QUANTITATIVE ANALYSIS OF CARDIOMYOCYTE MITOCHONDRIAL ULTRASTRUCTURE OF RATS ADAPTED TO ALTITUDE HYPOXIA

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In the study of adaptation of the myocardium to the various factors of hypoxic states ultrastructural changes have recently been described in different components of heart cells [6-8]. Meanwhile the structure of the organoid systems especially of the mitochondria during prolonged interrupted exposure to pressure chamber hypoxia at high altitudes has not yet been completely studied. Among the studies conducted on this problem, there have been only isolated quantitative ultrastructural investigations [10].

This paper describes a study of the time course of quantitative structural and functional parameters of mitochondria in different zones of cardiomyocytes in rats during long-term adaptation to altitude hypoxia. The techniques used are based on those of an investigation [4] which revealed the particular features of reactions of rat mitochondria to hypokinesia.

EXPERIMENTAL METHOD

Experiments were carried out on adult male albino rats (35 experimental and 5 control). Adaptation to altitude hypoxia was created by keeping the animals daily in a pressure chamber at an "altitude" of 9000 m for 90 days. During the first week of adaptation the period of exposure of the animals was increased successively from 2 h (1st day) to 6 h (7th day). Later during adaptation the animals were kept for 6 h in the pressure chamber daily. To support the life of the rats at a high "altitude" they were "dropped" every 2 h for 15-20 min to an "altitude" of 6000 m, and then raised to an "altitude" of 9000 m. The animals were killed on the 1st, 5th, 10th, 20th, 45th, 60th, and 90th days of the experiment. Myocardium from the right ventricle was fixed in 3% glutaraldehyde and 1% OsO_4 solution and embedded in a mixture of Epon and Araldite. Ultrathin sections were studied in the IEM-100B electron microscope. The following parameters were determined on photographs obtained with a final magnification of 10,000: the mean number of mitochondria per unit area (100 μ^2), the relative area occupied by mitochondria (in %), the mean number of cristae per mitochondrion, the mean length of all the cristae in a mitochondrion, the density index of the mitochondria (the ratio of the area of the mitochondria to the area of the myofibrils), and the density index of the mitochondrial cristae (the ratio of the mean length of the cristae in a mitochondrion to the mean area of mitochondrion). These parameters were chosen because they provide adequate information on the structure and function of the mitochondrial apparatus of the cardiomyocytes.

All parameters were studied separately in the myofibrillary, perinuclear, and subsarcolemmal zones, allowing for their functional heterogeneity (6).

EXPERIMENTAL RESULTS

The quantitative parameters of mitochondria from different zones of the cardiomyocytes of control and experimental rats are given in Table 1. After a single elevation of the animals to an "altitude" of 9000 m most mitochondria from all zones of the cardiomyocytes were swollen and enlarged, with widened spaces between their cristae. The mean values of the number of cristae, their total length, and the area of the mitochondria in the myofibrillary and perinuclear zones were increased. The increase in area of the mitochondria in these zones led to a decrease in the density index of the cristae.

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TABLE 1. Quantitative Parameters of Mitochondria from Cardiomyocytes of Control and Experimental Rats (M ± m)

Duration of experiment, days	Zone of cardiomyocyte	Number of mitochondria in 100 μ^2	Area of mitochondria%	Number of cristae in mitochondria	Total length of cristae in mitochondrion, μ	Density index of mitochondria	Density index of cristae
Control	Myofibrillary	9±1,23	25±2,16	8,1±0,31	8,16±0,01	0,33	3,11
	Perinuclear	10±1,31	22,4±3,02	7,1±0,29	7,9±0,90	0,28	3,51
	Subsarcolemmal	10±1,11	23,6±1,18	9,0±0,37	8,7±0,81	0,30	3,76
1	Myofibrillary	16±1,32*	45,6±2,29*	9,67±0,45*	7,79±1,21	0,83	2,76
	Perinuclear	11±1,58	33,7±3,77*	10,6±0,78*	8,79±1,03	0,50	2,88
	Subsarcolemmal	13±14,6	18,3±0,77	8,3±0,81	6,91±1,19	0,25	4,36
5	Myofibrillary	18±1,46*	40,8±3,62*	6,72±1,28	6,67±0,51	0,68	2,98
	Perinuclear	20±1,53*	38,6±3,05*	6,41±1,78	6,81±0,68	0,62	2,94
	Subsarcolemmal	19±1,75*	32,6±2,02*	6,91±1,53	6,57±0,7	0,48	3,7
10	Myofibrillary	22±1,44*	54,1±4,21*	5,36±0,44*	3,58±1,12*	1,17	1,45
	Perinuclear	23±2,61*	43,1±0,16*	6,18±0,91	4,12±1,07	0,75	2,21
	Subsarcolemmal	17±3,42*	49,3±1,23*	6,03±0,18*	5,11±0,83	0,97	1,76
20	Myofibrillary	23±2,89*	52,1±5,12*	4,41±0,36*	4,80±0,38*	1,08	2,05
	Perinuclear	18±4,97*	41,2±0,16*	6,13±0,80*	4,31±1,26	0,70	3,08
	Subsarcolemmal	26±3,46*	41,4±0,48*	6,4±0,40*	3,50±0,20*	0,71	1,76
45	Myofibrillary	13±2,15	52,5±5,54*	6,41±1,64	75,5±1,12	1,1	1,76
	Perinuclear	28±3,12*	65,5±5,86*	9,2±1,05	10,29±1,43	1,89	4,21
	Subsarcolemmal	16±1,51*	56,6±1,09*	7,8±0,65	10,0±1,51	1,3	2,77
60	Myofibrillary	19±1,75*	34,9±1,07*	6,36±0,7*	6,90±0,45	0,53	3,18
	Perinuclear	10±1,63	21,7±3,58	6,76±1,5	8,17±0,8	0,27	3,70
	Subsarcolemmal	14±1,15	30,5±2,22*	6,31±0,63*	8,70±0,77	0,43	3,66
90	Myofibrillary	11±1,22	25±3,14	4,6±0,33*	4,67±1,01*	0,33	2,05
	Perinuclear	14±1,15	23,6±1,28	5,1±0,13*	4,77±0,81	0,3	2,83
	Subsarcolemmal	12±1,03	24,8±2,1	4,77±0,38*	3,61±0,12*	0,32	1,75

*$P \leqslant 0.02$.

In the subsarcolemmal zone on the 1st day of the experiment the density index of the mitochondria was 20% lower than in the control, whereas the density index of the cristae was increased under these circumstances by 11.6%. By the 5th day of the experiment the number and relative area of the mitochondria were increased, but the number of cristae and the mean total length of the cristae per mitochondrion were reduced in all zones of the cardiomyocytes on average by 23.3%. On the 10th-20th days of the experiment most mitochondria were swollen, with destroyed cristae. The relative area of the mitochondria in the myofibrillary zone was increased by 2.1 times and in the perinuclear zone by 1.8 times. The mean length of the cristae was reduced by 1.7 and 2.5 times, respectively. The density index of the cristae was lowest in the subsarcolemmal zone.

By the 45th-60th days of the experiment the number of mitochondria in different zones of the cardiomyocytes could be either increased or reduced. The density index of the mitochondria reached its highest value in all zones by the 45th day of the experiment. Many small mitochondria with densely packed cristae were seen in the perinuclear zone. The relative area of the mitochondria was increased in this zone to 65.5% from 22.4% in the control. By the 60th day of the experiment the density index of the cristae in all zones of the cardiomyocytes reached the control level. With an increase in the length of stay of the animals under conditions of severe hypoxia the mitochondria in most cells were sharply changed. The time course of the integral morphological parameters showed a tendency for a decrease in the number and relative area of the mitochondria, the mitochondrial index, and the density index of the cristae in all zones of the cardiomyocytes. The maximal decrease in all these parameters occurred by the end of the experiment.

The results of quantitative analysis revealed certain general principles of structural and functional reorganization of the cardiomyocyte mitochondria in rats during long-term adaptation to extremely unfavorable conditions of altitude hypoxia. First, the response of the mitochondrial apparatus to a signal generated by the high functional stress on the heart cell is effected very rapidly. Manifestation of this process could be observed as early as on the 1st day of the experiment, in the form of an increase in the total number and relative area of the mitochondria in the myofibrillary and perinuclear zones and a decrease in the relative area of mitochondria in the subsarcolemmal zone. Heterogeneity of the configurational changes observed in the mitochondria can evidently be explained on the grounds that during a

period of intensive stress reactions of the heart cells functional changes take place in structures within the organoids themselves, with more intensive destructive changes affecting those mitochondria in the subsarcolemmal zone. According to published research, this fact is evidence of disturbances predominantly affecting the membranous structures of the sarcolemma in hypoxic states [9]. In the next stages of the experiment, structural and functional reorganization of the mitochondria in different zones of cardiomyocytes followed a cyclic course. Until the 20th day of the experiment the compensatory-adaptive reaction of the mitochondrial apparatus was reflected mainly in the more intensive formation of small populations of new organelles with an increase in their total number. By the 45th day of the experiment large mitochondria were mainly predominant, with an overall decrease in their quantitative parameters except in the perinuclear zone.

Toward the end of the experiment both the intensity of formation of new mitochondria and the relative area occupied by the mitochondria, and also the total number and length of the cristae were all sharply reduced. It can be concluded from ultrastructural criteria of mitochondrial functional activity [7] that the intermittent activation of individual mitochondrial structures, in the form of their hyperplasia and hypertrophy in different zones of the cardiomyocyte, is an essential condition for ensuring the materials required to maintain the viability of heart cells [3].

The facts described above are evidence that under severely hypoxic conditions at an "altitude" of 9000 m, when the arteriovenous oxygen partial pressure in the cardiomyocytes disappears [2], gradual exhaustion of the functional reserves of the intracellular organelles, especially the mitochondria, takes place and a state of adaptation to high altitudes does not arise.

The structural and functional changes observed in the mitochondrial apparatus of the cardiomyocytes play an important role in the pathogenesis of disturbances of protein synthesis and the reduction in contractility and the functional reserves of the heart in high altitude hypoxia [1, 5].

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