STEREOLOGIC ULTRASTRUCTURAL AND CYTOCHEMICAL STUDY OF MYOCARDIAL HYPERTROPHY DURING AGING

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UDC 612.172.014.2-06:612.67+616.127-007.61-053.9

KEY WORDS: aging; myocardial hypertrophy; electron-microscopic stereology and cytochemistry.

It has recently been shown that the myocardium of clinically healthy people and experimental animals undergoes hypertrophy with age [2, 5]. Considerable metabolic and ultrastructural changes in the principal organelles take place in the cardiac myocytes under these circumstances. However, the character of myocardial hypertrophy during aging has not been studied.

The aim of the present investigation was, by using methods of quantitative electron-microscopic and electron-cytochemical investigation, to undertake an ultrastructural analysis of the heart muscle cells of aging animals. It was considered to be important to compare the stereologic parameters thus determined with those in experimental myocardial hypertrophy.

EXPERIMENTAL METHOD

Ten male intact Wistar rats aged 4 months (3 animals) and weighing 213.3 ± 8.8 g, 24 months (three animals) weighing 240.0 ± 25.2 g, and 33 months (four animals), weighing 337.5 ± 5.0 g, were used. After decapitation of the animals the relative weight of each heart was determined. Samples of tissue from the left ventricle, treated in the usual manner with paraform–osmium fixation, were dehydrated with propylene oxide and embedded in a mixture of Epon with Araldite. Longitudinal sections were cut on the LKB ultrotome, stained with uranyl acetate and lead citrate, and examined in the JEM 100B electron microscope.

The mean diameter of the muscle fiber for each group of animals was determined in semithin sections under a magnification of 639 by means of the MOV-15 ocular micrometer. Stereometry of the cardiac myocytes was carried out under a final magnification of 18,000. The relative volume (V_V) of the myofibrils, mitochondria, sarcoplasmic reticulum, T system, and cytoplasmic matrix (including glycogen, ribosomes, and the amorphous substance of the cytoplasm, and also the relative surface area (S_V) of these ultrastructures, bounded by membranes, were estimated. A test system of short sections (n = 21, P = 42, I_L = 1 cm) was used. On the basis of the results, values of surface to volume ratios of the ultrastructures and ratios of volume of the principal cell organelles to the volume of the myofibrils were calculated.

Succinate dehydrogenase (SDH) activity was detected by the method in [12] and the bulk density of granules of copper ferrocyanide in the mitochondria of the myocardiocytes was calculated under a final magnification of 20,000; an open test system (P-121) was used.

The technique of the measurements, formulas for stereometry of the cell compartments, and the method of statistical analysis are given [1, 4].

EXPERIMENTAL RESULTS

In rats aged 24-33 months hypertrophy of the heart develops, as shown by an increase in the absolute weight of the organ and in the mean diameter of the cardiac myocytes (Table 1). The relative weight of the heart was increased only in animals aged 24 months.

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(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 101-104, July, 1981. Original article submitted December 30, 1980.

0007-4888/81/9207-0977$07.50

TABLE 1. Results of Morphometric and Stereologic Analysis of Rat Heart during Aging (M ± m)

Parameter	Age of animals			P_{1-2}	P_{1-3}	P_{2-3}
	4 months (1)	24 months (2)	33 months (3)			
Morphometric characteristics of heart and muscle fibers						
Body weight, g	213,3±8,8	240,0±25,2	337,5±5,0		0,001	0,01
Absolute weight of heart, mg	933,3±44,2	1550,0±0,0	1450,0±86,5	0,001	0,01	
Relative weight of heart, mg/g of body weight	4,37±0,08	6,20±0,85	4,31±0,30	0,05		0,05
Diameter of cardiac myocytes, μm	15,2±0,9	17,8±0,2	17,9±0,6	0,05	0,05	
Stereologic characteristics of cardiac myocytes						
Relative volume $V_{v_i}^{cyt}$, mm^3/cm^3:						
of mitochondria	312,3±10,5	256,5±15,7	286,9±10,0	0,05		
of myofibrils	475,7±14,7	558,0±23,5	565,3±11,6	0,05	0,01	
of sarcoplasmic reticulum	24,2±0,9	14,0±1,4	10,5±0,6	0,001	0,001	0,05
of T-system of remaining structures of cytoplasm	22,6±0,6	16,5±0,3	9,2±0,8	0,001	0,001	0,001
	165,2±11,3	154,9±15,5	128,1±12,3			
Relative surface area ($S_{v_i}^{cyt}$), m^2/cm^3:						
of mitochondria	1,706±0,055	1,581±0,112	1,266±0,036		0,001	0,05
of myofibrils	1,673±0,082	1,637±0,037	1,466±0,053			
of sarcoplasmic reticulum	0,455±0,043	0,267±0,033	0,258±0,028	0,05	0,05	
of T-system	0,341±0,032	0,212±0,014	0,121±0,032	0,01	0,01	
Surface to volume ratio (S_{v_i}/V_{v_i}):						
of mitochondria	5,5±0,3	6,2±0,4	4,5±0,2		0,05	0,01
of myofibrils	3,5±0,1	2,9±0,1	2,6±0,1	0,01	0,001	0,01
of sarcoplasmic reticulum	18,8±1,9	19,2±2,2	24,5±2,2			
of T-system	15,2±1,7	12,8±0,5	13,1±0,7			
Ratio of bulk density (V_{v_i}/V_{vbd}):	0,051±0,004	0,025±0,002	0,019±0,001	0,001	0,001	0,05
of sarcoplasmic reticulum and myofibrils	0,658±0,039	0,463±0,045	0,509±0,025	0,01	0,05	
of mitochondria and myofibrils	0,048±0,003	0,029±0,002	0,016±0,001	0,01	0,01	0,01
of T-system and myofibrils						
Bulk density of granules of chelate in mitochondria, %	20,7±3,4	11,9±1,5	10,4±1,1		0,05	

The results of stereologic analysis of the electron micrographs showed that during aging considerable changes take place in the ultrastructural organization of the cardiac myocytes. The relative volume of the mitochondria decreased in the aging animals (significantly only at the age of 24 months), probably in connection with a decrease in the numerical density of these organelles with age [11]. The surface density of the mitochondria decreased under these circumstances from 1.706 ± 0.055 to 1.266 ± 0.036 m^2/cm^3. This index determined the significant decrease in the surface to volume ratio of the mitochondria in animals aged 33 months. The mitochondria in the cardiomyocytes of these rats appeared larger and were surrounded by a larger quantity of glycogen (Fig. 1A); secondary lysosomes were often arranged side by side with the mitochondria (Fig. 1B).

It was shown electron-cytochemically that SDH activity in the mitochondria (Fig. 1C) fell by about half as a result of senile hypertrophy of the heart (Table 1). The decrease in the activity of this enzyme of the tricarboxylic acid cycle was linked with a fall in the degree of coupling of oxidation with phosphorylation and potentiation of the role of glycolysis in the energy metabolism of the cell [3]. Such a situation arises in cardiac hypertrophy because of compensatory hyperfunction.

During aging the relative volume of the myofibrils increased significantly (Fig. 1D). The highest value of this index did not ultimately exceed 60% of the volume of cytoplasm. A tendency also was observed for the surface density of the myofibrils to decrease with age, leading to a significant decrease in surface to volume ratios of these organelles.

In old animals fibers of myofibrils in the cardiomyocytes were thickened and the myofibrillary mass was increased. For normal functioning of the cell under these conditions an increase in the calcium inflow is necessary. The calcium inflow into the cell and its turnover are regulated by the sarcoplasmic reticulum and T-system. These transport systems

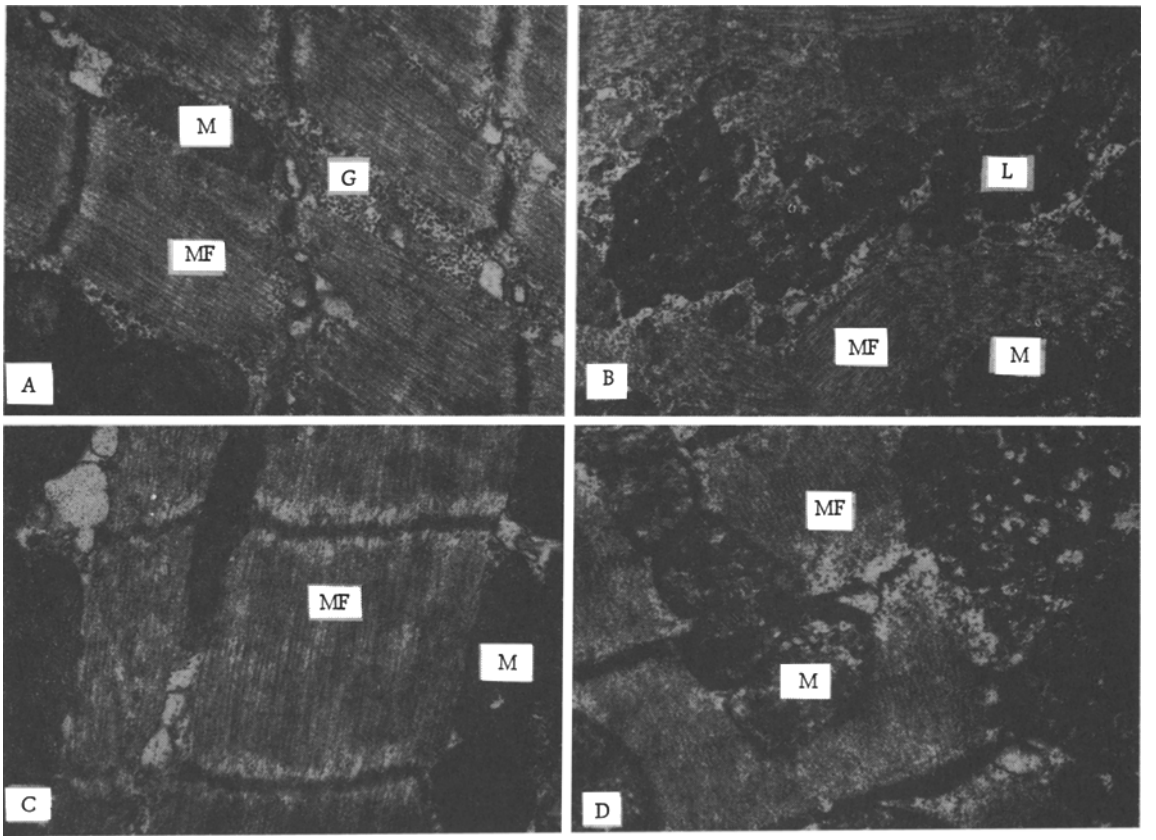

Fig. 1. Ultrastructural and electron-cytochemical characteristics of cardiac myocytes of hypertrophied myocardium of old rats. A) Increased glycogen content (G) around mitochondria (M), B) formation of secondary lysosomes (L), C) increased content of myofibrils (MF), D) granules of chelate in mitochondria. Magnification: A, C, D) 10,000, B) 8,300 ×.

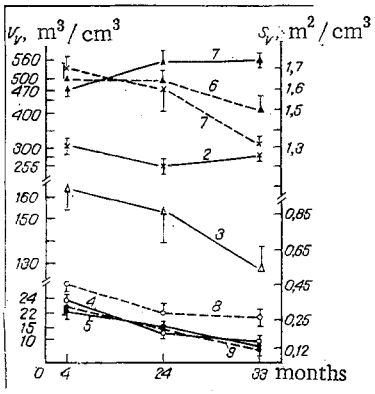

Fig. 2

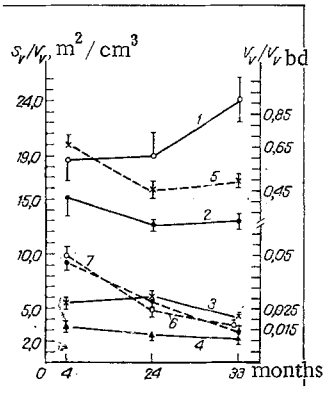

Fig. 3

Fig. 2. Results of measurements of primary stereologic parameters of organelles of cardiac myocytes during myocardial hypertrophy in old rats. Abscissa, age of animals (in months); ordinate: left — bulk density (in mm^3/cm^3), right — surface density (in m^2/cm^3). 1, 6) Myofibrils; 2, 7) mitochondria; 4, 8) sarcoplasmic reticulum; 5, 9) T-system; 3) remaining structures of cytoplasm.

Fig. 3. Results of calculations of secondary stereologic parameters of organelles of cardiac myocytes during myocardial hypertrophy in old rats. Abscissa, age of animals (im months); ordinate: left — surface to volume ratios of organelles (in m^2/cm^3), right — ratio of relative volumes of organelles and relative volumes of organelles and relative volume of myofibrils. 1) Sarcoplasmic reticulum, 2) T-system, 3) mitochondria, 4) myofibrils, 5) mitochondria/myofibrils, 6) sarcoplasmic reticulum/myofibrils, 7) T-system/myofibrils.

undergo considerable changes during aging (Fig. 2): the relative volume of the sarcoplasmic reticulum ultimately decreased by 57%, and that of the T-system by 59%. Meanwhile their surface density decreased: from 0.455 ± 0.043 to 0.258 ± 0.028 m^2/cm^3 for the sarcoplasmic reticulum and from 0.341 ± 0.032 to 0.121 ± 0.032 m^2/cm^3 for the T-system. A similar decrease in the relative volume of the sarcoplasmic reticulum and in its surface area per unit myofibrillary mass has also been found in the myocardium of aging Syrian hamsters [14].

The mechanisms lying at the basis of development of myocardial hypertrophy in old age and in experimental myocardial hypertrophy do not differ significantly but are universal for adaptive and compensatory reactions in the heart. This is shown by the decrease in the ratio of bulk density of the mitochondria and myofibrils during hypertrophy of the heart of varied genesis [6, 8-10, 13]. One of the distinguishing features of myocardial hypertrophy in old age is a decrease in the "saturation" of the myofibrils per unit volume with other cell organelles, as is shown by analysis of the volume ratios of the mitochondria, sarcoplasmic reticulum, T-system, and myofibrils (Figs. 3). An increase in the relative volume of the sarcotubular system [10] has been found in experimental models of working myocardial hypertrophy [7], in which it compensates for the reduced ability of the sarcoplasmic reticulum to bind calcium in the hypertrophied myocytes [15]. Myocardial hypertrophy in old age is characterized by an increase in myofibrillary mass and a decrease in the structural density of the transport systems, accompanied by relative constancy of quantitative indices of the mitochondrial compartment.

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