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STEREOLOGICAL ANALYSIS OF MYOCARDIUM REORGANIZATION UNDER THE INFLUENCE OF EXTREME ENVIRONMENTAL CONDITIONS

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ABSTRACT

The left ventricular myocardium of Wistar rats was studied by stereological methods after the following treatments: exposure to low temperature (-7°C), single overheating (at 43°C), adaptation to high altitudes (3200 m above sea level), and transfer to high latitudes (at 69° of the North). It was shown that the tissue spatial reorganization of the myocardium passes through two stages: at the first one the volume and surface-to-volume ratios of bulk tissue components (especially capillaries and cardiomyocytes) change considerably, at the second one a tendency toward the normalization of tissue architectonics is observed. This dynamics of tissue reorganization is likely to be conditioned by stress reaction and reveals some structural mechanisms of non-specific response to extreme impact. The changes observed were more pronounced in the rats exposed to low temperatures, as well as in rats transferred to high latitudes and did not depend on the character of changing the heart weight (increase or decrease). On the contrary, the ultrastructural spatial reorganization of cardiomyocytes was characterized by continuous disproportional changes of volume ratios of bulk organelles (especially mitochondria and myofibrils), which were evident during influence of environmental factors. These discrepancies in the dynamics of tissue and intracellular spatial reorganization of myocardium are supposed to reflect the fundamental peculiarities of spatial-temporal organization of regenerative processes in parenchymal cells and stromal components. Thus, the normalization of tissue spatial organization observed is not accompanied by the normalization of intracellular cardiomyocyte organization.

Key words: cardiomyocytes, extreme environment, myocardium, rats, stereology.

INTRODUCTION

The three-dimensional organization of tissues or cells is believed to reflect all kinds of cellular and intercellular interactions during differentiation, growth and development of adaptive-compensatory reactions, and its assessment under different conditions may provide important information about the nature and trends of the processes mentioned above (Legato, 1979; David et al., 1981; Schaper et al., 1985).

The myocardium consists mainly of three cell populations: parenchymal cells (cardiomyocytes), endothelial cells and connective tissue cells. In adult mammals the cardiomyocytes do not proliferate, and adaptive-compensatory or restorative growth of myocardium under unfavourable conditions is mainly a result of cardiomyocyte hypertrophy, as well as stromal cell hyperplasia. Such different regenerative strategies of main cell populations of myocardium are responsible for different qualitative and quantitative changes of these cells under the same conditions. A new morphofunctional status resulted from such changes could be estimated to a certain extent by using stereologic procedure, and some conclusions may be drawn about outcomes of the processes studied.

The myocardial spatial reorganization (tissue and subcellular) under the influence of extreme environmental conditions, such as low and high temperatures, unusual heliogeomagnetic exposure, long-term altitude hypoxia, was studied less than the one under the induced hypertension, physical training or administration of some biological mediators. For a few years we have studied by quantitative methods the rat myocardium under extreme environmental conditions (Nepomnyashchikh et al., 1988, 1994; Lushnikova et al., 1993a,b,c, 1994). Analyzing the data, we found some common trends in the development of adaptive-compensatory reactions in myocardium both at tissue and subcellular level. Assuming that the trends revealed may be an important morphofunctional criteria for prognosis we are trying in this paper to generalize our facts.

The present study has been undertaken to determine some general principles of tissue and intracellular spatial reorganization of the myocardium during adaptation to extreme environmental conditions.

MATERIAL AND METHODS

Adult male Wistar rats were used in all the experimental models. In the first model (model 1, intensive cooling) 71 rats (initial body weight: 213.3 ± 8.8 g) were exposed to a low temperature (-7°C) for 16 days. The animals were kept in individual cages all time except the time periods, when they got a feed and water. Control animals were kept at room temperature. The myocardial samples from experimental and control rats were taken at days 8 and 16.

In the second model (model 2, adaptation to unusual heliogeomagnetic conditions) 109 rats (initial body weight: 232.0 ± 13.8 g) were transferred by air to the high latitudes (at the 69th parallel, Alykel), where they were kept in the vivarium for 37 days. Control animals were transported with in middle latitudes under the same flight conditions (for assessment of flight-induced stress). Then control rats were kept under the same conditions. The myocardial samples were synchronously taken from experimental and control animals at days 1, 12, 27 and 37.

In the third model (model 3, adaptation to high altitudes) 53 rats (initial body weight: 355.1 ± 12.2 g) were placed at an altitude of 3200 m (Tien Shan, Tuya-Ashu pass) and were kept for 10 months in the vivarium. Control animals were maintained during the same time period at an altitude of 720 m (Bishkek City). The myocardial samples were studied after 5- and 10-month stay in the mountains.

In the fourth model (model 4, single overheating) 28 rats (initial body weight: 112.6 ± 5.4 g) were exposed to a single overheating at 43°C for 45 min. As in the case of intensive cooling, the exposure time was dictated by the vitality of the animals and corresponded to the time limit when large-scale mortality occurred. The myocardial samples from experimental and control animals were taken at days 3 and 7 after exposure.

At the end of the experiments, body weight were measured, and hearts were excised. After weighing the hearts the left ventricular myocardial samples (approx. 1 mm^3) were removed and immediately fixed by immersion in 4% paraformaldehyde buffered with 0.1 M sodium phosphate (4°C, pH 7.4) for 4 more hours. After rinsing with 0.1 M sodium phosphate buffer (4°C, pH 7.4) postfixation by immersion for 1 hour in 1% osmium tetroxide buffered with 0.1 M sodium phosphate (4°C, pH 7.4) was carried out. After rinsing with the same phosphate buffer, the tissue samples were dehydrated in a grated ethanol series. Substitution of ethanol by acetone was followed by incubation of the tissue in a 1:1 mixture of acetone and epoxy resins (epon and araldite) at room temperature. After 12 hours, the tissue samples were embedded in mixture of epon and araldite and polymerized for 24 hours at 60°C. Simultaneously the sections from each heart were prepared for light microscopic examination.

The stereologic analysis was performed on semithin longitudinal sections of myocardium for tissue level of structural organization and on ultrathin sections for intracellular level. Fragments of cardiomyocytes were photographed at an initial magnification of 5000, and then the negatives were projected on the table surface at a 18000-fold magnification. The following quantitative variables were evaluated for tissue level: the volume and surface densities of cardiomyocytes, capillaries, endothelial cells, connective tissue cells (in sum), and the volume density of interstitial spaces, as well as the volume and surface-to-volume ratios of bulk tissue components. For intracellular level we evaluated the volume and surface densities of myofibrils, mitochondria, smooth sarcoplasmic reticulum (SSR) and T-tubules, and the volume density of sarcoplasm (including glycogen granules, lipid droplets, lysosomes, etc.), as well as the volume and surface-to-volume ratios of bulk cardiomyocyte organelles. The stereologic procedure applied was available for anisotropic samples (Sitte, 1967; Weibel, 1969; Mobley, Page, 1972; Eisenberg et al., 1974).

Mean value and standard error were calculated, and statistical analysis (t-test) was performed to test differences between the values for each experimental group and those of the respective control group.

RESULTS

Body weights and heart weights changed differently under extreme environmental conditions selected. In the first model body weight was at least a 42% lower ($p < 0.05$) at the 16th day of experiment compared with control rats; heart weight at the same time period decreased by 14%, but this difference was not significant. In the second model body weight of rats transferred to high latitudes increased by 40% ($p < 0.01$), whereas heart weight increased by 24% ($p < 0.01$). The body weight of rats transported with in middle latitudes increased by 34% ($p < 0.01$) only at the 7th and 12th day of experiment, and the heart weight did not change significantly. In the third model body weight of rats after 10 months stay in the mountains decreased by 47% ($p < 0.01$), and heart weight increased by 28% ($p < 0.01$) compared with respective control groups. In the fourth model body weight at the 7th day after a single overheating decreased by 12% (NS), and heart weight decreased by 24% ($p < 0.05$).

The light microscopic examination of the myocardium of control rats revealed a nearly normal structure. Some cardiomyocyte alterations, such as contracture lesions, as well as moderate hemodynamic disturbances and perivascular sclerosis were observed in the myocardium of 11-month old rats, which were control for "high altitude" animals. The mosaic character of cardiomyocyte alterations was revealed in most animals exposed to the influence of extreme environmental conditions. Simultaneously contracture lesions (eosinofilic muscle segments) and dystrophic changes of cardiomyocytes were found. During the early stages the

contracture lesions of cardiomyocytes prevailed, but at the later ones the amount of dystrophic cells increased. In all cases the hemodynamic abnormalities were also observed. In the fourth model an atrophied cardiomyocytes and necrosis of individual muscle segments in some myocardial zones were recorded at the 7th day after exposure.

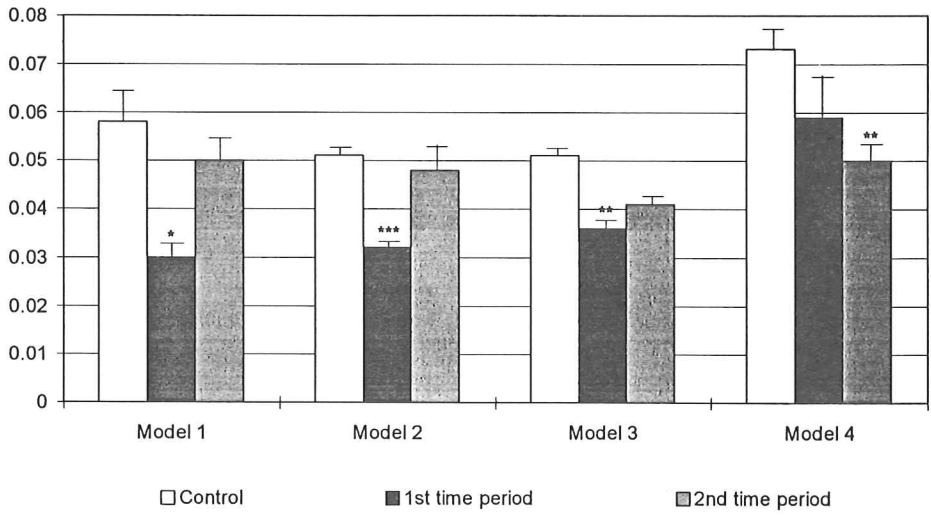

*Fig. 1. Volume ratios of capillaries to cardiomyocytes at different time periods of experiments. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.*

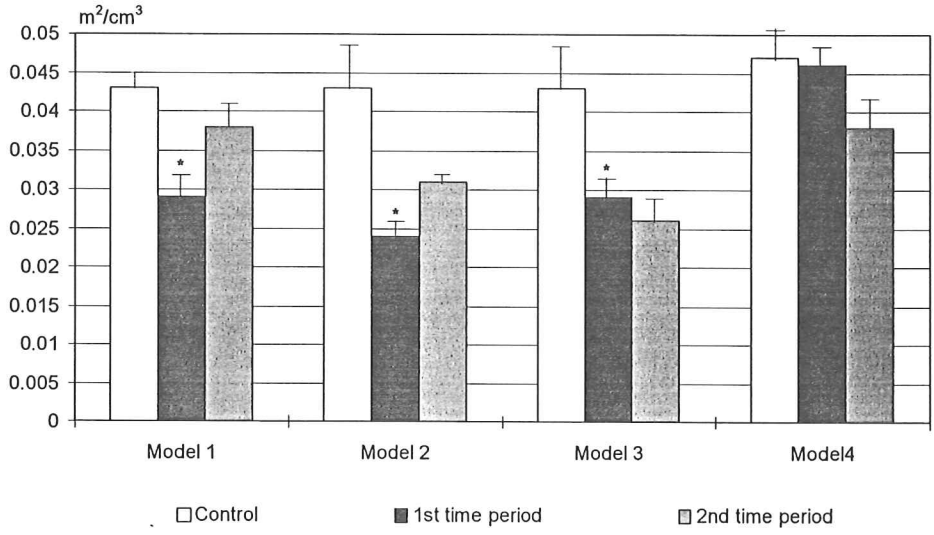

*Fig 2. Surface-to-volume ratios of capillaries to cardiomyocytes at different time periods of experiments. * - $p < 0.05$.*

Analysis of the parenchymal-stromal interactions in myocardium under the extreme environmental conditions revealed some general regularities of tissue spatial reorganization. The considerable decrease of the volume and surface densities of capillaries was the most pronounced feature of such reorganization. The volume density of cardiomyocytes did not change significantly. As a result of these changes, the volume and surface-to-volume ratios of capillaries to cardiomyocytes decreased remarkably (Fig. 1, 2).

The changes observed were more pronounced in the rats exposed to low temperature, as well as in the rats transferred to high latitudes. For example, the volume and surface densities of capillaries in myocardium of rats exposed to low temperature were dropped by 50% ($p < 0.05$) and 36% ($p < 0.01$), respectively, up to the 8th day of exposure than those in control groups, and 19% and 11% at the 16th day. The volume and surface-to-volume ratios of capillaries to cardiomyocytes were decreased by 48% ($p < 0.05$) and 33% ($p < 0.05$) respectively at the 8th day and did not differ significantly at the 16th day of exposure. In the case of adaptation to high latitudes the volume and surface densities of capillaries were decreased by 34% ($p < 0.05$) and 41% ($p < 0.05$) at the 12th day of experiment, and 4% and 26% ($p < 0.05$) at the 27th day. The volume and surface-to-volume ratios of capillaries to cardiomyocytes were decreased by 37% ($p < 0.001$) and 44% ($p < 0.05$), respectively, at the 12th day and changed slightly at the 27th day.

The volume and surface densities of capillaries decreased by 30% ($p < 0.05$) and 32% ($p < 0.05$), respectively, after 5 months stay at high altitudes, and 23% and 30% after 10 months. The volume and surface-to-volume rations of capillaries to cardiomyocytes dropped by 29% ($p < 0.01$) and 33% ($p < 0.05$) after 5 months and a 18% and 32% after 10 months, respectively. After single overheating the volume and surface densities of capillaries were reduced by 31% ($p < 0.01$) and 18% respectively at the 7th day after exposure. The volume and surface-to-volume ratios of capillaries to cardiomyocytes decreased by 32% ($p < 0.01$) and 19% at the same period of time.

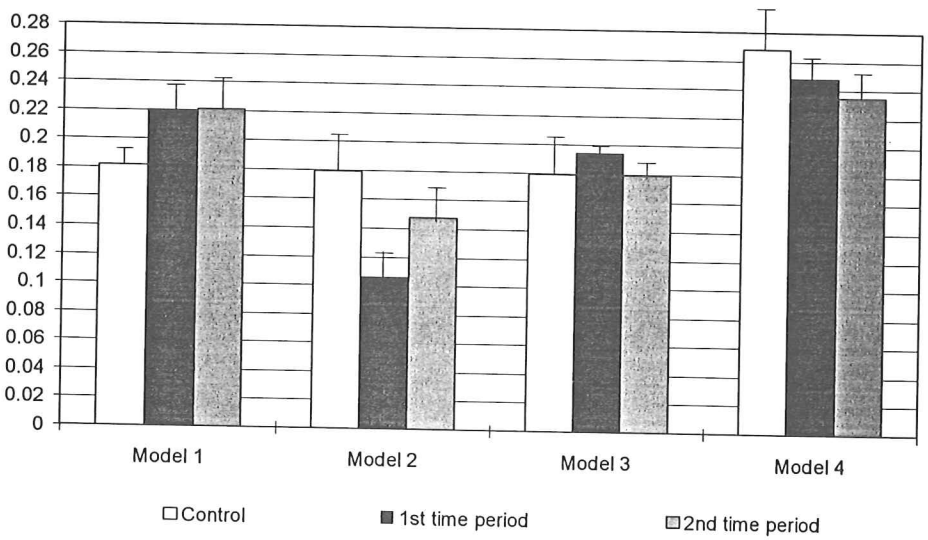

Fig. 3. Volume ratios of stroma to parenchyma in rat myocardium at different time periods of experiments.

The quantitative parameters of the connective tissue components increased in models 1, 2 and 3, and did not change significantly in model 4. This increase was most pronounced during intensive cooling (64% and 35% at days 8 and 16, respectively) and after transfer to high latitudes (39% and 19% at days 12 and 27, respectively). Thus, the volume ratio between stroma and parenchyma (Fig. 3) remained the same in the course of adaptation to high altitudes and after overheating, but increased during intensive cooling (21%) and decreased after the transportation to high latitudes (41% and 17% at days 12 and 27, respectively).

It should be noted that more considerable changes in quantitative values occurred at the first stages of experiments, and then a tendency toward normalization of tissue architectonics was observed except for model 4.

The electron microscopic examination of cardiomyocytes demonstrated a nearly normal ultrastructure in all control groups. Ultrastructural examination revealed marked abnormalities of the cardiomyocytes in all models studied. Reduced biosynthesis and increased lysis of ultrastructures were the crucial factors of intracellular reorganization of cardiomyocytes under extreme conditions. Clearing of the sarcoplasmic ground, fraying and thinning of myofibril bundles, and foci of lysis of myofilaments were also observed. Lytic changes of the sarcoplasm were most pronounced in the perinuclear and subsarcolemmal zones. The myelin-like structures were often seen. Such changes were preserved throughout the course of the experiments and were even more pronounced at their end. It should be mentioned that all the times the mitochondria appeared to be the most stable organelles, although their number in the cells markedly declined, notably in the perinuclear zone. At the later stages of experiments the polysomes were noted in sarcoplasm of cardiomyocytes, especially, in the foci of lysis of myofilaments, which was indicative of enhanced processes of intracellular regeneration. Despite this fact, the number of lytic and atrophied cardiomyocytes increased at the end of experiments.

Analysis of stereologic parameters of cardiomyocytes under the conditions studied revealed some regularities of their spatial reorganization. The volume density of myofibrils raised in all experimental models, whereas the volume density of mitochondria lowered. As a consequence, the volume-to-volume ratios of mitochondria to myofibrils decreased. These changes were more pronounced after transfer to high latitudes (33% lower) and under the intensive cooling (28% lower). The increase in myofibril surface densities and, as a rule, in their surface-to-volume relationships was also observed in all experimental models. And the decrease in mitochondria volume densities was accompanied by the increase in their surface densities and in surface-to-volume relationships.

The changes of quantitative values of SSR and T-systems were different. For example, the volume density of SSR raised by 46% after transfer to high latitudes, did not change under the intensive cooling and after single overheating, and lowered by 30% during the adaptation to high altitudes. The volume density of T-tubules also decreased by 28% during adaptation to high altitudes, but raised by 47% after single overheating, and did not change under the extreme cooling and after the transfer to high latitudes.

Analysis of the volume ratio of the major organelles of cardiomyocytes (mitochondria, SSR, T-tubules) to myofibrils revealed a stable tendency toward a decrease of this parameter under the conditions studied (Fig. 4). The reliable reduction of this index was recorded after the transfer to high latitudes (by 30%) and during the intensive cooling (by 28%).

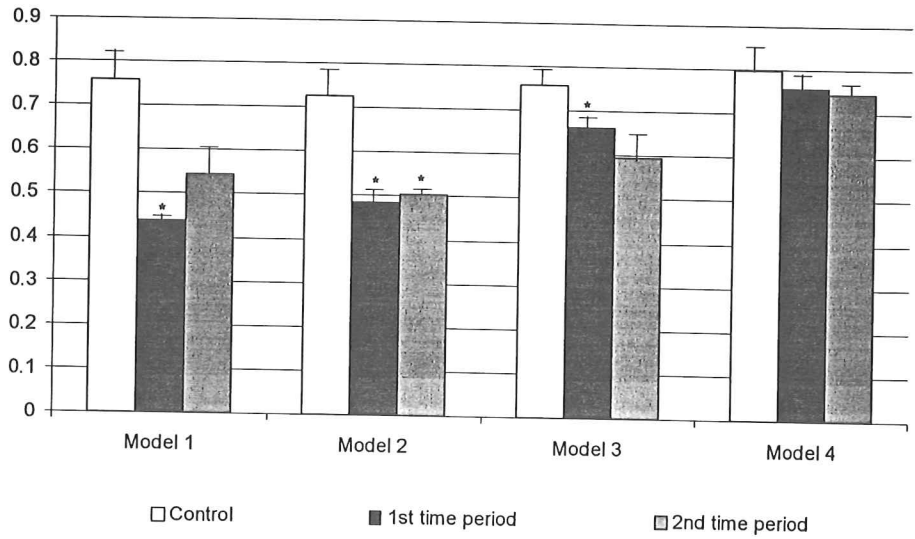

*Fig. 4. Volume ratios of bulk cardiomyocyte organelles (in sum) to myofibrils at different time periods of experiments. * - $p < 0.05$.*

It should be emphasized that disproportional changes of volume ratios of major cardiomyocyte organelles were evident throughout the course of the experiments and were even more pronounced at their end.

DISCUSSION

The present study combines light and electron microscopic measurements derived from longitudinal myocardial sections taken from the left ventricular free wall to determine some general principles of myocardial reorganization at tissue and subcellular levels under the extreme environmental conditions. The environmental conditions considered (intensive cooling, living in unusual heliogeomagnetic conditions, long-term altitude hypoxia, overheating) were extreme and often caused an animal death. The heart weight changed differently under these conditions, but a similar myocardial reorganization (tissue and subcellular) was observed.

At the tissue level, the decrease in the volume and surface densities of capillaries, and as a result of these changes, the decrease in the volume and surface-to-volume ratios of capillaries to cardiomyocytes were the most important events of such reorganization. The decrease of these quantitative values was also shown in heart hypertrophy induced by hypertension (Anversa et al., 1979, 1980; Dammrich, Pfiefer, 1983; Engelmann et al., 1987) and was indicative of unbalanced increase in the volume and surface of capillaries and cardiomyocytes in hypertrophied myocardium. The decrease in these parameters in our experiments means that unbalanced changes of these tissue components occur not only in heart hypertrophy, but also in heart atrophy. A mechanism that may cause such a profound myocardial reorganization is supposed to be more general, e.g. stress.

Extreme environmental conditions are well known to result in development of stress, which is characterized by specific hormonal pattern and a number of structural-functional

changes of major tissue systems and organs. Catecholamines and corticoids play an important role in the development of stress and are believed to affect cardiomyocyte functioning. The high concentration of catecholamines in blood is the cause of increased number of contracture-damaged cardiomyocytes. This type of cardiomyocyte alterations was reported by a number of authors studying the morphological changes of myocardium under the influence of endogenous and exogenous catecholamines (Nepomnyashchikh, 1991). We also observed contracture-damaged cardiomyocytes in all experimental models and believed that they were indicative of stress reaction. These cardiomyocyte alterations were more pronounced at the first stages of experiments, when stress was evident. The endothelial cells are likely to be the next target structures of myocardium, which are essentially altered during the development of stress reaction. The progressive decrease in capillary volume and surface densities resulted from extreme impact restricted the transport of oxygen and metabolic substrates from blood to cardiomyocytes and thus led to the development of their plastic insufficiency.

The present investigation also demonstrates that unbalanced adaptive-compensatory response of parenchymal and stromal components was more evident at the first stages of experiments and then a tendency toward normalization of tissue architectonics was observed excepting model 4. In this model the time period of myocardium restoration was very small to reveal such a tendency. A similar capillary reaction pattern was demonstrated in short-term and long-term experiments of renovascular hypertension (Mall et al., 1990; Fisher et al., 1992). It was shown that the capillary supply tended to become normalized after long-term renovascular hypertension (Fisher et al., 1992) as opposed to short-term experiment, when stereologic parameters of capilarization were decreased (Mall et al., 1990).

It is worth to mention that relative decrease of capillary mass was approximately compensated by increased interstitial mass (connective tissue components). Such changes favoured the preservation of the volume ratios of stroma to parenchyma almost at all time periods.

At the subcellular level, the composition of cardiomyocytes was significantly modified by extreme environmental factors. In all models the volume density of myofibrils was increased, and the volume density of mitochondria was decreased. As a consequence, the mitochondria-to-myofibril volume ratios were considerably reduced. Similar changes were found in experimental myocardium hypertrophy induced by pressure-overload (Anversa et al., 1978, 1980; Dammrich, Pfiefer, 1983; Mall et al., 1987), in hypobaric hypoxia (Lund, Tomanek, 1980), and during fasting (Vandewoude, Buyssens, 1992). It is generally accepted that a decreased volume ratio of mitochondria to myofibrils deteriorates the energy supply of the cardiomyocytes and may be an important factor limiting the compensatory capacity of cardiac muscle cells. Therefore, the stable reducing of relative mitochondrial mass, and as a consequence energy production, resulted in deterioration of intracellular regenerative processes.

It should be emphasized that the character of cardiomyocyte alterations under extreme environmental factors studied was conditioned by two main events: (1) high level of catecholamines in blood resulting in contracture lesions and (2) disturbances of intracellular regenerative processes followed by destruction and lysis of cell organelles, as well as sarcoplasm ground. It was also mentioned that the contracture lesions were more evident at the first stages of experiments. At the later stages the catabolic processes were enhanced particularly in perinuclear and subsarcolemmal zones. The focal lysis of myofibrils and sarcoplasm ground (sarcoplasm clearing), destruction of a part of mitochondria, increase in the number of myelin-like structures were the main features of cardiomyocyte reorganization observed.

Similar results were reported in other experimental models of plastic cardiac insufficiency, e.g. in anthracycline cardiomyopathy of rats (Nepomnyashchikh et al., 1984; Semenov et al., 1988). The only discrepancy with the present findings was the diffuse character of lytic lesions of myofibrillar bundles. Almost all cardiomyocytes were affected by anthracycline antibiotics. In the present experiments the lytic changes of myofibrils and sarcoplasm, as well as organelle destruction, were mainly focal, and a mosaic pattern of cardiomyocyte damages was observed.

On the whole, the stable reduction of the volume ratio of cardiomyocyte organelles (mitochondria, SSR, T-tubules) to myofibrils was found in all models. This reduction was more pronounced in intensive cooling and after transferring to high latitudes, as well as in the case of the volume and surface-to-volume ratios of capillaries to cardiomyocytes. Taking into account these stereological changes we may conclude that these environmental conditions are most extreme and cause the deterioration of cardiac performance.

To summarize we can say that extreme environmental conditions cause the pronounced tissue and subcellular spatial reorganization of myocardium. From our point of view, the most informative criteria of morphofunctional state of myocardium during the development of adaptive-compensatory processes are the volume and surface-to-volume ratios of capillaries to cardiomyocytes (for tissue level) and the mitochondria-to-myofibril volume ratio (for subcellular level). These stereologic parameters must be estimated simultaneously to determine the structural reaction pattern and draw some conclusions about the outcomes of processes studied, because an absence pronounced changes or normalization of tissue composition does not mean that cardiomyocyte composition is normal.

REFERENCES

Anversa P, Loud AV, Giacomelli F, Wiener J. Absolute morphometric study of myocardial hypertrophy in experimental hypertension. II. Ultrastructure of myocytes and interstitium. Lab Invest 1978; 38: 597-609.

Anversa P, Olivetti G, Melissari M, Loud AV. Morphometric study of myocardial hypertrophy induced by abdominal aortic stenosis. Lab Invest 1979; 40: 341-49.

Anversa P, Olivetti G, Melissari M, Loud AV. Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 1980; 12: 781-95.

Dammrich J, Pfeifer U. Cardiac hypertrophy in rats after supravalvular aortic constriction. I. Size and number of cardiomyocytes, endothelial and interstitial cells. Virchow's Arch B Cell Path 1983; 43: 265-86.

David H, Bozner A, Meyer R, Wassilew G. Pre- and postnatal development and ageing of the heart. Ultrastructural results and quantitative data. Exp Path Suppl 7. Jena: VEB Gustav Fisher Verlag, 1981. 176 p.

Eisenberg BR, Kuda AM, Peter JB. Stereological analysis of mammalian skeletal muscle. I. Soleus muscle of the adult guinea pig. J Cell Biol 1974; 60: 732-54.

Engelmann GL, Vitullo JC, Gerrity RG. Morphometric analysis of cardiac hypertrophy during development, maturation and senescence in spontaneously hypertensive rats. Circ Res 1987; 60: 487-94.

Fisher M, Wiest G, Tekesin I. et al. Effects of combined renovascular hypertension and diabetes mellitus on myocardial cells, non-vascular interstitium and capillaries: a stereological study on rat hearts. Virchow's Arch A Path Anat 1992; 420: 499-506.

Legato M. Cellular mechanisms of normal growth in the mammalian heart. I. Qualitative and quantitative features of ventricular architecture in the dog from birth to five months of age. Circ Res 1979; 44: 250-62.

Lund DD, Tomanek RJ. The effects of chronic hypoxia on the myocardial cells of normotensive and hypertensive rats. Anat Rec 1980; 196: 421-30.

Lushnikova EL, Mazhbich BI, Nepomnyashchikh LM. Stereological analysis of myocardial tissue organization in long-term adaptation to high altitude. Bull Exp Biol Med (Engl Tr) 1993a; 116(12): 1526-30.

Lushnikova EL, Nepomnyashchikh LM, Klinnikova MG, Molodykh OP. Quantitative analysis of rat myocardial tissue for general overheating of the organism. Bull Exp Biol Med (Engl Tr) 1993b; 116(7): 866-71.

Lushnikova EL, Nepomnyashchikh LM, Mazhbich BI. Quantitative ultrastructural analysis of rat cardiomyocytes after a prolonged stay in the mountains. Bull Exp Biol Med (Engl Tr) 1994; 117(6): 669-73.

Lushnikova EL, Nepomnyashchikh LM, Molodykh OP, Klinnikova MG. Stereological ultrastructural analysis of rat cardiomyocytes after total hyperthermia. Bull Exp Biol Med (Engl Tr) 1993c; 117(1): 99-103.

Mall G, Klingel K, Hasslacher GH. et al. Synergistic effects of diabetes mellitus and renovascular hypertension on the rat heart – stereological investigations on papillary muscles. Virchow's Arch A Path Anat 1987; 411: 531-42.

Mall G, Zimmer G, Baden S, Mattfeldt T. Capillary neoformation in the rat heart – a stereological study on papillary muscles in hypertrophy and physiologic growth. Basic Res Cardiol 1990; 85: 531-40.

Mobley BA, Page E. The surface area of sheep cardiac Purkinje fibres. J Physiol 1972; 220: 547-63.

Nepomnyashchikh LM. Morphogenesis of the main processes in heart general pathology. Novosibirsk: Nauka, Sibirian Division, 1991. 352 p. (in Russian).

Nepomnyashchikh LM, Lushnikova EL, Nepomnyashchikh GI. et al. Stereohistologic analysis of the myocardium of homoiothermic animals during cooling. Bull Exp Biol Med (Engl Tr) 1988; 104(11): 1620-25.

Nepomnyashchikh LM, Lushnikova EL, Nepomnyashchikh GI. Intracellular reorganization of rat cardiomyocytes following transfer to high latitudes. Bull Exp Biol Med (Engl Tr) 1994; 117(5): 539-44.

Nepomnyashchikh LM, Semenov DE, Tumanov VP, Tsellarius Yu G. Stereologic analysis of myocardial structures in plastic cardiac insufficiency (abnormal ultrastructural cardiomyocyte regeneration). Bull Exp Biol Med (Eng Tr) 1984; 96(9): 1217-21.

Schaper J, Meiser E, Stammler G. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice, and from human hearts. Circ Res 1985; 56: 377-91.

Semenov DE, Nepomnyashchikh LM, Semenova LA. Morphological manifestation of anthracycline cardiomyopathy in the right and left ventricular myocardium of rats. Bull Exp Biol Med (Engl Tr) 1988; 104(7): 1018-22.

Sitte H. Morphometrische Untersuchungen an Zellen. In: Weibel ER, Elias H, eds. Quntitative Methods in Morphology. Berlin - Heidelberg - New York: Springer Ver., 1967: 167-97.

Vandewoude MFJ., Buyssens N. Effect of ageing and malnutrition on rat myocardium. I. The myocyte. Virchow's Arch A Path Anat 1992; 421: 179-88.

Weibel ER. Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol 1969; 26: 235-302.

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