Morphofunctional Changes in Mitochondria during Stress

S. A. Lobanov, A. V. Danilov, E. V. Danilov, S. K. Asaeva, G. F. Arslanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 12, pp. 699-702, December, 2007
Original article submitted April 3, 2007

Electron microscopy revealed morphofunctional changes in mitochondria of rats under stress conditions. Reparative processes and pathomorphological changes were monitored. Heterogeneous ultrastructural changes in mitochondria were revealed.

Key Words: *cerebellum; mitochondria; neurons; ultrastructure*

Cells of the organism are characterized by high plasticity. Functional overload and treatment with various agents and bioactive substances can induce overstrain, which is followed by the development of pathological processes [1-3,5]. They primarily include structural and functional changes in the cell energy system. Many subcellular mechanisms of the cell response are poorly understood [4-6]. Studying the role of mitochondria in compensatory and adaptive processes in cells under various functional conditions is of considerable theoretical and practical importance.

Here we studied structural and functional characteristics of mitochondria in cerebellar neurons under the influence of stress factors.

MATERIALS AND METHODS

Experiments were performed on 23 outbred albino rats weighing 195-205 g. The study was conducted in the daytime (winter period) for 30 days. During hyperdynamia, the rats were placed in a special cage. This cage was put on a treadmill. Each session lasted for 45 min and the rest period was 15 min (12 sessions per day were performed). Structural characteristics of mitochondria were evaluated during hyperdynamia. The cerebellum was isolated 1, 14, and 30 days after the start of the study, fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.2-7.4), placed in OsO_4 for 1 h, dehydrated in increasing concentrations of alcohols and acetone, and embedded into Epon-812. Ultrathin sections were prepared on a LKB-III ultratome and contrasted with lead salts and uranyl acetate. Sections were examined under a JEM-100S electron microscope at an accelerating voltage of 90 kV. Normalization of the state was evaluated on day 14 after treatment. Intact rats were maintained in a vivarium under standard conditions and served as the control. Each group consisted of 5 animals.

RESULTS

The cerebellum of rats is presented by neurons. The number and quality of mitochondria in neurons depend on their functional state. The majority of mitochondria in rat cerebellar neurons looked like small round grains or thin rods with a diameter of 0.1 μ. The shape and size of mitochondria varied in different cells. There were round or elongated rod-shaped mitochondria with a diameter ≥0.3-0.5 μ. The length of thread-like mitochondria reached several microns. Branched mitochondria were often found in preparations (Fig. 1, *a*, *b*). Ultrastructural study of neurons showed that 18.5% mitochondria in bodies of rat cerebellar neuronal are presented by branched forms. Branching of mitochondria was

M. Aktulla Bashkirian State Pedagogical University, Ufa. ***Address for correspondence:*** lobanovprof@yandex.ru. S. A. Lobanov

0007-4888/07/1446-0849

not necessarily observed in individual sections. In several regions of the cytoplasm, small profiles of mitochondria were adjacent to one or two large profiles. These data suggest the existence of mitochondria with a complex spider-like shape. The majority of mitochondria in neuronal processes had a rod-like shape. The diameter and length of these mitochondria were 0.1 and ≥1 μ, respectively. Thin mitochondria prevailed in dendrites and axons. In neuronal regions with synaptic terminals, these mitochondria looked like small spherical structures. Very long and thin mitochondria were identified in fine preterminal parts of axons and terminal regions of dendrites. The length of these mitochondria reached 12-20 μ. Mitochondria were distributed over the cytoplasm of the perikaryon. Study of electron microphotographs showed that mitochondria are absent in the Nissl substance. They were usually localized along the outer boundary of this substance. Mitochondria were identified near parallel microtubules and neurofilaments and elements of the smooth endoplasmic reticulum. Under normal conditions, mitochondria of cerebellar neurons had a smooth elementary membrane. The folded membrane was situated near the inner surface of the smooth membrane. This membrane covered the so-called inner mitochondrial space with a dense matrix. The intermembrane space is the outer mitochondrial space with the contents of a much lower density. The outer and inner membranes significantly differ in the structure, chemical composition, and function.

After stress exposure, the number of peduncular round particles with a diameter of 80-95 nm increased on the surface of the inner membrane, which was directed toward the space of the organelle. However, the outer and inner surface of the outer mitochondrial membrane remained smooth.

Study of electron microphotographs showed that mitochondria of cerebellar neurons have longi-

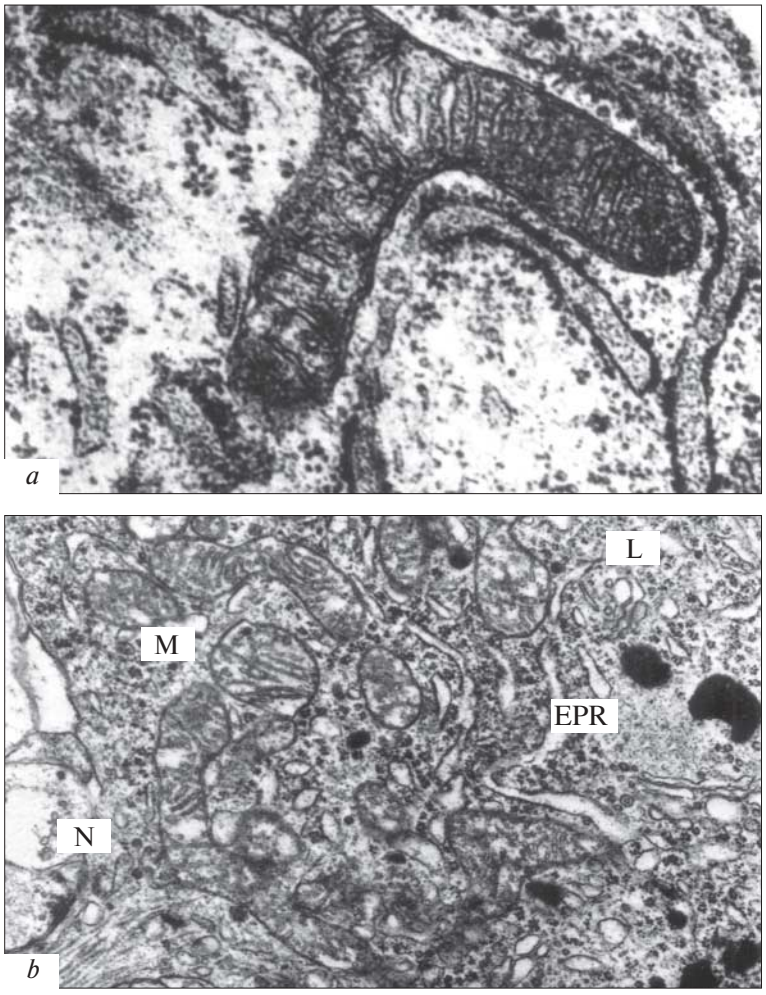

Fig. 1. Mitochondria of Purkinje cells from rat cerebellum under normal conditions (*a*) and 24 h after hyperdynamia (*b*). Branched mitochondria with the matrix of moderate electron density and well-defined cristae (*a*, ×48,000). Mitochondria of different shape and size (*b*, ×40,000). EPR, endoplasmic reticulum; M, mitochondria; N, neurofilaments; L, lysosomes in the zone of degeneration.

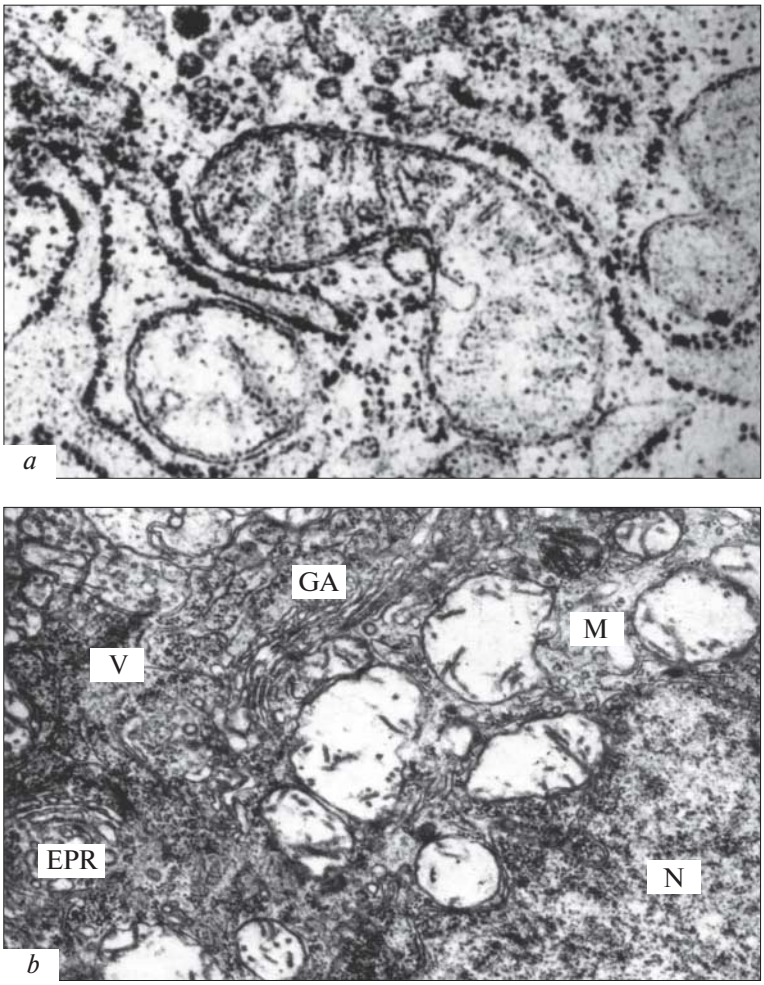

Fig. 2. Mitochondria of Purkinje cells from rat cerebellum 14 (*a*) and 30 days after hyperdynamia (*b*). Branched mitochondria with the lightened matrix (*a*, ×55,000). Mitochondria with damage to the inner membrane (*b*, ×37,000). N, nucleus; M, mitochondria; GA, Golgi apparatus; EPR, endoplasmic reticulum; V, vesicles.

tudinal cristae. This specific feature of the internal structure was most typical of long thin mitochondria. They were mainly found in processes of cerebellar neurons. Since these mitochondria were very thin, the crista often occupied the entire length of the organelle. On the transverse section of mitochondria, this crista was "suspended" in a dense matrix. The crista was not connected to the inner membrane from which it arose.

It should be emphasized that mitochondria first responded to the influence of stress factors. Mitochondria were characterized by severe swelling, formation of giant forms, significant destruction, and accelerated disintegration (Fig. 2, *a*, *b*). Temporal changes in cellular metabolism and decrease in structural and functional activity of organelles (primarily of mitochondria) were observed in the follow-up period. Changes in the inner mitochondrial membrane and mitochondrial matrix were also revealed in neurons of the cerebellar cortex. Ultrastructural characteristics of several mitochondria were recovered when the animals returned to normal vital activity.

As differentiated from control animals, mitochondria of hyperdynamic rats included numerous small promitochondria. The formation of promitochondria was related to reparative regeneration of the inner membrane in mitochondria with the intact outer membrane. Massive proliferation of coated vesicles in the Golgi apparatus had a positive effect on intracellular processes of reparative regeneration during this period.

Another characteristic of mitochondria in cerebellar neurons from stressed animals is the absence of dense granules in the matrix. Small number of mitochondrial granules probably results from low energy reserves and high demands in glucose and oxygen, which is typical of brain cells.

Our results indicate that neuronal mitochondria are closely related to organelles, which require mi-

tochondrial ATP. However, mitochondria of cerebellar neurons are not closely related to the Nissl substance. They do not constitute the endoplasmic reticulum, which has a considerable number of ribosomes. Mitochondria of nerve cells are rarely found in deep layers of the Nissl substance.

Stress is accompanied by structural and functional changes in mitochondria, which primarily concerns the mitochondrial matrix and inner membrane. The compensatory and adaptive response of neurons to stress is characterized by strain and reconstruction of the cell energy system.

REFERENCES

1. I. P. Ashmarin and P. V. Stukalov, *Neurochemistry* [in Russian], Moscow (1996).
2. S. A. Lobanov and T. F. Emeleva, *Cerebellum and Stress* [in Russian], Ufa (2005).
3. S. A. Lobanov, T. F. Emeleva, A. V. Danilov, *et al*., *Med. Vestn. Bashkortostana*, No. 1, 72-75 (2006).
4. S. V. Solov'ev and S. P. Gerasin, *Ros. Med. Biol. Vestn*., Nos. 1-2, 183-186 (2000).
5. P. J. Attwell, S. F. Cooke, and C. H. Yeo, *Neuron*, **34**, No. 6, 1011-1020 (2002).
6. G. Kroemer and J. Reed, *Nat. Med*., **6**, No. 5, 513-519 (2006).