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QUANTITATIVE ULTRASTRUCTURAL CHARACTERISTICS OF RAT CARDIOMYOCYTE MITOCHONDRIA DURING HYPOKINESIA

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UDC 612.172.6.014.21-06:612.766.2

KEY WORDS: hypokinesia; myocardium; mitochondria — adaptive structural changes.

As a result of many ultrastructural studies the nature and dynamics of changes in the various components of the myocardium during hypokinesia have been described [1, 6, 8]. Nevertheless, the structure of the organelle systems, such as the mitochondrial apparatus of the cardiomycocytes, in hypokinesia, has not yet been fully investigated. Among investigations devoted to this problem [1, 2], there have been only isolated quantitative studies [6].

The object of this investigation was to study quantitative structural and functional indices of cardiomyocyte mitochondria of rats kept for 30 days under conditions of hypokinesia.

EXPERIMENTAL METHOD

Experiments were carried out on rats (9 control and 18 experimental) aged 6-8 months. To create conditions of hypokinesia the rats were placed in restraining cages. The rats were killed on the 10th, 20th and 30th days of the experiment. Pieces of left ventricular myocardium were fixed in 1% osmium tetroxide solution and embedded in Araldite. Sections were cut on UMTP-I and KV-III microtomes and photographed in UEMV-100B and JEM-7A electron microscopes. The following indices were determined on photographic prints under a magnification of 30,000: 1) the mean number of mitochondria per unit area (100 μ^2) of cardiomyocyte, 2) the relative area (in %) occupied by mitochondria, 3) the mean number of cristae per mitochondrion, 4) the mean length of all cristae in a mitochondrion, 5) the mean area of a mitochondrion, 6) the density index of the mitochondrial cristae (the ratio of the mean length of all cristae to the mean area of a mitochondrion. Indices 3-6 were studied separately in the perinuclear, myofibrillary, and subsarcolemmal zones of the perikaryon and overall mean indices for the cardiomyocytes were calculated. This approach was based on the assumption that corresponding to functional differences between these zones [4] the mitochondria may perhaps differ in function, resistance, and reactivity, and may react differently to conditions of hypokinesia. The indices stated above were chosen because they were sufficiently informative on the structure and function of the cardiomyocyte mitochondrial system [7].

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 231-233, February, 1981. Original article submitted July 22, 1980.

0007-4888/81/9102-0229$07.50

EXPERIMENTAL RESULTS

Indices of perinuclear, intermyofibrillary, and subsarcolemmal mitochondria in the control rats did not differ statistically significantly (Table 1). By the 10th day of the experiment most mitochondria from all zones were swollen, with a decrease in density of the matrix and reduction and fragmentation of the cristae. The overall mean indices for the number of cristae, the length of all cristae, and the area of the mitochondria were increased (Fig. 1A, C). Changes in the first two indices took place on account of mitochondria in the myofibrillary zone (Table 1). The increase in area of the mitochondria observed in all zones (Table 1) was due to a decrease in the density index of the cristae (Fig. 1D; Table 1). The number of mitochondria per unit area of perikaryon was reduced by 30.7% but the relative area occupied by the mitochondria increased to 61.5% (43.4% in the control). On the 20th day swollen mitochondria predominated as before. On the 30th day both swollen mitochondria and those with dense packing of the cristae were found equally frequently. At all times destroyed mitochondria were rare. The number of mitochondria per unit area of perikaryon was held at a level close to that observed after 10 days, namely 64.1% on the 20th day and 66.7% on the 30th day relative to the control.

The number of cristae in the mitochondria increased progressively (Fig. 1A). The mean length of all cristae in a mitochondrion fell on the 20th day to near the control level, but by the 30th day it was again increased (Fig. 1B). The mean area of a mitochondrion on the 20th and 30th days of the experiment remained significantly greater than in the control (Fig. 1C). The density index of the cristae remained low and reached a minimum on the 20th day (Fig. 1D). The relative area occupied by mitochondria in the perikaryon showed a tendency to return to normal, but on the 30th day it was again higher than the control level, at 51.8%. The dynamics of the overall metric indices for each zone of the perikaryon separately followed the same pattern (Table 1).

On the basis of these results some conclusions can be drawn regarding structural and functional reorganization of the cardiomyocyte mitochondrial system during hypokinesia. In the first place, an overall decrease in the number of mitochondria will be noted. This was evidently due to the fact that on the first days of the experiment (until the 10th day), i.e., in the period of most marked stress reactions, degeneration and destruction of some mitochondria took place. Manifestations of this process continued to be observed in single mitochondria until the end of the experiment. To compensate for the degeneration and destruction, compensatory and adaptive structural changes developed in the remaining mitochondria, evidence of which could be clearly seen on the 10th day of the experiment and continued to develop until its end. These structural changes developed synchronously in mitochondria of all zones of the cardiomyocyte perikaryon and were reflected in a progressive increase in the number of cristae, an increase in the total length of all cristae and an increase in the area of the mitochondria, as well as an increase in the relative area of the perikaryon occupied by mitochondria.

In reports of investigations which have so far been published, one of the compensatory and adaptive reaction of the cardiomyocyte mitochondrial system in the early stages of hypokinesia is an increase in the formation of mitochondria *de novo*, leading to an increase in the population of small organelles and in their total number [1, 6]. By the 30th day the mitochondria have increased in size but decreased in number [6]. Formation of mitochondria *de novo* also was observed in the present investigation, but on too small a scale to compensate for their destruction. The dynamics of the indices studied in the present experiment points to a hypertrophic character of the compensatory and adaptive structural changes in the cardiomyocyte mitochondrial system during hypokinesia. Taking ultrastructural criteria of mitochondrial function as a guide [5], it can be concluded that the hypertrophic structural changes now observed are evidence of intensive functioning and exhaustion of functional reserves in many mitochondria. This conclusion is confirmed by the low density index of the mitochondrial cristae of the experimental rats. Some of the results now obtained are in disagreement with those published previously [6]. On the whole, however, in conjunction with the results of previous investigations it can be concluded that at the periods of hypokinesia studied, the most marked compensatory and adaptive structural changes take place in the rat cardiomyocyte mitochondrial system, but a state of adaptation is not reached. Such a state is formed later [6]. Finally, it should be noted that an important role in the pathogenesis of the metabolic disturbances in the reduction of contractile power and diminution of the

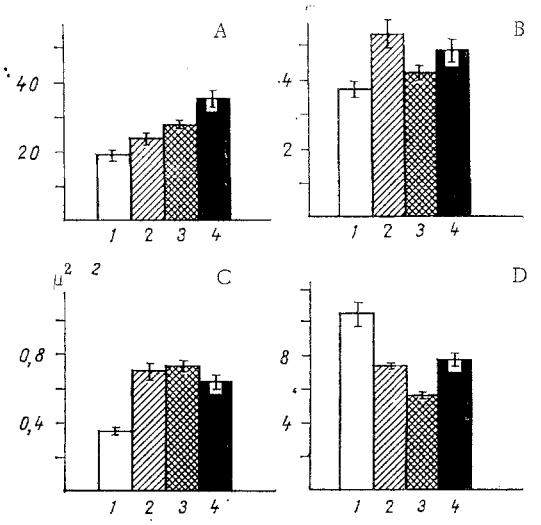

Fig. 1. Overall mean quantitative indices for cardiomyocyte mitochondria of control and experimental rats. A) Mean number of cristae per mitochondrion; B) mean length of all cristae in a mitochondrion; C) mean area of mitochondrion; D) density index of mitochondrial cristae; 1) control; 2,3,4) hypokinesia for 10, 20 and 30 days, respectively.

TABLE 1. Quantitative Indices for Mitochondria in Different Zones of Perikaryon in Cardiomyocytes of Control and Experimental Rats.

Group of animals	Duration of hypokinesia	Zone of perikaryon of cardiomyocyte	Number of cristae in mitochondrion	Mean length of all cristae in mitochondrion, μ	Mean area of mitochondrion, μ²	Density index of mitochondrial cristae
Control		Perinuclear	18,0±1,8	3,47±0,40	0,37±0,04	9,34
		Myofibrillary	17,9±1,9	3,26±0,41	0,31±0,02	10,42
		Subsarcolemmal	20,5±1,9	4,23±0,53	0,36±0,04	11,67
Experimental (hypokinesia)	10 days	Perinuclear	19,2±1,9	4,71±0,51	0,64±0,06*	7,34
		Myofibrillary	27,8±2,8*	6,19±0,65*	0,83±0,08*	7,46
		Subsarcolemmal	23,0±2,1	4,93±0,58	0,66±0,05*	7,37
	20 days	Perinuclear	29,9±2,7*	4,42±0,43	0,80±0,05*	5,49
		Myofibrillary	25,9±2,0*	3,72±0,43	0,67±0,05*	5,53
		Subsarcolemmal	26,7±1,8*	4,21±0,38	0,71±0,04*	5,87
	30 days	Perinuclear	31,6±4,1*	4,18±0,75	0,56±0,03*	7,37
		Myofibrillary	35,1±2,4*	5,24±0,46*	0,63±0,03*	8,24
		Subsarcolemmal	37,3±5,0*	5,02±0,71	0,67±0,09*	7,48

*Indices of experimental rats significantly higher than corresponding indices for control rats at $P \leqslant 0.05$ level.

functional reserves of the heart during hypokinesia [3], is played, not only by changes in the protein-synthesizing and contractile systems [1], but also by structural and functional changes in the cardiomyocyte mitochondrial system described above.

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PATHOMORPHOLOGICAL CHANGES IN THE TESTES OF RATS FED ON PRODUCTS IRRADIATED WITH γ RAYS

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UDC 616.686-091-092.9-02:612.392.014.482.4

KEY WORDS: rat testes; γ rays.

Information on the harmful effects of prolonged consumption of products preserved by exposure to γ rays has accumulated in the Soviet and western literature. In particular, it has been shown that prolonged feeding with irradiated meat and fish causes disturbances of protein and lipid metabolism, a decrease in the rate of gain of body weight, and a decrease in the number of offspring in experimental animals [2]. Previously [1] the writers described significant morphological changes of the membranous glomerulonephritis type in the kidneys of rats fed for 20 months with irradiated products.

This paper describes the results of a morphological study of the testes of the same animals.

EXPERIMENTAL METHOD

Experiments were carried out on 120 mature male rats aged 1 month kept in all experiments together with the same number of females. The animals of group 1 received the standard animal house diet [1], irradiated in a dose 10 times higher than the optimal dose used in practice for food preservation (0.25-5.6 megarads). The animals of group 2 received food products irradiated in the optimal dose of 25-500 kilorads, and the food given to animals of group 3 was irradiated in a dose one-tenth as high as in group 2 (2.5-56 kilorads). The rats of group 4 (control) received identical food products, but not exposed to γ rays. The food products were irradiated on the K-300 (All-Union Food Conservation Research Institute) γ-ray apparatus with cobalt-60.

All the animals were decapitated 20 months later. The testes were weighed on analytical scales, fixed in neutral 10% formalin, and sections were cut and stained with hematoxylin–eosin, by Masson's method, with Scharlach red, methyl green–pyronine, and by the PAS reaction. To study the dynamics of spermatogenesis, the frequency of discovery of stages of the cycle in the spermatogenic epithelium (CSE) was determined in sections. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The coefficient of variation of the absolute weight of the testes in animals receiving food irradiated in doses of 0.25-5.6 megarads (group 1) and 25-500 kilorads (group 2) was significantly higher than normally. A marked difference was observed in the size and weight of the right and left testes, which could be either increased (up to 2.7 g) or reduced (down to 0.5 g). However, the difference between the ratio of the weight of the larger testis to the weight of the smaller testis was significant only for the males of group 1 ($P < 0.05$), fed with products irradiated in a dose 10 times higher than optimal. Meanwhile, edema of the stroma was observed microscopically in animals of all three experimental groups to a varied degree, accompanied by a high content of PAS-positive substances, enlargement and coarsening of the collagen fibers and, in a few cases, accumulation of labrocytes. Collections of lym-

Laboratory of Pathomorphology, Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 233-236, February, 1981. Original article submitted November 13, 1980.

 0007-4888/81/9102-0232$07.50