ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF ABSOLUTE PARAMETERS OF CARDIOMYOCYTES IN MYOCARDIAL HYPERTROPHY

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KEY WORDS: myocardial hypertrophy; ultrastructure of cardiomyocytes; stereologic analysis.

To understand the nature of the compensation and adaptation which take place during myocardial hypertrophy it is important to assess absolute total volumes and surface areas of tissue and cellular structures [4]. Relative parameters, which have most frequently been considered during the study of myocardial hypertrophy [3, 6, 12-14], provide information on the character of relations between the various tissue and cellular components. More recently, the attention of investigators has been increasingly drawn to the analysis of absolute volumes and surface areas, numbers, and linear dimensions of various tissue and cellular structures during hypertrophy of the heart [4, 7-11], because these parameters give some idea of the degree of changes in particular structures in the cell or in the whole organ. Changes in volume, surface area, and number of the organelles in the "average" cardiomyocyte have been described in the greatest detail, and these parameters in the whole organ or in the ventricles of the heart have been assessed less frequently.

The aim of this investigation was to study the character of changes in absolute parameters of the organelles of the cardiomyocytes during the development of hypertrophy of the hearts.

EXPERIMENTAL METHOD

Ten male Wistar rats weighing initially 200.9±20.3 g, in which arterial hypertension was produced by constricting the lumen of the abdominal aorta by Selye'e method [5], were used. The absolute total volume and surface area of the organelles of the cardiomyocytes were calculated from the left ventricle for intact animals and for rats on the 35th day after constriction of the lumen of the abdominal aorta. For this purpose, the rats were decapitated and the heart removed and placed in a cold chamber until it completely stopped beating. After fixation in 4% paraformaldehyde for 30 min, the heart and each ventricle were accurately weighed (the left ventricle was weighed together with the centricular septum). Tissue samples from the left ventricle were again fixed in 4% paraformaldehyde, postfixed in 2% OsO_4, dehydrated, and embedded in a mixture of Epon and Araldite.

The volume of tissue of the left and right ventricles (V_{lv} and V_{rv} respectively) was calculated by dividing their mass by the specific gravity of the myocardium, namely 1.06±0.006 g/cm^3 [7]. Absolute values were calculated by using relative values calculated for the tissue. The bulk and surface density of the organelles of the cardiomyocytes in the tissue were calculated by the equations

$$V_{v_j} = V_{v_j}^{cyt} \cdot V_{v\,cyt}^{t},$$
$$S_{v_j}^{t} = S_{v_j}^{cyt} \cdot V_{v\,cyt}^{t}.$$

The values of $V_{v_j}^{cyt}$ and $S_{v_j}^{cyt}$ were calculated during electron-microscopic investigation, whereas those of $V_{v_{cyt}}^{t}$ were calculated during a light-optical study of semithin sections, by methods described previously [1, 4].

The absolute total volume and surface area of the organelles of the cardiomyocytes in tissue of the left ventricle were determined by the equations

$$V_j = V_{v_j}^{t} \cdot V_{lv},$$
$$S_j = S_{v_j}^{t} \cdot V_{lv}.$$

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TABLE 1. Results of Calculations of Absolute Stereologic Parameters of Cardiomyocytes of Hypertrophied Myocardium in Wistar Rats with Arterial Hypertension Due to Constriction of Abdominal Aorta (M±m)

Parameter	Control	35th day after operation	Change, in %
Weight of left ventricle, g	0,687±0,018	0,980±0,015***	+43
Weight of right ventricle, g	0,108±0,004	0,142±0,005**	+31
Volume of left ventricle, cm^3	0,648±0,016	0,924±0,014***	+43
Volume of right ventricle, cm^3	0,102±0,004	0,134±0,005**	+31
Relative volume in tissue, cm^3/cm^3, of:			
myofibrils	0,426±0,005	0,512±0,014**	+20
mitochondria	0,298±0,014	0,208±0,003	—30
SSR	0,012±0,0008	0,018±0,002*	+50
T system	0,011±0,0008	0,011±0,0005	—
Surface area per unit volume of tissue, m^2/cm^3, of:			
myofibrils	0,978±0,029	1,177±0,074	+20
mitochondria	0,962±0,044	0,911±0,051	—5
SSR	0,228±0,003	0,412±0,012***	+81
T system	0,122±0,007	0,122±0,019	—
Absolute volume, cm^3, of:			
myofibrils	0,276±0,009	0,473±0,005***	+71
mitochondria	0,193±0,006	0,192±0,005	—
SSR	0,008±0,0007	0,017±0,002**	+113
T system	0,007±0,0006	0,010±0,0003*	+43
Surface area, m^2, of:			
myofibrils	0,633±0,016	1,086±0,052***	+72
mitochondria	0,622±0,013	0,842±0,050*	+35
SSR	0,148±0,005	0,381±0,005***	+157
T system	0,079±0,006	0,122±0,014	+54

Legend. *P < 0.05, **P < 0.01, ***P < 0.001.

The volume and surface area were calculated for myofibrillary bundles, mitochondria, the smooth sarcoplastic reticulum (SSR), and the T system. The results of the calculations were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During hypertrophy of the heart under conditions of arterial hypertension due to constriction of the lumen of the abdominal aorta, the weight and volume of the left ventricle increased significantly (by 43%; Table 1). The right ventricle also was involved in the process of hypertrophy, but the increase in its weight and volume were smaller (by 31%). The predominant increase in size of the left ventricle and in its mass during the development of hypertension of the systemic circulation was demonstrated previously [9, 15]. It was accordingly considered that tissue samples from the wall of the left ventricle would be representative when assessing the dynamics and the degree of the changes in the parenchymatous and stromal structures in the hypertrophied myocardium. When the heart hypertrophied by 41% the total volume of the cardiomyocytes increased (from 0.550±0.018 cm^3 in the control to 0.777±0.005 cm^3 on the 35th day of the experiment, $P < 0.001$). The total volume of the structural components of the interstitial connective tissue increased by a greater degree (by 50%; from 0.098±0.004 to 0.148±0.013 cm^3, $P < 0.05$); an important contribution to this increase was made by the cells, fibers, and ground substances of the connective tissue. These quantitative changes in the parenchyma and stroma reflect intensification of sclerotic changes in the tissue of the hypertrophied myocardium. Stereologic analysis also revealed a significant increase in the relative volume of the myofibrils (by 20%) and of SSR (by 50%) in the heart tissue, whereas the total relative volume of the mitochondria decreased somewhat (Table 1). Similar changes took place in the volume of these organelles per unit volume of cytoplasm of the cardiomyocytes [2]. The bulk density of the T-system was unchanged in the tissue with the development of hypertrophy. The surface density of the organelles showed a similar change to their total relative volume.

Calculation of the absolute values showed that in the hypertrophied myocardium the total volume (Fig. 1) and surface area (Fig. 2) of the myofibrillary bundles increased significantly (by 71 and 72% respectively). The absolute total volume of the mitochondria remained virtually unchanged, but the total surface area of these organelles increased (by 35%). This time course of the changes was found during the development of cardiac hypertrophy under conditions of hypobaric hypoxia [7], and it probably reflects an increase in the number and a decrease in size of the mitochondria, associated with increased efficiency of their work. It is also considered that during a rapid increase in weight of the myofibrils, this kind of reorganization of the energy-producing apparatus helps to maintain the reserves of structural materials for the muscle cells of the heart.

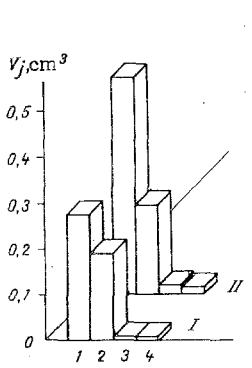

Fig. 1

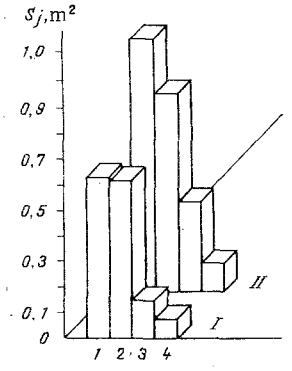

Fig. 2

Fig. 1. Absolute volume of organelles of cardiomyocytes in left ventricle of control animals (I) and on 35th day after constriction of abdominal aorta (II). Here and in Fig. 2: 1) myofibrils, 2) mitochondria, 3) SSR, 4) T-system.

Fig. 2. Surface area of organelles of cardiomyocytes in left ventricle of control animals (I) and on 35th day after constriction of abdominal aorta (II).

The total volume and surface area of SSR (by 113 and 157% respectively) and of the T system (by 43 and 54% respectively) also increased significantly during myocardial hypertrophy. It is evident that in myocardial hypertrophy as a result of arterial hypertension the controllable parameters are not only the total volume of the myofibrils and the area of the outer membranes of the mitochondria [7], but also the volume and surface area of structures maintaining ion exchange.

Similar changes in the absolute volume and surface area of the principal intracellular organelles (but calculated per cardiomyocyte) were found when myocardial hypertrophy was studied on the 8th day after subdiaphragmatic aortic stenosis in Wistar rats [9], evidence that the compensatory and adaptive reactions which develop in these two models of myocardial hypertrophy are common. The greater increase in the absolute volume of the mitochondria [9] than in the present experiments can be explained on the grounds that the authors cited used younger animals (initial body weight 90 g), in which physiological growth has not yet ceased.

Analysis of the dynamics of changes in the relative and absolute total parameters of the organelles of the cardiomyocytes during hypertrophy of the heart revealed certain differences. The increase in weight and volume of the left ventricle was accompanied by an increase in the volume and surface area of all the organelles. However, these processes were more marked in relation to the myofibrils of SSR, and this reflects the character of development of cardiac hypertrophy under the experimental conditions used.

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STRUCTURAL AND HISTOCHEMICAL CHARACTERISTICS OF EXPERIMENTAL PERIMUSCULARIZATION OF THE FEMORAL VEIN IN DIFFERENT WAYS

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KEY WORDS: vein; blood flow; vessel-tissue relations.

Attempts have recently been made to prevent the reflux of blood in the altered valveless deep veins of the lower limbs by creating artificial valves [2, 4, 6, 8], and also by extravasal constriction by the fascia lata [1] or by perivenous cuffs made of tantalum wire, 0.3 mm in diameter, in the form of a coil [3]. Unfortunately, intravascular valve mechanisms create a turbulent blood flow, which leads to thrombus formation, whereas permanent perivenous stenosing devices, while reducing retrograde reflux of blood, lead to disturbance of the centripetal blood flow.

Accordingly, in the investigation described below, an attempt was made to create partially acting perivenous mechanisms, using autogenous tissue for this purpose.

EXPERIMENTAL METHOD

Operations were performed on 32 mongrel dogs under general anesthesia (intrapleural injection of 10% hexobarbital solution in a dose of 0.5 mg/kg body weight). Two methods of perimuscularization were used, followed by comparative study of the histological changes in the structure of the musculo-vasal complex in order to choose the optimal version.

An anterior flap was cut in the frontal plane from the medial head of the quadriceps femoris muscle, located posteriorly to the femoral vein, and after division of its lateral edge it was transposed to the anterior surface of the mobilized femoral vein behind the artery (18 dogs). The edges of the excised piece of muscle were then sutured together and the vein was buried in its mass.

A tunnel was formed in the thickness of the medial head of the quadriceps femoris muscle (14 dogs). After division of the mobilized femoral vein distally to the level of the tunnel, the proximal segment of the divided vein was passed through the tunnel. Continuity of the femoral vein was restored by a vascular suture apparatus, by the end to end technique.

A segment of the perimuscularized vein was chosen during life under general anesthesia for histological investigation of the musculo-vasal complex 2 weeks, 1, 3, and 6 months, and 1 year after the operation. Material was fixed in 10% neutral formalin and in Carnoy's fluid, and then taken up through absolute alcohols. For the same preparation two or three experiments were undertaken by each method and at each time.

Serial paraffin histological sections were stained with hematoxylin and eosin and by Van Gieson's and Weigert's methods. Staining for histochemical study was carried out by the methods of McManus, Hale, Ritter and Oleson, and Brachet. The experimental results were analyzed in total for all experiments at each time of observation, but separately for each preparation.

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