ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF THE MYOCARDIUM OF HOMOIOTHERMIC ANIMALS DURING COOLING

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Prolonged exposure of homoiothermic animals to low temperatures gives rise to a complex series of structural and functional changes in the cardiovascular system [1, 3, 4, 10, 13]. The most complete description in this connection has been given of the character and trend of changes taking place at the tissue level of organization. In particular, it has been shown that volume relationships of capillaries to cardiomyocytes are considerably modified in the myocardium of animals during cooling [1, 8]. The spatial reorganization of the myocardium at the tissue microregion level, together with neuroendocrine factors, determines ultrastructural changes in the cardiomyocytes under the influence of low temperatures. However, the character of these changes, the stages through which they pass, and the general principles of spatial reorganization of heart muscle cells under these conditions have been inadequately studied, and this makes it difficult to understand the essence of the developing adaptive processes or to predict their outcome.

The aim of this investigation was to study the intracellular spatial reorganization of cardiomyocytes in homoiothermic animals exposed to the action of low temperatures.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male Wistar rats weighing 120-150 g. The animals were subjected to continuous cooling (except during feeding) in a temperature chamber at —7°C. The rats were decapitated after 2 and 4 h, and 8 and 16 days; the heart was quickly removed and placed in a cold chamber to stop it from beating. Pieces of tissue from the left ventricle for electron-microscopic investigation were fixed in 4% paraformaldehyde solution, postfixed in a 1% osmium tetroxide solution, and embedded in epoxide resins. Semithin sections were cut with a Tesla ultramicrotome and stained with azure II. Ultrathin sections were cut on an LKB III ultramicrotome, stained with uranyl acetate and lead citrate, and examined in the JEM 100B electron microscope.

The relative volume of the myofibrils, mitochondria, smooth sarcoplasmic reticulum (SSR), T-system, and sarcoplasm (the term here is applied to the sarcoplasmic matrix, glycogen, ribosomes, lipid droplets, and lysosomes), and the surface density of the main sarcoplasmic organelles were determined. Using primary stereologic parameters, secondary parameters were calculated: surface volume ratios of the organelles, volume and surface—volume ratios of the principal organelles to myofibrils. The results of the stereologic analysis were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

As we showed previously [8], in rats under the chosen conditions of whole-body cooling (—7°C), there was a marked decrease (by 42%) in body weight and in the weight of the heart (by 14%) on the 16th day. As a result of the greater decrease in body weight, there was a significant increase in the relative weight of the heart (by 50%). Nevertheless, the decrease in the absolute weight of the heart points to the presence of atrophy.

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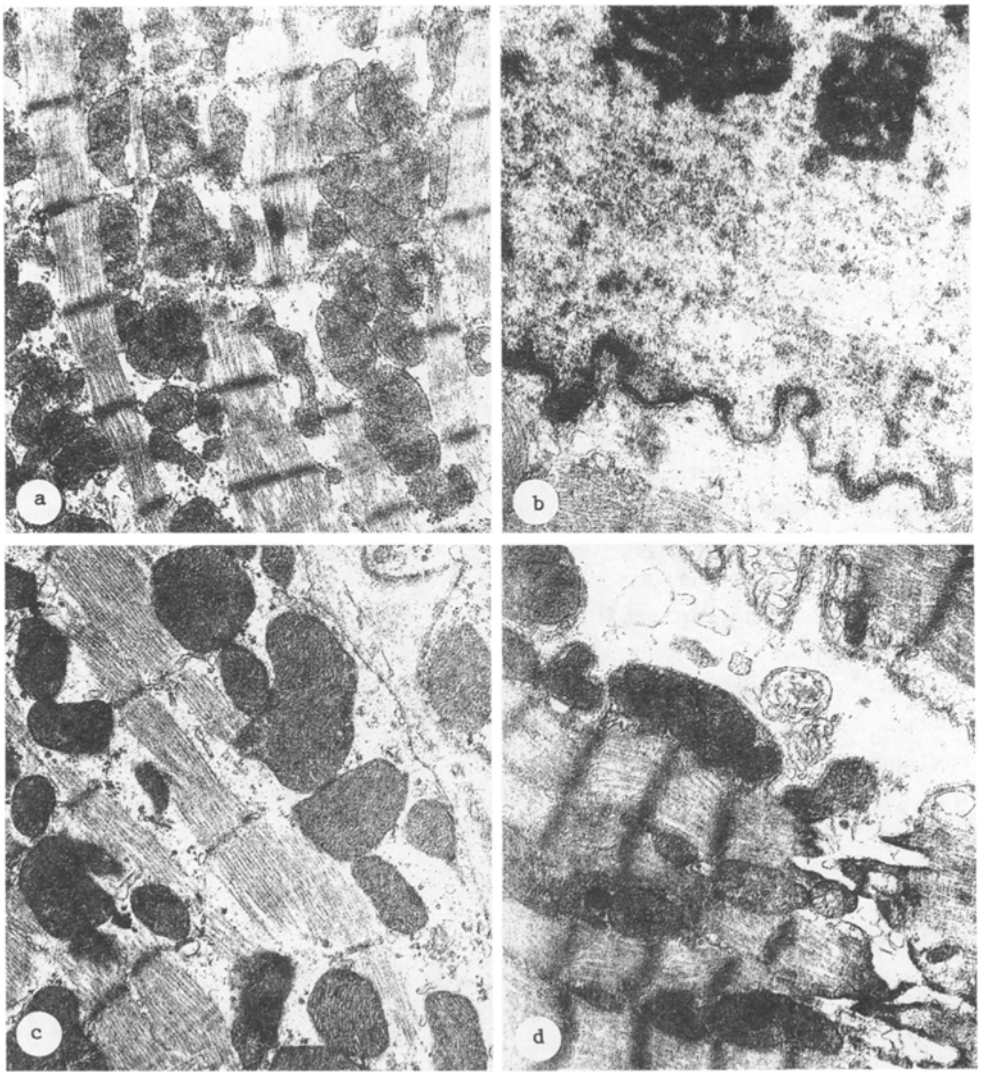

Fig. 1. Ultrastructural changes in rat cardiomyocytes during exposure of animals to low temperatures: a) rarefaction of bundles of myofibrils and partial lysis of myofilaments, 5000×; b) disappearance of granular component from nucleolus, 8300×; c) focal lysis of myofibrils and complete disappearance of glycogen granules from sarcoplasm. Concentrations of polysomes visible between myofibrils and under sarcolemma, 6500×; d) widening of space between cells in region of intercalated disk and appearance of myelinlike structures in it, 6600×; a) after 4 h of general cooling; b, c, d) after 8 days of general cooling.

The character of the ultrastructural changes in the cardiomyocytes of rats under the influence of low temperatures is determined by two principal factors: the development of a general adaptation syndrome (stress reaction) and stimulation of contractile and noncontractile thermogenesis. Acute short-term cooling is regarded, by the mechanism of its development, as a stress reaction, including sympathetic and adrenal humeral mechanisms, leading to activation of thermogenesis [2]. Elevation of the catecholamine level in the heart during the first few hours (2 h-4 h) of the experiment causes contractual injuries to the myofibrils, characteristic of metabolic damage to heart muscle [5]. Meanwhile, in nearly all the cardiomyocytes along the course of the bundles of myofibrils regions of rarefaction of myofilaments and of their partial lysis are found along the periphery of the bundles (Fig. 1a). At all these times, as a result of the intensification of noncontractile thermogenesis, which also is mediated primarily through noradrenalin [14], glycogen reserves in the cardiomyocytes are considerably exhausted. Meanwhile instability

of the mitochondrial membranes is noted, some of the mitochondria undergoing complete or partial destruction. In virtually all cells around the nuclei a small border of "devastated" sarcoplasm appears.

Qualitative changes in the cardiomyocytes correlate with changes in the quantitative ratios of the principal cytoplasmic organelles. As early as 4 h after the beginning of cooling there is a tendency for the bulk density of the myofibrils to increase and for that of the mitochondria to decrease. The bulk density of the T-system is appreciably lowered (by 31%), with an increase in the bulk density of the sarcoplasm (by 34%). The increase in the latter parameter was probably caused by moderate edema of the sarcoplasm. The surface density of the myofibrils and their surface to volume ratio increased significantly, evidence of loosening of the structure of the bundles of myofibrils. The surface density and surface to volume ratio of the mitochondria show similar changes, reflecting the appearance of smaller organelles by this time.

Opposite changes in the bulk density of the organelles are responsible for a decrease in the volume ratio of the mitochondria and T-system to the myofibrils. The ratio of the total volume of the principal sarcoplasmic organelles to the volume of the myofibrils also decreases.

During the long-term action of low temperatures, during the first week stimulation of the sympathetic innervation is preserved [2], which is responsible for activation of noncontractile thermogenesis. During this period, as before, contractual injuries to the myofibrils take place in the cardiomyocytes as a response to involvement of the heart in noncontractile thermogenesis, but morphological features of disturbance of intracellular regeneration [11] predominate, caused by a decrease in the inflow of structural materials to the cells of the parenchyma.

The high metabolic activity of the tissues and organs involved in noncontractile (brown adipose tissue and liver) and contractile (skeletal muscles) thermogenesis requires an influx of large quantities of structural materials. As a result of redistribution of the reserves of these structural materials in the body, the heart experiences a relative deficiency of them, and this brings about qualitative and quantitative structural changes in the cardiomyocytes during long-term exposure to cold. Morphological changes observed in the cardiomyocytes, namely disappearance of the granular component from the nucleoli, focal lysis of bundles of myofibrils (Fig. 1b, c), destructive changes in mitochondria, and an increase in the number of myelinlike structures (residual bodies), correspond to those observed in the heart muscle cells in the presence of a deficiency of structural materials [12].

Autophagy is intensified in the cardiomyocytes. The more numerous myelinlike structures are probably products of autophagy of the mitochondria, for they are located where these organelles are concentrated. As a reflection of these processes in the interstitial tissue concentrations of young and mature forms of macrophages, which also include lymphocytes, are formed.

The intercalated disks undergo considerable changes and assume a zigzaglike external appearance. Widening of the spaces between two neighboring cells is observed, with the appearance of myelinlike structures in them (Fig. 1d). In some cases rupture of the cells can be found at the intercalated disks.

After 8 days of general cooling the most marked changes are found in the quantitative spatial organization of the cardiomyocytes. The bulk and surface density of the myofibrils increase significantly (by 25.6% and 75% respectively). The bulk density of the mitochondria is significantly reduced (by 28%), but their surface density and surface to volume ratio are increased (by 40% and 95% respectively), evidence that newly formed mitochondria (small forms) predominate during this period. The volume and surface characteristics of the SSR are virtually unchanged whereas the surface to volume ratio of the T system is significantly increased.

At this time there is an even greater decrease in the volume ratio of mitochondria to myofibrils (by 43%), of the T-system to myofibrils (by 48%), and of the sarcoplasm to myofibrils (by 40%). A significant decrease in bulk density of the principal sarcoplasmic compartments is responsible for a marked decrease (by 42%) in the ratio of their total volume to the volume of the myofibrils, i.e., a decrease in the unit of "saturation" of the myofibrils by other organelles. A similar spatial reorganization of the cardiomyocytes is found during atrophy of the heart, arising during total alimentary starvation and hypokinesia [9], and also during hypertrophy of the heart, developing in response to a sharp increase in the functional load and during aging [7].

After 16 days of cooling of the animals contractual injuries to the cardiomyocytes are appreciably more severe (contractures of the II and III degree are found). Subsegmental contractures are observed in some cardiomyocytes. The mitochondria in such large concentrations, and sometimes these organelles show signs of destruction. During prolonged exposure (more than 1 week) to cold, just as during exposure to other extremal factors giving rise to a deficiency of structural materials in parenchymatous cells, morphological signs of regenerative reactions appear clearly in the cardiomyocytes: the appearance of polysomes in foci of lysis of the myofibrils, and hyperplasia of elements of the rough sarcoplasmic reticulum. However, the intensity of these processes is not sufficient to restore the normal morphological and functional composition of the cardiomyocytes. By the 16th

day of the experiment signs of lysis of the ultrastructures in the heart muscle cells are not so distinctly visible, but the general character of their spatial reorganization and quantitative relations of the organelles under these circumstances do not differ significantly from those at the previous time of the experiment.

Thus long term exposure to low temperatures causes the development of a deficiency of structural materials for heart muscle cells of rats. The cardiomyocytes develop a combination of qualitative morphological changes characteristic of the syndrome of deficiency of structural and repair materials. The most important factor in spatial (quantitative) reorganization of the cardiomyocytes is reduction of the unit of "saturation" of the myofibrils with other intracellular organelles — a stereotyped reaction in response to a sharp increase or decrease in the functional load, and due to a decrease in the supply of plastic materials to the cells.

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