrane, forming deep focal invaginations. Electron density of the axoplasm greatly varied. Neurofilaments and microtubules predominated in it; oval, elongated, bent mitochondria were seen, adjacent to the axolemma. The mitochondrial membranes were retained, in some cases the matrix was clarified; focal lysis of cristae was observed. Moderate interstitial edema was noted.

The detected differences in the type of ultrastructural changes in different populations of LRN neurons are confirmed by the data indicating that chronic stress exposure leads, on the one hand, to a decrease in the content of α_{2A}-adrenoreceptor RNA [13] in the LRN neurons and on the other, to an increase in enkephalin content [11] and α_{2A}-adrenoreceptor RNA level in large glutamatergic LRN neurons [7], which is regarded as a sign of increased functional activity of some LRN neurons during adaptation to chronic stress exposure. Considering that the vasomotor center is located in the ventrolateral nuclei of the reticular formation of the medulla oblongata [8,9,12], the ultrastructural changes detected in LRN neurons of prepubertal rat can promote the formation of arterial hypertension in long-term stress.

Thus, the detected ultrastructural changes can be regarded as a manifestation of great morphological variability of the reticular formation neurons during the formation of long-term adaptation of the

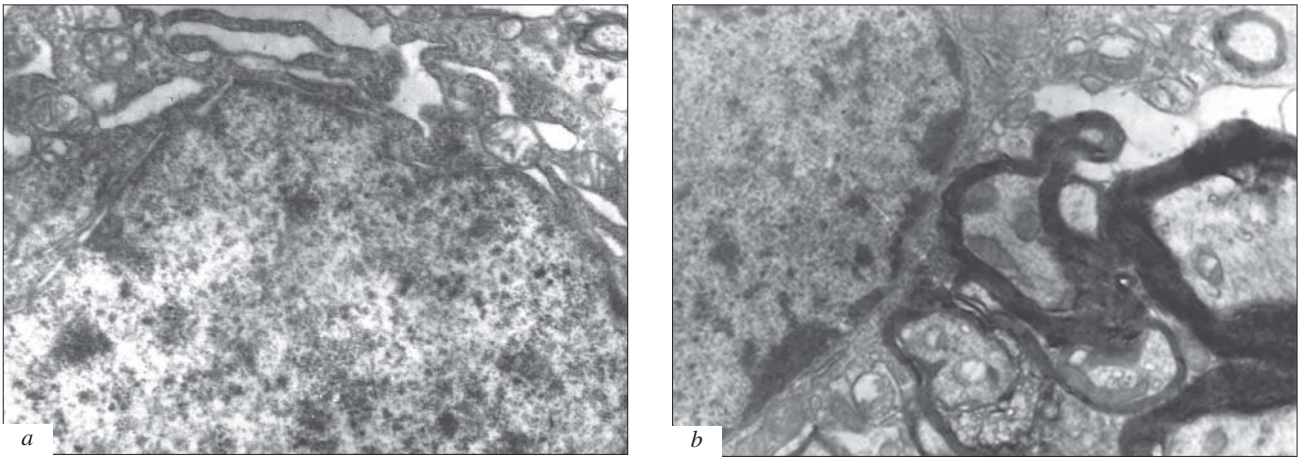

Fig. 1. Ultrastructural changes in LRN of the medulla oblongata in rats exposed to MPS. *a*) neuronal perikaryon with sharply dilated rough endoplasmic reticulum cisterns on day 15 of MPS (×10,000); *b*) neuropile with axoplasm vacuolation, focal loosening of myelin on day 30 of MPS (×10,000).

prepubertal CNS. Characteristic features of this stage of common adaptation syndrome are augmentation of the ultrastructural signs of reversible injuries, exhaustion of the intracellular reserves in the perikaryons of predominantly small and medium-sized LRN neurons in the presence of more or less intact ultrastructural elements in the majority of large neurons. These ultrastructural differences can lead to changes in functional activity of LRN neurons and, presumably, reflect disordered histogenesis of the reticular formation nuclei in the postnatal ontogeny during long-term stress exposure.

REFERENCES

1. P. A. Motavkin, *Manual of Histology* [in Russian], Vol. 2, St. Petersburg (2001), pp. 553-563.
2. V. B. Pisarev, A. V. Smirnov, P. A. Khloponin, and V. P. Tumanov, *Byull. Eksp. Biol. Med.*, **140**, No. 8, 215-217 (2005).
3. A. V. Smirnov, G. L. Snigur, and D. Yu. Gurov, *Vestn. Volgograd. Gos. Univer.*, No. 2, 8-11 (2004).
4. E. A. Yumatov, *Mental Stress: Theoretical and Clinical Aspects* [in Russian], ed. by K. V. Sudakov and V. I. Petrov, Volgograd (1997), pp. 23-28.
5. V. B. de Graaf-Peters and M. Hadders-Algra, *Early Hum. Dev.*, **82**, No. 4, 257-266 (2006).
6. M. A. Nasser Hajibagheru, ed., *Electron Microscopy Methods and Protocols*, Totowa, New Jersey (1999).
7. G. Flugge, M. van Kampen, H. Meyer, and E. Fuchs, *Eur. J. Neurosci.*, **17**, No. 5, 917-928 (2003).
8. T. Kishi, Y. Hirooka, Y. Kimura, *et al.*, *Circulation*, **109**, No. 19, 2357-2362 (2004).
9. A. Korsak and M. P. Gilbey, *Neuroscience*, **124**, No. 3, 709-717 (2004).
10. P. Levitt, *J. Pediatr.*, **143**, No. 4, Suppl., S35-S45 (2003).
11. J. A. Mansi, S. Laforest, and G. Drolet, *J. Neurochem.*, **74**, No. 6, 2568-2575 (2000).
12. D. N. Mayorov, G. A. Head, and R. De Matteo, *Hypertension*, **44**, No. 1, 101-106 (2004).
13. H. Meyer, M. Palchaudhuri, M. Sheinin, and G. Flugge, *Brain Res.*, **880**, No. 1-2, 147-158 (2000).
14. S. Nosaka, K. Murata, M. Kobayashi, *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **279**, No. 3, 1239-1247 (2000).