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Stereological Ultrastructural Analysis of Rat Cardiomyocytes after Total Hyperthermia

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Hyperthermia results in the suppression of intracellular regeneration in cardiomyocytes, manifested as intensified lysis and destruction of organelles, and leads to myocardial atrophy. Intracellular reorganization of cardiomyocytes is characterized by increases in the volume and surface density of myofibrils, sarcoplasmic reticulum, and T system.

Key Words: *cardiomyocytes; intracellular organization; total hyperthermia; stereology*

The ultrastructural changes occurring in the cells of homoiothermic animals for total hyperthermia have not been investigated in sufficient detail. Attention has been focused on the effect of hyperthermia on cultured cells, predominantly on tumor cells [1,11]. As a result of these studies, hyperthermia has found application in anticancer therapy, since tumor cells have a low resistance to heating. It has been demonstrated that hyperthermia induces irreversible structural changes in the plasma membrane leading to disturbance of membrane functions, for example, the enzyme activity and passive membrane transport [8-10]. Specific proteins with putative protective activity against heat shock are being synthesized [12,13]. Generally speaking, the direct effect of hyperthermia on cell populations has been studied. In fact, however, total hyperthermia, to which humans or animals are usually exposed, more often than not produces an indirect effect on the internal organs. Pronounced morphofunctional changes have been observed in the myocardium of animals exposed to hyperthermia [6,7], namely necrobiotic and atrophic alterations in some cardiomyocytes [3]. A clear understanding of the processes of intracellular regeneration taking place in cardiomyocytes after hyperthermia makes it possible to predict the direction in which the adaptive-compensatory reactions in the myocardium will proceed and to elucidate some patterns of intracellular reorganization in cardiomyocytes induced by unfavorable environmental factors.

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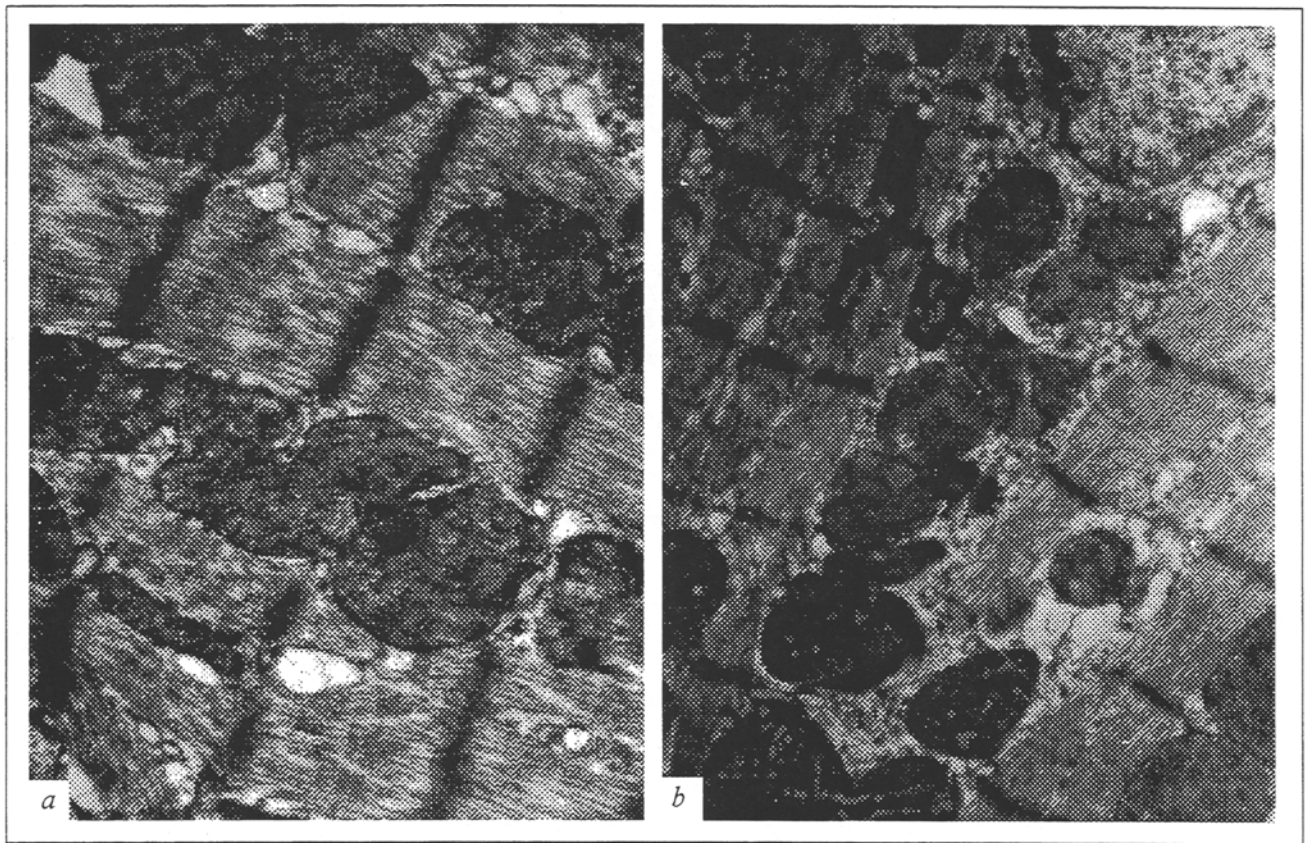

Fig. 1. Ultrastructure of rat cardiomyocytes 3 days after a single total hyperthermia. *a*) widened vesicles and tubules of agranular sarcoplasmic reticulum, partial lysis of myofilaments, ×6000; *b*) reduced perinuclear zone and secondary lysosomes in it, ×5000.

The objective of this study was to examine stereologically the reorganization of cardiomyocyte ultrastructure in the dynamics of post-hyperthermia restitution.

MATERIALS AND METHODS

The experiments were performed on 28 male Wistar rats subjected to a single total hyperthermia (43°C, 45 min) in a thermal chamber [3]. Samples for electron microscopy were collected on day 3 and day 7 after the procedure. Control rats of the same age and the experimental rats were maintained in the vivarium on standard laboratory rations and given free access to water.

Specimens from the left ventricle myocardium and left papillary muscle were fixed with 4% paraformaldehyde and postfixed with 1% osmium tetroxide. After dehydration and soaking, the specimens were embedded in Epon-Araldite. Ultrathin sections were cut in an LKB III ultratome, contrasted with uranyl acetate and lead citrate, and examined in a JEM-100 electron microscope at a beam voltage of 60 kV. Stereological analysis of the intracellular organization was performed on negatives, using a multipurpose test system [4]. Fragments of cardiomyocytes were photographed at an initial magnification of 5000, and then the negatives were projected on the table surface at a 18,000-fold magnification. The volume densities of myofibrils, mitochondria, agranular sarcoplasmic reticulum, T tubules, and sarcoplasm were evaluated. The surface density was determined for myofibrils, mitochondria, sarcoplasmic reticulum, and T tubules. The surface-volume ratio of the organelles and volume ratio of the major organelles to myofibrils were calculated. Statistics included calculation of the mean values and standard error of the mean and comparison of the data using Student's t test.

RESULTS

Hyperthermia caused a statistically significant decrease (24%) in the heart weight, a decrease which was predominantly the result of myocardial atrophy.

Ultrastructural analysis of rat cardiomyocytes on day 3 after a single total hyperthermia revealed a compact localization of intracellular organelles. The structure of myofibril bundles was generally preserved; however, thinned and disintegrated myofibrils in individual cells and sometimes small foci of myofibrillar lysis were seen. Ultrastructurally the

mitochondria remained virtually unchanged, although some contained small vacuoles filled with a floccular mass. The vesicles and tubules of the agranular sarcoplasmic reticulum (Fig. 1, *a*) and T tubules were widened. The nuclei contained predominantly euchromatin; nucleoli were often fragmented and consisted of a fibrillar component. The perinuclear zone was substantially reduced and often contained secondary lysosomes (Fig. 1, *b*).

On day 7 after total hyperthermia the changes in the cardiomyocyte ultrastructure were more pronounced. There were lytic alterations in the cytoplasm, particularly in the subsarcolemmal zone (Fig. 2, *a*). Myofibrillar bundles were often disintegrated and myofilaments were partially lysed. The changes in the mitochondria occurred mainly in the cristae: they were widened and contained vesicles. There were foci of lysis and crista destruction. Degradation of the mitochondria occurred practically in all cardiomyocytes, this being morphologically manifested in the presence of myelin-like structures and residual bodies (Fig. 2, *b, c*) localized between the mitochondria and in the subsarcolemmal zone. The vesicles of the sarcoplasmic reticulum (Fig. 2, *d*) and T tubules were widened considerably, which lent a foamy appearance to the cytoplasm. It should be mentioned that the cytoplasm contained numerous polysomes, indicating the initiation of intracellular regeneration. Nevertheless, some cardiomyocytes were atrophied. These cells were markedly thinned; they contained far fewer organelles, and the nucleus and the organelles of the perinuclear zone were localized eccentrically, immediately under the sarcolemma.

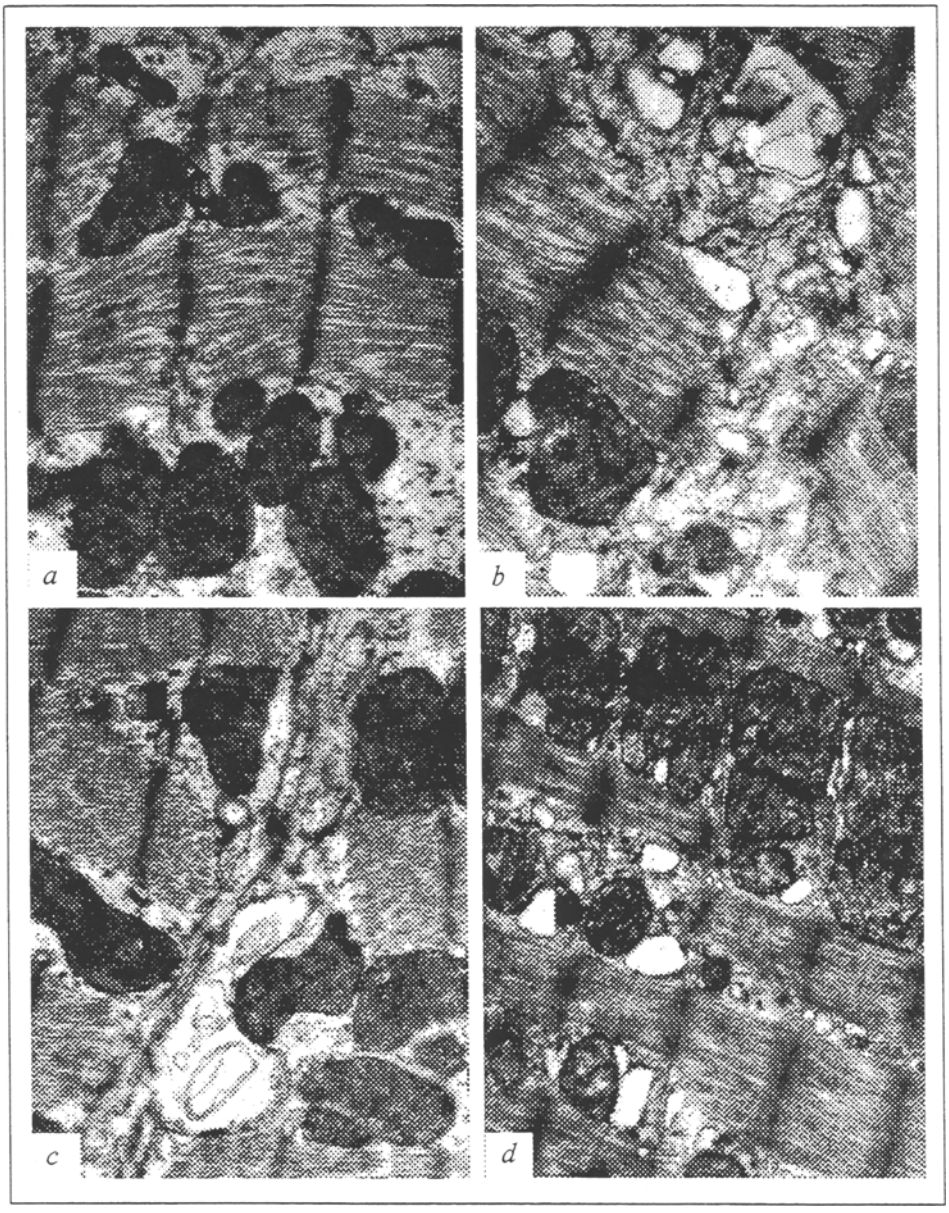

Fig. 2. Ultrastructure of rat cardiomyocytes 7 days after a single total hyperthermia. *a*) disintegration of myofibril bundles and pronounced lysis of the sarcoplasmic matrix; *b*) residual bodies in the subsarcolemmal zone and widened vesicles in the sarcoplasmic reticulum; *c*) residual bodies in the intercellular space; *d*) widened vesicles of the sarcoplasmic reticulum, focal lysis of the mitochondrial matrix.

TABLE 1. Stereological Analysis of the Intracellular Organization of Rat Cardiomyocytes after Total Hyperthermia ($M \pm m$)

Parameter	Control	Time after hyperthermia, days	
		3	7
Volume density, mm^3/cm^3			
of myofibrils	510.1±8.0	529.1±11.8	538.2±0.8**
of mitochondria	372.3±24.8	358.4±19.5	355.9±6.3
of sarcoplasmic reticulum	23.1±4.1	27.7±4.9	28.5±4.0
of T system	10.9±1.4	14.0±1.6	16.0±0.5**
of sarcoplasm	83.6±13.7	70.8±14.9	61.4±9.6
Surface density, m^2/cm^3			
of myofibrils	1.9621±0.0682	2.2438±0.1417	2.2742±0.0908*
of mitochondria	1.6719±0.0960	1.8648±0.0226*	1.9911±0.0189
of sarcoplasmic reticulum	0.3307±0.0707	0.5944±0.2141	0.5948±0.0593
of T system	0.1410±0.0210	0.2411±0.0675	0.2161±0.0164*
Surface–volume ratio, m^2/cm^3			
of myofibrils	3085±0.12	4.25±0.36	4.23±0.17
of mitochondria	4.51±0.22	5.23±0.22*	5.60±0.10**
of sarcoplasmic reticulum	14.1±0.48	20.10±3.74	21.34±2.23*
of T system	12.92±0.22	16.84±4.18	13.44±0.58
Volume ratio, number of:			
mitochondria to myofibrils	0.731±0.059	0.679±0.044	0.661±0.012
sarcoplasmic reticulum to myofibrils	0.045±0.008	0.052±0.010	0.053±0.008
T tubules to myofibrils	0.021±0.003	0.027±0.003	0.030±0.001*
total number of mitochondria, sarcoplasmic reticulum, and T tubules to myofibrils	0.798±0.051	0.758±0.037	0.744±0.019

Note. One asterisk indicates significance of differences at $p<0.05$; two asterisks indicate significance of differences at $p<0.01$.

Stereological analysis of the intracellular organization of the cardiomyocytes on day 3 after total hyperthermia revealed a tendency toward an increase in the volume and surface densities of myofibrils, agranular sarcoplasmic reticulum, and T tubules. The volume density of the mitochondria was somewhat reduced, but their surface density was significantly increased (12%), which led to a significant increase (16%) in the surface-volume ratio of the organelles.

An increase in the volume (6%) and surface (16%) densities of myofibrils was recorded on day 7 after hyperthermia. There were no significant changes in the volume density of the mitochondria; however, their surface density and surface-volume ratio increased significantly (by 19 and 24%, respectively), which indicated the predominance of small, newly formed mitochondria. Reliable increases were also noted for the volume and surface densities of the agranular sarcoplasmic reticulum (23 and 80%, respectively) and of the T tubules (47 and 53%, respectively). The more pronounced increase in the surface density of the sarcoplasmic reticulum compared with the volume density led to a statistically significant increase (51%) in the surface-volume ratio.

It should be mentioned that the volume ratio of the major organelles to myofibrils decreased, while the volume ratio of the organelles to the sarcoplasmic reticulum and T tubules increased (43%), resulting in a negligible decrease in the total volume ratio of organelles to myofibrils.

Thus, the ultrastructural changes occurring in cardiomyocytes after hyperthermia were due predominantly to inhibition of intracellular regeneration, which induced destructive changes in the organelles and enhanced lytic processes in the sarcoplasm. The stereological intracellular organization of cardiomyocytes was characterized by increased volume and surface densities of myofibrils, T tubules, and agranular sarcoplasmic reticulum. Despite of these changes, there was a stable tendency toward a decrease in the total volume density of the major organelles to myofibrils. We observed a similar dynamics of qualitative and quantitative changes in cardiomyocyte ultrastructure after hypothermia [2,5]. The main difference consisted in the changes in T tubules and sarcoplasmic reticulum: after total hypothermia the volume and surface densities of these structures did not change, while the volume ratio of T system to myofibrils was changed. The increase in volume and surface density of the membrane structures responsible for ion transport and regulation of contraction-relaxation may be regarded as a specific feature of the adaptive-compensatory reactions developing in cardiomyocytes after total hyperthermia. This increase largely reflects the spatial organization of these

structures (probably, shape changes), since it proceeds against the background of suppressed regeneratory-plastic processes and myocardial atrophy. It should be mentioned that exposure of an animal to a single total hyperthermia during a critical time period after which the animal can die induces such far-reaching disturbances in the intracellular regeneration in the cardiomyocytes that the consequences of these disturbances - changes in the cell architectonics - are permanent and become more pronounced by the 7th day of the experiment.

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The Effect of Morphine on RNA Synthesis in Some Brain Structures of Rats with Various Narcological Resistance

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Different incorporation of ^{3}H-uridine in RNA and increased RNA synthesis after the addition of morphine are demonstrated in all brain structures of resistant rats, as well as in the cortex, nucleus accumbens, griseum centrale, and nucleus ventriculus hypothalami of prone rats.

Key Words: *morphine; narcological resistance; transcription activity of nerve cells*

The predominant involvement of certain brain structures of the central nervous system (CNS), namely the sensorimotor cortex (SmC), cerebellar cortex (CbC), CA3 zone of the hippocampus (CH), amygdala (A), nucleus raphe magnus (NRM) griseum centrale (GC), nucleus ventromediali hypothalami (NVmH), and nucleus accumbens (NA) has been demonstrated in the development of drug and alcohol addiction [3,4]. With the exception of CbC, these structures have high contents of endogenous opioids and opiate receptors and play a

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