MORPHOLOGY AND PATHOMORPHOLOGY

Stereological Analysis of Absolute Parameters of Rat Myocardium Exposed to Contrasting Temperature Effects

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It is shown that different regimens of general cooling and overheating of the organism produce different changes of the volume and area of the major tissue components of the myocardium. The most pronounced alterations in the volume and area of the tissue components are recorded after overheating. A marked decrease of the volume and area of the microcirculatory bed is a regular feature of tissue reorganization in the myocardium under the influence of cooling and heating.

Key Words: *general cooling; general overheating; myocardium; stereology; absolute indexes*

Analysis of the patterns of changing parenchyma-stroma relationships or in tissue reorganization is essential for understanding the nature and direction of the adaptive-compensatory processes developing under various extreme factors [2,7,9]. Such analysis is particularly important for the myocardium, where cell systems interact with intercellular (cardiomyocytes) and primarily cellular (endotheliocytes and connective tissue cells) forms of regeneration at the level of the tissue microregion [10,11]. Alterations of the regenerative capacity of these cell systems exposed to extreme factors will dictate the nature of parenchyma-stroma relationships and will manifest themselves integrally in the spatial tissue organization of the myocardium, the assessment of which is of prognostic significance. Analysis of the spatial tissue and intracellular organization of the myocardium is performed according to the relative indexes of volume and area of the tissue and cell structures [1,15], which yield information on the type of relationships between structural components. The absolute indexes must be analyzed in order to gauge the alterations of volume and area of structures within cells or in the whole organ. Such investigations were previously performed to study the development of myocardial hypertrophy of various genesis [4,12-14]. However, data concerning the nature of changes in the myocardial volume and cell components in the whole organ exposed to extreme factors are practically nonexistent.

The goal of the present study was to elucidate the nature of myocardial tissue reorganization under extreme temperature effects on the basis of estimation of the changes in total volume and area of tissue components in the myocardium.

MATERIALS AND METHODS

An assessment of changes in volume and area of the main myocardial tissue components in Wistar rats subjected to contrasting temperature effects was performed using the following models: a moderate general cooling at 3-4°C for 8 weeks, a general supercooling at -7°C for 16 days, and a one-time general overheating at 43°C for 45 min (in this

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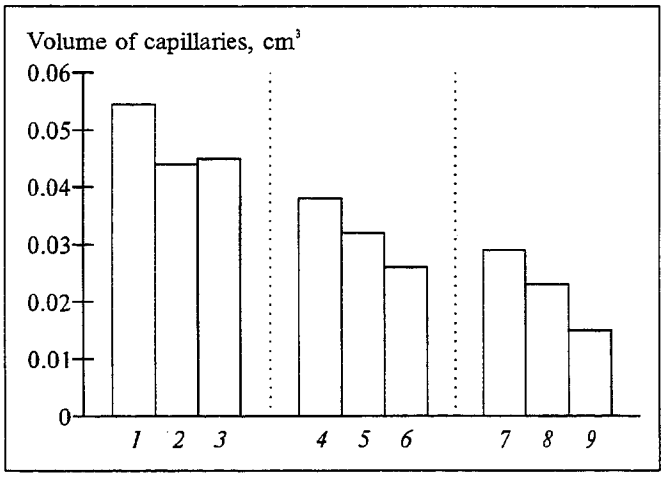

Fig. 1. Changes of total volume of capillaries in the rat myocardium upon exposure to contrasting temperatures. Here and in Fig. 2: control (*1*), 6 weeks (*2*), and 8 weeks (*3*) in experiments with moderate general cooling; control (*4*), 8 days (*5*) and 16 days (*6*) in experiments with general supercooling; control (*7*), 3rd day (*8*) and 7th day (*9*) in experiments with general overheating.

model the tissue samples were examined on the 3rd and 7th day).

The weight of the rat heart and of the left ventricle (LV) was determined at the beginning and at the end of the experiment for each experimental group. The material was then treated by the methods described previously [9] to obtain paraffin and semithin sections. The volume of the left ventricle tissue (V_{LV}) was determined by dividing its mass by the specific gravity of the myocardium, namely 1.06±0.006 g/cm³.

The absolute total volume (Vi) and area (Si) of the myocardial tissue components were calculated by the equations: $Vi = V_{LV} \times V^{tc}_{vi}$ and $Si = V_{LV} \times S^{tc}_{vi}$, where V^{tc}_{vi} and S^{tc}_{vi} the volume and surface densities of the corresponding tissue structures [6]. For this purpose a tissue stereological analysis was performed on the semithin sections. The volume and surface density of the cardiomyocytes, their nuclei, capillaries, endotheliocytes, cells, fibers, and matrix of connective tissue were assessed using a multipurpose test-system.

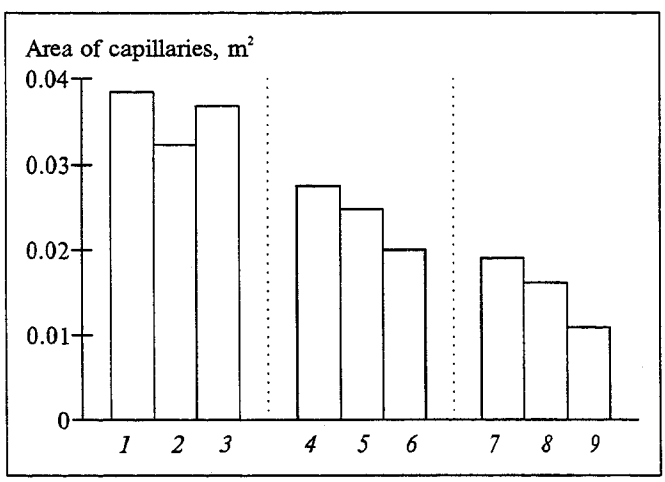

Fig. 2. Changes of total area of capillaries in the rat myocardium upon exposure to contrasting temperatures.

The results of the investigation were subjected to statistical treatment and the reliability of differences was determined by the Student test.

RESULTS

Analysis of the weight and volume characteristics of the left ventricle myocardium revealed a reliable decrease in these indexes for general supercooling and after the one-time general overheating (by 15 and 27%, respectively) and a 10-14% increase of them under the moderate general cooling (Tables 1-3) in all experimental models.

The quantitative changes in volume and area of the myocardial tissue components was found to be most pronounced after general overheating of the organism. A decrease of the volume and area in all the main myocardial components (by 25-48%, Table 3) was noted with this type of temperature action. The volume and area of the cardiomyocyte nuclei decreased significantly (by 44 and 33%, respectively) as well as the same parameters of capillaries (by 48 and 42%, respectively). The volume and surface characteristics of endothelial and connective tissue cells were reduced substantially. It should be noted that the decrease of the total volume of cardiomyocytes correlated with the diminution of LV mass, while the total capillary volume decreased practically 2-fold.

The increase of mass and volume of the left ventricle occurring for moderate general cooling of the animals was due to an elevation of the total volume of cardiomyocytes, fibers, and connective tissue matrix (Table 1). The general cooling was found to cause a decrease of the total volume and area of capillaries (by 15 and 5%, respectively) and a still more marked fall in the volume and surface characteristics of the endotheliocytes and connective tissue cells (on the average by 40%).

The decrease of mass and volume under general supercooling was brought about by the reliable reduction of the total volume of cardiomyocytes (by 18%) and their nuclei as well as of the total volume of capillaries (by 32%, Tabl. 2). In contrast, the total volume of endotheliocytes, cells, fibers, and connective tissue matrix increased toward the 16th day of the experiment. Nevertheless, the absolute value of this growth was insignificant and it could not compensate for the more considerable decrease of the volume of cardiomyocytes and microcirculatory bed (MCB).

TABLE 1. Absolute Stereological Parameters of Wistar Rat Myocardium Exposed to Moderate General Cooling ($M \pm m$)

Index	Control	Time of cooling	
		6 weeks	8 weeks
Body weight, g	275.0±7.1	284.0±11.5	417.0±24.8***
Heart weight, mg	1300.0±54.8	1480.0±49.0*	1428.0±69.5
Weight of LV, mg	1121.0±43.0	1276.0±43.0*	1236.0±64.4
Volume of LV, cm^3	1.058±0.041	1.204±0.041*	1.166±0.061
Total volume, cm^3:			
cardiomyocytes	0.937±0.026	1.066±0.050	1.014±0.054
cardiomyocyte nuclei	0.014±0.003	0.011±0.002	0.015±0.003
capillaries	0.053±0.001	0.044±0.004	0.045±0.007
endothelial cells	0.023±0.003	0.021±0.004	0.017±0.002
connective tissue cells	0.010±0.001	0.011±0.001	0.007±0.001*
connective tissue fibers and matrix	0.050±0.011	0.153±0.021*	0.080±0.012
Total area, m^2:			
cardiomyocytes	0.105±0.009	0.102±0.011	0.109±0.008
cardiomyocyte nuclei	0.009±0.003	0.007±0.001	0.009±0.001
capillaries	0.039±0.003	0.032±0.004	0.037±0.003
connective tissue cells	0.010±0.0003	0.009±0.001	0.006±0.001

Note. Here and in Tables 2 and 3: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Comparative analysis of the dynamics of absolute indexes of the myocardial tissue components under the contrasting temperature shows that the bulk of mass and LV volume alterations consisted of changes in the total volume and mass of cardiomyocytes comprising about 80% of the myocardial volume under the experimental conditions. It should be noted that, in spite of the inverse changes in heart mass and LV volume at different temperatures, a decrease of the total volume (Fig. 1) and area (Fig. 2) of the MCB was found in all cases. These parameters dropped most significantly under general supercooling and after general overheating, the alterations being due to a marked decrease of the total volume of endothelial cells. Despite a slight increase in the total endotheliocyte volume toward the 16th day of general supercooling, this fell short of the recovery of the MCB initial volume.

A relative decrease of the capillary volume and area in a volume unit of tissue was shown previ-

TABLE 2. Absolute Stereological Parameters of Wistar Rat Myocardium Exposed to General Supercooling ($M \pm m$)

Index	Control	Time of cooling	
		8 days	16 days
Body weight, g	213.3±8.8	210.0±20.2	123.3±8.8*
Heart weight, mg	933.3±44.1	979.3±54.5	800.0±0.1
Weight of LV, mg	813.3±38.4	851.7±46.4	691.7±1.7*
Volume of LV, cm^3	0.767±0.036	0.803±0.044	0.653±0.002*
Total volume, cm^3:			
cardiomyocytes	0.641±0.028	0.674±0.043	0.527±0.009*
cardiomyocyte nuclei	0.008±0.0002	0.009±0.002	0.007±0.001
capillaries	0.038±0.006	0.032±0.006	0.026±0.002
endothelial cells	0.015±0.002	0.011±0.001	0.016±0.001
connective tissue cells	0.009±0.002	0.010±0.001	0.010±0.001
connective tissue fibers and matrix	0.056±0.002	0.069±0.007	0.065±0.004
Total area, m^2			
cardiomyocytes	0.083±0.008	0.091±0.001	0.075±0.001
cardiomyocyte nuclei	0.005±0.0004	0.006±0.0006	0.007±0.001
capillaries	0.028±0.003	0.025±0.002	0.020±0.0003*
connective tissue cells	0.010±0.002	0.008±0.0003	0.009±0.001

TABLE 3. Absolute Stereological Parameters of Wistar Rat Myocardium after a One-Time General Overheating ($M \pm m$)

Index	Control	Time of cooling	
		8 days	16 days
Body weight, g	112.6±5.4	118.0±5.7	98.6±8.8
Heart weight, mg	610.0±22.7	600.0±46.4	466.0±47.6*
Weight of LV, mg	525.0±20.2	522.0±39.8	383.0±23.5**
Volume of LV, cm^3	0.495±0.019	0.493±0.038	0.361±0.022**
Total volume, cm^3:			
cardiomyocytes	0.384±0.026	0.353±0.020	0.288±0.030*
cardiomyocyte nuclei	0.009±0.001	0.007±0.001	0.005±0.0005
capillaries	0.029±0.002	0.022±0.004	0.015±0.002*
endothelial cells	0.010±0.001	0.009±0.001	0.007±0.001*
connective tissue cells	0.007±0.001	0.005±0.0002	0.005±0.001
connective tissue fibers and matrix	0.059±0.009	0.054±0.005	0.042±0.003
Total area, m^2			
cardiomyocytes	0.052±0.002	0.052±0.004	0.043±0.003
cardiomyocyte nuclei	0.006±0.001	0.005±0.001	0.004±0.0004
capillaries	0.019±0.003	0.017±0.002	0.011±0.002
connective tissue cells	0.006±0.0006	0.004±0.0005	0.004±0.0004

ously in stereological studies of the myocardium under the same experimental conditions [5,8]. Calculation of the absolute indexes attests to a total decrease of the MCB volume in the LV myocardium exposed to extreme temperatures. The results obtained suggest a marked inhibition of the regenerative capacity of endotheliocytes during the organism's adaptation to inappropriate temperature conditions. A similar reduction of regenerative capacity may be identified in connective tissue cells under conditions of moderate cooling and general overheating of the animals, because their total volume dropped by around 30%. Considering the absence of body temperature changes in these experimental models, we can assume that the weakened intracellular regeneration in cardiomyocytes and the inhibition of proliferative activity in the cambial elements of endotheliocytes and connective tissue cells did not result from any direct temperature drop in the tissue and cell systems, but were due to reorganization of the plastic and energetic resources to attain the optimal level of heat production.

Thus, extreme temperatures cause significant alterations in the parenchyma-stroma relationships in the myocardium and destroy previously established correlations between structural elements of the parenchyma and stroma at the level of the tissue microregion. The type of myocardial regeneration processes causing changes of spatial tissue organization may be classified as a peculiar kind of substitution or atypical regeneration [3], whereby the tissue architectonics is destroyed.

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