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Electron Microscopic Study of Left Ventricular Cardiomyocyte Mitochondriom in Rats Subjected to Head-Down Tilt

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The mitochondriom of left-ventricular cardiomyocytes in rats subjected to head-down tilt was studied at the electron microscopic level. The ultrastructure of individual mitochondria was disturbed and the number of intermitochondrial contacts in the perinuclear zone increased after 30-day head-down tilt. These parameters did not return to normal over 30 days after 30-day tilt. Repeated 14-day head-down tilt led to a more pronounced increase in the number of intermitochondrial contacts, mitochondria with abnormal ultrastructure were still observed.

Key Words: *mitochondriom; intermitochondrial contacts; cardiomyocyte; head-down tilt*

Cardiomyocytes (CMC) contract constantly and rhythmically and therefore they require incessantly functioning system of energy production. Energy production system in CMC is presented by mitochondriom, a network formed by numerous mitochondria united via intermitochondrial contacts (IMC) [2,5]. The mitochondriom is well studied. The ultrastructure of IMC is described in detail: on sections these structures are seen as zones of maximum approximation of mitochondrial membranes with a drastic local increase in electron density of membranes and intermembrane spaces [2]. The role of IMC consists in the transfer of energy in the form of electric potential through membranes of the united mitochondria [6], which allows us to regard mitochondria united by IMC as an intracellular membrane cable [9]. The number of IMC increases with increasing of functional load on CMC and *vise versa* [5,7]. This is paralleled by changes in other parameters of the mitochondriom (organelle ultrastructure, number, size, *etc.*) [5].

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We studied the behavior of left ventricular CMC mitochondriom in rats during head-down tilt. This method is widely used for simulation of the effect of zero gravity [8,10]. We previously described changes in other CMC parameters of animals subjected to head-down tilt [3]. The cardiovascular system is most liable to restructuring under conditions of true and simulated zero gravity [1,10], which determines not only theoretical, but also practical interest to these studies. It is difficult to predict changes in the left ventricular CMC mitochondriom during head-down tilt, because it is not clear whether it increases or decreases functional load to these cells. On the one hand, load to the left ventricle decreases due to hypokinesia and atrophy of skeletal muscles of hind paws during tilt [8] and hence, to lower need in blood supply. On the other hand, the function of the left ventricle in a head-down position is difficult because of necessity to pump a greater stroke volume of blood upward (against the gravitation force) [1].

We studied changes in the left ventricular CMC mitochondriom in head-down tilted animals, 30 days after tilt, and during repeated tilt.

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MATERIALS AND METHODS

Male Wistar rats were subjected to head-down tilt by the standard method [8]. The animals were divided into 5 groups: 1) 14-day head-down tilt; 2) 30-day head-down tilt; 3) 30 days in a normal position (rest) after 30-day head-down tilt; 4) repeated 14-day head-down tilt (after the first 30-day head-down tilt followed by 30-day rest); and 5) control.

Fragments of the left ventricular myocardium from the heart apex were fixed in 4% glutaraldehyde, post-fixed in 1% OsO_4, dehydrated by the standard method for electron microscopy, and embedded in epon. Longitudinally oriented CMC were cut on an LKB III ultratome. Ultrathin sections were additionally contrasted in aqueous solution of uranyl acetate and lead citrate after Reynolds and examined under a JEM-100C electron microscope at 80 kV. Location of the mitochondria in CMC zones (interfibrillar, perinuclear, subsarcolemmal perivascular) was analyzed on electron microphotographs by numerical density of mitochondrial profiles (number per unit area) and the number of IMC per 100 mitochondria was counted.

The data were statistically processed using Student's *t* test and nonparametrical Mann—Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

No changes were observed after 14-day head-down tilt: in all zones of the cell the numerical density of mitochondria, their ultrastructure and number of IMC were within the normal range (Fig. 1).

After 30-day head-down tilt the numerical density of mitochondria did not differ from the control (Fig. 1, *a*), but in some CMC mitochondria local clarification of the matrix and local destruction of cristae were noted (lysis; Fig. 2, *b*). Mitochondria with these disorders were more often seen in the perinuclear and subsarcolemmal perivascular zones. The number of IMC increased by 27% in comparison with the control in the perinuclear zone, showed a trend to increase in the interfibrillar zone, and did not change in the subsarcolemmal perivascular zone (Fig. 1, *b*). According to published data [4], mitochondria divide in the perinuclear zone; mitochondria with impaired ultrastructure were seen in this zone. These facts indicate restoration of mitochondrial number in this zone for maintaining their pool under conditions of head-down tilt. Increased number of IMC in the perinuclear zone is presumably provides additional energy for these processes. On the other hand, the detected increase in the number of IMC in CMC at this term can be due to increased energy requirement for pumping the blood upwards against the gravitation force.

The numerical density of mitochondria did not change after 30-day rest (Fig. 1, *a*). Abnormal mitochondria (with local destruction of cristae) were seen, *i. e.* the changes were similar to those observed at the previous stage of the experiment. The number of CMC decreased in comparison with the previous stage: to the control level in the perinuclear zone, below the control level in the subsarcolemmal perivascular zone, and only tended to decrease in the interfibrillar zone (Fig. 1, *b*). Hence, normalization of ultrastructure of mitochondria and number of IMC require a longer period than the period of head-down tilt during which changes in these parameters developed. Slow recovery of the mitochondriom after the end of exposure was also observed under other experimental conditions [7].

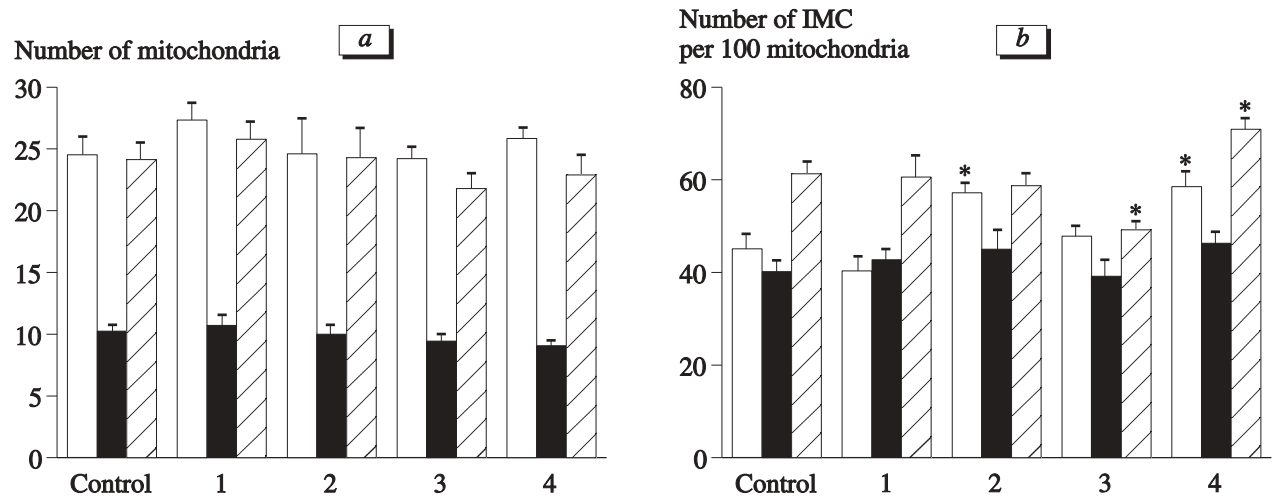

Fig. 1. Morphometric parameters of rat left ventricular cardiomyocyte mitochondria during head-down tilt. *a*) numerical density of mitochondria per 10 μ²; *b*) number of intermitochondrial contacts (IMC). Light bars: perinuclear zone; dark bars: interfibrillar zone; cross-hatched bars: subsarcolemmal perivascular zone. *1*) 14-day tilt; *2*) 30-day tilt; *3*) 30-day tilt+30-day rest; *4*) repeated 14-day tilt. *$p<0.05$ compared to the control.

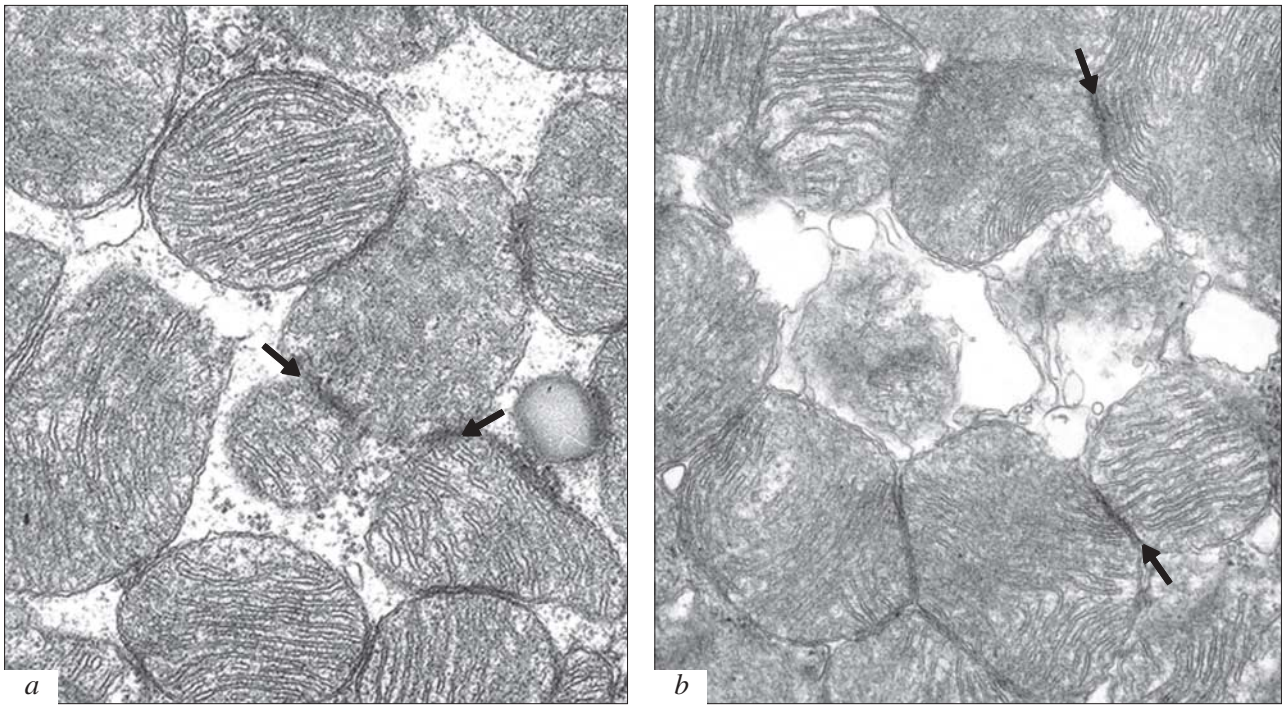

Fig. 2. Ultrastructure of left ventricular cardiomyocytes. *a*) control; *b*) experiment; 30-day head-down tilt. Mitochondria with impaired ultrastructure are seen in the center, ×36,700. Arrows show intermitochondrial contacts.

The numerical density of mitochondria remained unchanged in comparison with the control after 14-day repeated head-down tilt (Fig. 1, *a*). The ultrastructure of some mitochondria was changed and these changes were similar to those observed at the previous stages of the experiment: local clarification of the matrix and destruction of cristae. It should be noted that these changes were more abundant in the perinuclear area. The number of IMC increased more than during the previous stages (Fig. 1, *b*): by 30 and 16% of control and by 22 and 44% of the levels during the previous stage in the perinuclear and subsarcolemmal zones, respectively. In the interfibrillar zone the number of IMC tended to increase in comparison with the control and previous term. Hence, this experiment again demonstrated different reaction of mitochondria located in different zones of CMC, which is in line with previous reports [5,7]. Increased number of IMC against the background of unchanged numerical density of mitochondria confirms the dependence of IMC number on functional load to CMC, but not on the density of mitochondria. More pronounced increase in the number of IMC after repeated exposure can be explained by persisting changes in CMC (e.g. altered mitochondria) developed during the first exposure.

Hence, 30-day head-down tilt induced changes in the ultrastructure of some mitochondria in CMC and increased the number of IMC in the perinuclear zone of these cells. These parameters did not return to normal after 30-day rest. Repeated 14-day head-down tilt led to more pronounced increase in the number of IMC, the ultrastructure of CMC mitochondria remained impaired.

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