STRUCTURAL RESPONSE OF SKELETAL MUSCLE MITOCHONDRIA TO EXPERIMENTAL DEEP HYPOTHERMIA

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The homoiothermic organism gives a powerful response to exposure to acute cold in the form of contractile thermogenesis, which is aimed at maintaining temperature homeostasis. The level of metabolism of the skeletal musculature under these circumstances may be raised by as much as 40 times [4, 5]. The intensity of the processes of thermogenesis is largely determined by the morphological and functional state of the mitochondrial apparatus of the muscles and their supply of energy substrates [9]. The dynamics of changes in activity of the enzymes of glycolysis and oxidative discussed in the literature [7, 8, 11]. However, the structural basis of readjustment of the mitochondrial apparatus of the muscle fibers in response to cold has virtually not been studied.

The aim of this investigation was to undertake a qualitative and quantitative study of the mitochondrial apparatus of the muscle fibers of a locomotor muscle in deep hypothermia in experimental animals.

EXPERIMENTAL METHOD

Experiments were carried out on 24 noninbred male albino rats weighing 200-250 g. The state of deep hypothermia was obtained in the animals, immobilized in constraining cages, by external cooling. The rats were killed immediately their rectal temperature reached 15°C (series I), 4 h after prolonged deep hypothermia (series II), and on emergence from a state of deep-hypothermia when the body temperature was 35.5°C (series III). Intact animals served as the control. Samples of the trapezius muscle were fixed in a mixture of 2.5% glutaraldehyde solution and 2% paraformaldehyde solution in 0.1 M phosphate buffer, followed by postfixation in 1% OsO_4 solution, and embedded in a mixture of Epon and Araldite resins by the usual technique of electron microscopy. Ultrathin sections were cut on the LKB 8800 Ultramicrotome and examined in the JEM-100SX electron microscope. Quantitative parameters of the mitochondria were determined morphometrically: the relative volume, surface density, and number of profiles per 10 μ^2 area of section. The data were subjected to statistical analysis by Student's test on the BSM-6 computer.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the trapezius muscle of the animals of series I in the intial period of deep hypothermia revealed considerable changes in mitochondrial ultrastructure in the muscle fibers. Many mitochondria had signs of a varied degree of swelling: translucency of the matrix and widened cristae. Swelling of the organelles was often accompanied by disorientation and fragmentation of the cristae, which had acquired the appearance of short, wide tubules. Morphometric analysis at this period of the experiments revealed a decrease in the number of mitochondrial profiles, and this was especially marked at the periphery of the fibers. The relative volume of the mitochondria remained the same as in the control (Table 1).

In the animals of series II the structure of the mitochondrial apparatus underwent further qualitative and quantitative changes during prolonged deep hypothermia. The overwhelming majority of mitochondria showed signs of considerable swelling of the matrix and destruction of the inner membranes. Vacuolated mitochondria with a highly translucent matrix, completely without cristae, were found. According to the results of morphometric

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TABLE 1. Morphometric Parameters of Mitochondria of Muscle Fibers at Different Stages of Deep Hypothermia (M ± m)

Parameter	Series of experiments	Periphery of fiber	Center of fiber	Mean value
Relative volume, %	Control	7,50±0,65	5,47±0,47	6,46±0,36
	I	7,11±0,61	5,18±0,44	6,15±0,38
	II	7,73±0,74	6,27±0,26	7,10±0,43
	III	6,30±0,64	4,46±0,30	5,39±0,36*
Number of profiles per 10 μ^2 of section	Control	7,00±0,38	4,51±0,34	5,78±0,24
	I	5,64±0,31*	4,70±0,30	5,13±0,21*
	II	5,19±0,44*	4,72±0,33	4,92±0,24*
	III	5,78±0,35*	4,42±0,38	5,10±0,23*
Surface density, μ^{-1}	Control	1,13±0,09	0,95±0,10	1,07±0,09
	I	0,97±0,06	1,00±0,06	0,96±0,06
	II	1,08±0,09	1,10±0,09	1,08±0,06
	III	1,01±0,09	0,75±0,06	0,83±0,07*

Legend. *$p < 0.05$ Compared with control.

analysis, the decrease in the number of mitochondrial profiles at the periphery of the fibers at this period of the experiments was even greater than in the previous series (Table 1).

As regards the relative volume and surface density of the mitochondria, the values of these parameters were close to the control levels, and considering the reduced number of organelles, this points to an increase in their size, undoubtedly due to swelling.

On emergence from the state of deep hypothermia, structural heterogeneity of the mitochondrial apparatus increased. Just as in the previous series of experiments, numerous swollen organelles with distinct degenerative features were found. Meanwhile mitochondria with a matrix of increased electron density and with densely packed cristae were frequently observed. The large number of autophagosomes of different sizes at the periphery of the fibers, under the sarcolema, and in the perinuclear zones, are noteworthy. Foci of destruction of the contractile myofibrillary apparatus, resembling myocytolysis, were observed in the muscle fibers in which changes in the mitochondria were most significant. Morphometric analysis revealed a decrease in the number of mitochondrial profiles at the periphery of the fibers, and also a significant decrease in the average values of relative volume and surface density of these organelles compared with the control (Table 1).

Analysis of the ultrastructural organization of the mitochondria at different periods of deep hypothermia revealed that the most typical changes, and virtually the only changes in the animals of series I, were a varied degree of translucency of the matrix and widening of the cristae. Structural changes of this kind, according to Bulychev [2], are characteristic of these organelles during a change in cationic membrane permeability and in the activity of their enzyme systems. They are evidently reversible in character and can be regarded as a manifestation of considerable functional strain on the energy-producing apparatus of the muscle fibers associated with activation of contractile thermogenesis.

According to data obtained by other workers, a high level of functional strain [6], and also the influence of cold itself [1, 3, 10], are factors stimulating lipid peroxidation, and thus enhancing the toxic action of its products on the lipid components of membranes, and in particular, mitochondrial membranes. It may perhaps be the effect of these factors which led, in the animals of series II and III, to aggravation of the signs of destruction of mitochondria, associated with the irreversible loss of their functions.

The results of morphometric analysis also indicate reduction of the power of the mitochondrial apparatus. The tendency toward a decrease in the number of mitochondrial profiles, observed in the period of cooling of the animal, became more marked in the next series of experiments. Besides this, in the animals of series III on emerging from the state of deep hypothermia all the average quantitative parameters of these organelles decreased significantly. The fact that, despite the decrease in the number of mitochondria, their relative volume in the rats in the experiments of series I and II was unchanged, can be attributed to an increase in size of the organelles as a result of swelling. The presence of disturbances of the contractile myofibrillary apparatus and their correlation with destructive changes in the mitochondria are convincing evidence of the functional insufficiency of the energy pro-

viding apparatus of the cell, which is unable to satisfy completely the increased metabolic demands of a muscle which is working intensively during reheating of the body.

LITERATURE CITED

1. A. M. Belous and V. A. Bondarenko, Structural Changes in Biological Membranes during Cooling [in Russian], Kiev (1982).
2. A. G. Bulychev, Structure and Functions of Biological Membranes [in Russian], Moscow (1975), p. 59.
3. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
4. K. P. Ivanov, The Muscular System and Chemical Temperature Regulation [in Russian], Moscow–Leningrad (1965).
5. K. P. Ivanov, Bioenergetics and Temperature Homeostasis [in Russian], Leningrad (1972).
6. E. M. Kreps, Lipids of Cell Membranes [in Russian], Leningrad (1981).
7. D. I. Ryzhakov, A. V. Molodyuk, and N. V. Murav'eva, Hypothermia in Clinical and Experimental Medicine [in Russian], Gor'kii (1979), p. 29.
8. N. N. Timofeev, Artificial Hypobiosis [in Russian], Moscow (1983).
9. V. V. Khaskin, Physiology of Temperature Regulation [in Russian], Leningrad (1984), p. 237.
10. A. P. Shepelev, Fiziol. Zh. SSSR, 63, No. 3, 442 (1977).
11. T. F. Davison, Comp. Biochem. Physiol., 38, 21 (1971).

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF CLARA CELLS IN ANOXIC RATS BASED ON DATA OF SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

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The characteristics of structural and metabolic changes taking place in the lung in various anoxic states have been dealt with sufficiently widely in the literature [1-4], whereas only isolated publications have been devoted to the ultrastructure of the Clara cells in anoxia [7-9].

The aim of this investigation was to study ultrastructural changes in Clara cells of the terminal bronchioles and to evaluate their secretory activity in chronic anoxia with the aid of transmission (TEM) and scanning (SEM) electron microscopy.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. Anoxia was produced in a pressure chamber in which the air pressure was reduced to 310-340 mm Hg, equivalent to an altitude of 7000 m above sea level; exposure lasted 2 h. The rats were taken from the experiments by an injection of phentobarbital 5, 15, 30, and 60 days from its beginning. For SEM the lung was fixed by intratracheal injection of a 2.5% solution of glutaraldehyde under a pressure of 10 cm water. The material was dried at the critical point in liquid CO_2. After spraying with gold the specimens were examined in a "Hitachi S-500" scanning electron microscope. Secretory activity of the Clara cells was judged from the state of the apical surface [6]. With respect to this feature all the cells were divided into four groups: I) the

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