THREE-DIMENSIONAL REORGANIZATION OF PARENCHYMATOUS-STROMAL STRUCTURES OF THE MYOCARDIUM IN SPONTANEOUS GENETIC HYPERTENSION

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KEY WORDS: spontaneous genetic hypertension; hypertrophy of the heart; parenchyma and stroma of the myocardium; morphometry and stereology.

The study of parenchymatous-stromal relations in the myocardium in the course of its hypertrophy [2, 3, 7, 9] is very important, for the concept of a single morpho-functional reaction of parenchymatous and stromal structures during the development of general pathological processes provides more complete information on the character of the changes taking place and enables the outcome of various pathological states to be judged [10, 12]. However the diagnosis and prognosis of hypertrophic states of the myocardium requires a detailed investigation of this phenomenon in animals of different species and under different experimental conditions, as well as comparative investigations at the tissue [13] and cellular [11] levels by up-to-date morphological methods. One promising method of evaluating parenchymatous-stromal interactions is quantitative morphological analysis [8, 14].

The object of this investigation was to study the structural three-dimensional reorganization of muscle and connective tissue of the myocardium in rats with spontaneous genetic hypertension.

EXPERIMENTAL METHOD

The tissue organization of myocardium was studied in 19 inbred male rats with spontaneous genetic hypertension of the SHR (spontaneously hypertensive rats) line aged 1-11 months. Male Wistar rats aged 4-6 months (six animals) served as the control. The blood pressure (BP) was measured by means of a transducer with rubber cuff, placed on the tail of the animals superficially anesthetized with ether. The signal from the transducer was recorded on a Mingograph-34 (Elema-Schönander, Sweden). After decapitation of the rats the heart was placed in a cold chamber until it completely stopped beating and its absolute and relative weight was determined. For histological study the heart was fixed in a 20% solution of neutral formalin. Paraffin sections were stained with hematoxylin and eosin in conjunction with Perls' reaction, and by Van Gieson's method with counterstaining of elastic structures. The PAS reaction was set up with enzymic and chemical controls. Microscopic preparations were studied and photographed in direct and polarized light. Samples of tissue of the left papillary muscles for electron-microscopic investigation were fixed in a 4% solution of paraformaldehyde, postfixed in 2% OsO_4 solution, and after standard treatment the tissue was embedded in a mixture of Epon and Araldite. Semithin (thickness 1 μm) sections were cut on the LKB III ultrotome, stained with azure II, and examined in the Docuval universal biological photomicroscope (Carl Zeiss, East Germnay). The quantitative morphological investigation of the myocardium was undertaken on five rats aged 1 month, three rats aged 4 months, and four rats aged 11 months as well as on six normotensive Wistar rats. By means of the morphometric and stereologic methods described previously [1, 8], the relative volume (bulk density) of the cardiomyocytes, their nuclei, capillary lumen, endothelial cells, connective-tissue cells, fibers, and ground substance, and the relative surface area (surface density) of the cardiomyocytes and their nuclei,

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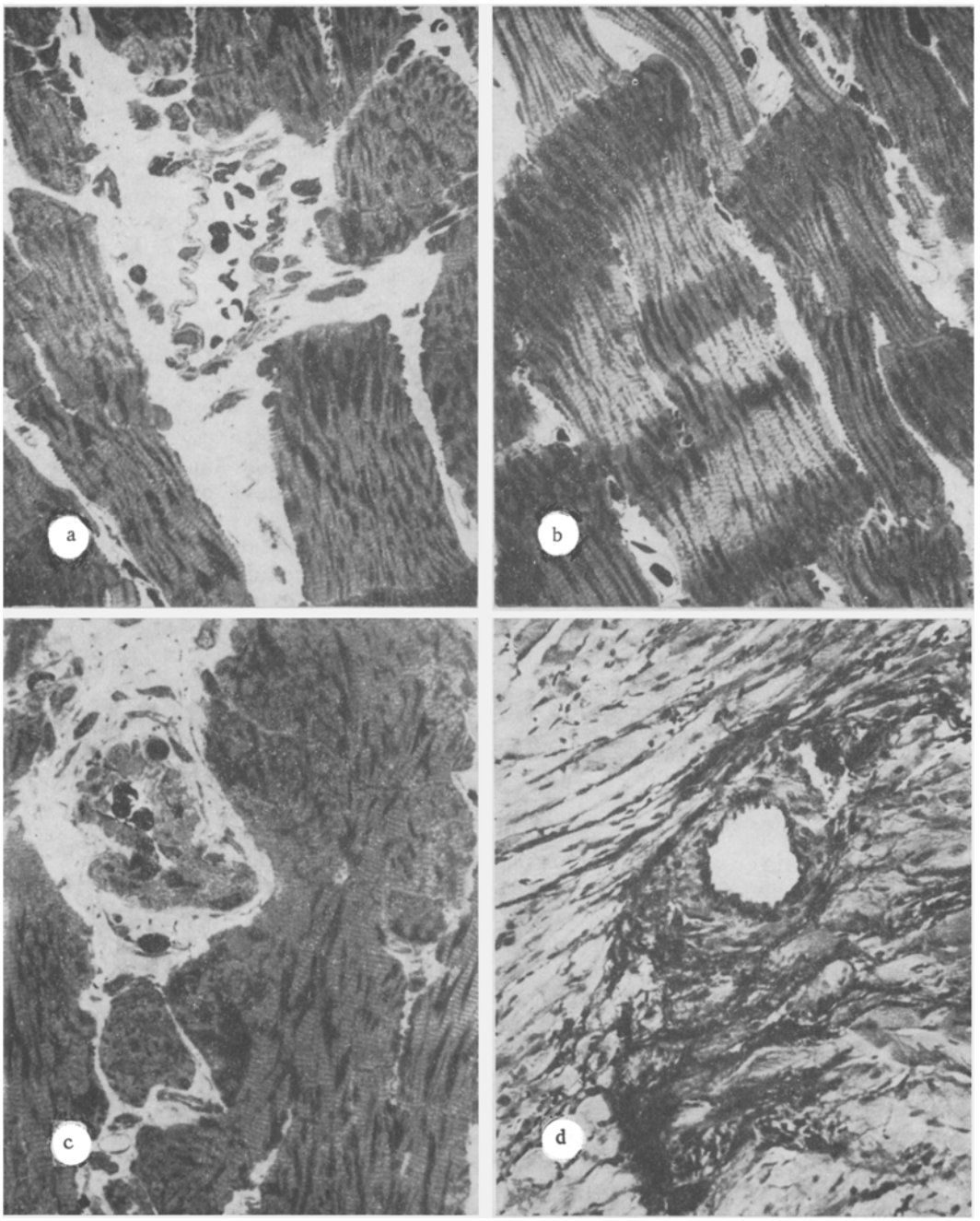

Fig. 1. Morphological changes in parenchyma and stroma of hypertrophied myocardium of SHR rats: a) marked hypertrophy of cardiomyocytes in left papillary muscle, pericapillary, perivascular, and interstitial sclerosis; b) irregular contraction of cardiomyocytes; c) hypertrophy and hyperplasia of smooth-muscle cells in middle layer of wall of intramural artery; d) constriction of lumen of arteriole and marked perivascular sclerosis in myocardium. Rats aged: b, c) 4 months, a, d) 11 months. In a, b, d) Semithin sections stained with azure II, 1250 ×; c) Van Gieson's stain, 400 ×.

the inner surface of the capillaries, and connective-tissue cells were estimated in semithin sections under a magnification of 1000. The relative volume of the parenchyma was calculated as the total bulk density of the cardiomyocytes and their nuclei, and the relative volume of the stroma as the total density of the capillaries, endothelial cells, and connective-tissue cells, fibers, and ground substance. Surface–volume ratios of the structures and volume and surface–volume ratios of some structures compared with others also were calculated. A screw-adjusted MOV-1-15 ocular micrometer was used and for each group of animals the mean diameter

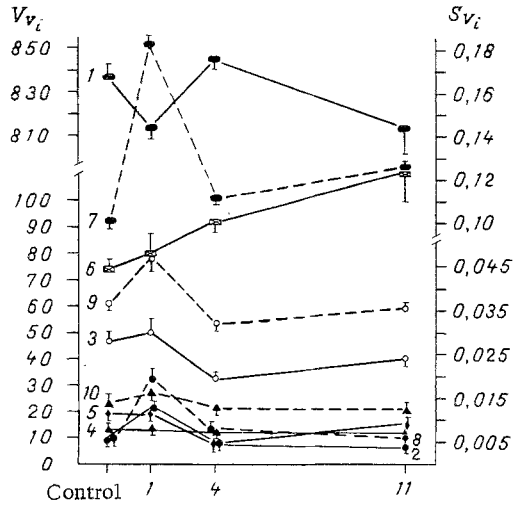

Fig. 2. Results of measurement of primary stereologic parameters of rat myocardial tissue structures during development of spontaneous hypertension. 1, 7) Cardiomyocytes, 2, 8) cardiomyocyte nuclei, 3, 9) capillaries, 4, 10) connective-tissue cells, 5) endothelial cells, 6) ground substance and fibers of connective tissue. Abscissa, age of animals (in months); ordinate: on left — bulk density (in mm^3/cm^3), on right — surface density (in m^3/cm^3).

of the cardiomyocytes was determined (the variability of the diameter of the cardiomyocytes was determined by the use of information theory) [5, 15]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

BP was raised in the spontaneously hypertensive rats after reaching the age of 1 month, and hypertrophy of the heart developed at the same time. For instance, the absolute weight of the heart in SHR rats aged 4 and 11 months was increased by 19 and 43% respectively, and the diameter of the cardiomyocytes was 1.7 times greater than that of the normotensive control.

Cardiomyocytes in SHR rats aged 1 month preserved their structure. In some cases cardiomyocytes with acidophilic staining of their sarcoplasm and with pycnotic nuclei were observed. On polarization microscopy cross-striation was clearly visible in these cells: Anisotropy of the A disks was intensified without any significant shortening of the isotropic disks.

The capillary walls were thin. In some cases numerous outgrowths of cytoplasm of the endotheliocytes facing the capillary lumen were formed, often curiously shaped. The intramural vessels of the myocardium sometimes had signs of moderate spasm with disarrangement of the structural elements of the intima and media.

In the myocardium of SHR rats aged 4 and 11 months the muscle fibers were thicker than in SHR rats aged 1 month (Fig. 1a) and polymorphism of the nuclei was increased. The number of acidophilically stained muscle segments, corresponding to contractures of the myofibrils, was increased (Fig. 1b). Cells were seen in which the longitudinal orientation of the myofibrils was disturbed [6, 9, 16, 17]. In these age groups destructive changes were found in the mitochondria, with considerable hyperplasia of the lamellar complex and an increase in the number of lysosomes.

In the course of development of hypertrophy of the heart, changes took place in the microcirculation (Fig. 1c); dystrophically changed capillary endotheliocytes appeared. Some of them had transparent cytoplasm of reduced density and haphazardly arranged and infrequent organelles, often with a damaged plasmalemma. Other endotheliocytes were characterized by an electron-dense matrix and poorly distinguishable ultrastructure. With age, progressive thickening of the walls of all intramural arteries developed, leading to constriction of the lumen of the vessels (Fig. 1d). Thickening of the wall took place on account of hyperplasia and hypertrophy of the smooth-muscle cells of the middle layer; thickening and multiplication of the elastic membranes also occurred. Gradually bundles of collagen fibers formed between

TABLE 1. Quantitative Morphological Characteristics of Parenchymatous-Stromal Relations of Myocardium of Rats with Spontaneous Genetic Hypertension

Parameter	Normotensive Wistar rats (aged 4-6 months)	Age of hypertensive SHR rats, months		
		1	4	11
Morphometric investigations				
Body weight, g	210,0±9,5	62,0±0,36***	273,3±23,3*	233,3±32,8
Absolute weight of heart, mg	948,0±33,9	431,0±19,5***	1126,7±148,6	1353,3±140,5*
Relative weight of heart, mg/g body weight	4,53±0,13	6,96±0,22***	4,09±0,22	5,87±0,35**
Diameter of cardiomyocytes, μm	15,5±0,5	13,5±0,5*	25,7±1,4***	26,0±1,0***
Stereologic investigations				
Relative volume ($V_{V_1}^{mc}$), mm^3/cm^3, of:				
cardiomyocytes	837,3±6,1	813,7±6,5	845,7±4,3	813,1±12,6
cardiomyocyte nuclei	9,8±0,6	21,8±0,3***	7,5±1,1	6,1±0,6**
capillaries	46,5±3,6	50,0±5,1	32,9±1,1*	40,8±1,7
endothelial cells	19,3±1,2	20,0±0,8	9,2±1,1**	16,1±0,5
convective-tissue cells	12,7±0,9	14,0±0,9	11,8±1,0	12,8±2,6
ground substance and fibers of convective tissue	74,4±2,8	80,5±8,2	92,9±3,8**	111,1±11,2**
Relative surface area ($S_{V_1}^{mc}$), m^2/cm^3, of:				
cardiomyocytes	0,1048±0,0056	0,1824±0,0162**	0,1115±0,0054	0,1266±0,0008*
cardiomyocyte nuclei	0,0067±0,0002	0,0197±0,0018***	0,0069±0,0017	0,0063±0,0005
capillaries	0,0359±0,0013	0,0475±0,0042*	0,0321±0,0016	0,0357±0,0008
connective-tissue cells	0,0148±0,0017	0,0164±0,0017	0,0134±0,0010	0,0129±0,0013
Surface-volume ratio (S_{V_1}/V_{V_1}), m^2/cm^3, of:				
cardiomyocytes	0,1252±0,0067	0,2243±0,0208**	0,1318±0,0059	0,1557±0,0019*
cardiomyocyte nuclei	0,6950±0,0377	0,9062±0,0959	0,8953±0,1321	1,0367±0,0239**
capillaries	0,7857±0,0588	0,9779±0,1356	0,9778±0,0706	0,8795±0,0477
connective-tissue cells	1,1545±0,0654	1,1702±0,1343	1,1381±0,0231	0,8814±0,0945
Ratio of bulk density of stroma to bulk density of parenchyma ($V_{V_{str}}/V_{V_{cmc}}$)	0,180±0,008	0,197±0,009	0,172±0,007	0,221±0,020
Ratio of bulk density of capillaries to bulk density of cardiomyocytes ($V_{V_{cap}}/V_{V_{cmc}}$)	0,056±0,005	0,060±0,006	0,038±0,002*	0,050±0,003
Ratio of surface density of capillaries to bulk density of cardiomyocytes ($S_{V_{cap}}/V_{V_{cmc}}$), m^2/cm^3	0,043±0,003	0,057±0,005*	0,038±0,002	0,044±0,0002

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

TABLE 2. Parameters of Information Analysis of Myocardium of Normotensive Wistar Rats and Spontaneously Hypertensive (SHR) Rats

Parameter	Normotensive Wistar rats (age 4 months)	Age of hypertensive SHR rats, months		
		1	4	11
Entropy (H), binary units	3,11	2,62	3,51	3,41
Relative entropy (h)	0,816	0,789	0,842	0,834
Excess (R), %	18,4	21,1	15,8	16,6

Legend. Diameter of cardiomyocytes was analyzed.

the cells of the middle layer of the arteries, with the development of intramural and, later, perivascular sclerosis.

In SHR rats of all age groups activation of the stromal cells, mainly active forms of fibroblasts, was observed; lymphocytes, macrophages, and mast cells also were seen. As myocardial hypertrophy developed, the degree of the cellular reaction of the stroma decreased a little, and bundles of collagen fibers, mainly with a pericapillary localization, appeared between the muscle cells.

During the development of myocardial hypertrophy no significant change took place in the relative volume of the cardiomyocytes in SHR rats compared with normotensive rats (Table 1).

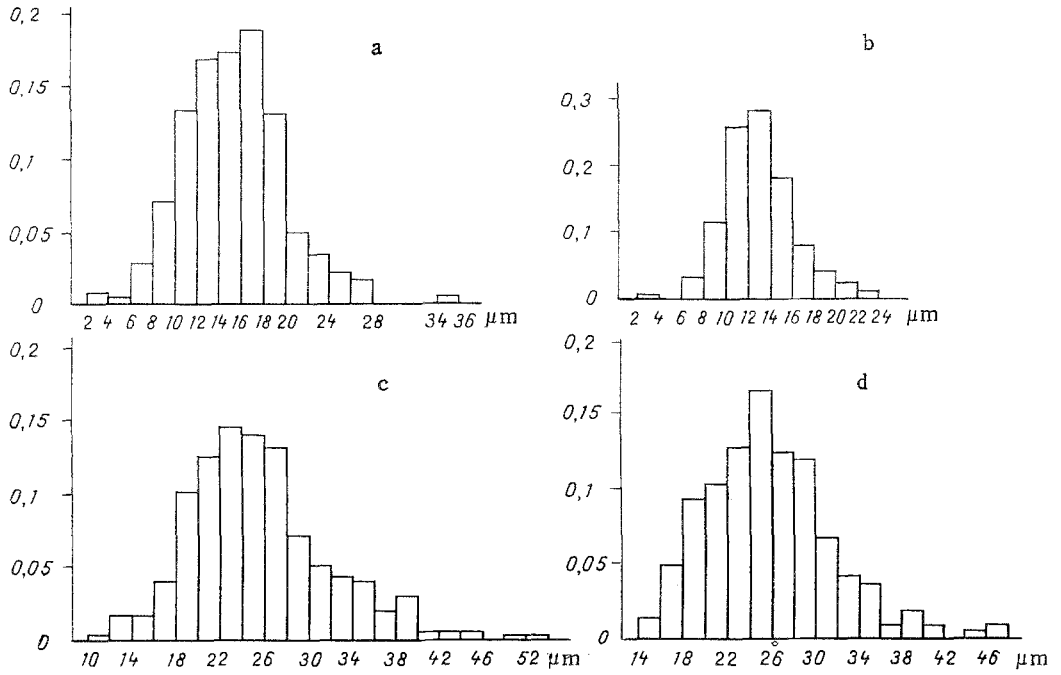

Fig. 3. Histograms of distribution of cardiomyocytes by diameter. Abscissa, diameter of myocytes (in μm); ordinate: a) normotensive Wistar rats aged 4 months, b) SHR rats aged 1 month, c) SHR rats aged 4 months, d) SHR rats aged 11 months.

The bulk density of the cardiomyocyte nuclei decreased in SHR rats with age. This time course of the relative volume of the cardiomyocytes and their nuclei is evidence that hypertrophy of the heart develops mainly on account of hypertrophy of the muscle cells, and under these circumstances their total number and the number of nuclei remain the same. This conclusion is supported also by the decrease in the relative surface area of the cardiomyocytes and their nuclei in SHR rats during the development of hypertrophy of the heart, although the surface density of the cardiomyocytes in animals aged 1 and 11 months was significantly higher than this parameter in normotensive rats (Figs. 2).

Significant changes took place in the stroma of the hypertrophied myocardium. The bulk and surface densities of the capillaries decreased considerably. These parameters were lowered by a greater degree in SHR rats aged 4 months (by 29 and 11% respectively compared with the normotensive control). The surface–volume ratio of the capillaries did not change significantly.

In the process of myocardial hypertrophy in spontaneously hypertensive rats the volume and surface–volume ratios of the capillaries to cardiomyocytes which, in our view, characterize most completely relations between these two components of the tissue microregion [4], decreased. The ratio of the bulk surface density of the capillaries to the bulk density of the cardiomyocytes was reduced most appreciably in 4-month-old SHR rats. In 11-month-old animals these parameters were higher than in the previous age group, evidence that structural changes in the hypertrophied myocardium take place in stages [9]. The bulk density of the endothelial cells also was more appreciably reduced in 4-month-old SHR rats (by 52% relative to the control). Hypertrophy of the heart in SHR rats is connected with a significant increase in the relative volume of the interstitial connective tissue (combining cells, fibrous structures, and ground substance) by 30%. This increase took place on account of noncellular components (chiefly collagen), for the bulk density of the connective-tissue cells was virtually unchanged. Accumulation of collagen in the stroma of the hypertrophied myocardium is explained by the increased rate of synthesis of this protein in SHR rats [18] which, in turn, may be connected with the presence of many actively synthesizing fibroblasts.

In the hypertrophied myocardium of SHR rats no significant change was found in parenchymatous-stromal relations, as shown by analysis of the ratio of bulk density of the stroma to

bulk density of the parenchyma. Nevertheless, it must be pointed out that this parameter was increased in SHR rats aged 11 months (with stable hypertension).

During the development of myocardial hypertrophy, analysis of changes in the diameter of the cardiomyocytes is of definite interest, for the increase in the number of size groups of muscle cells may be a manifestation of their structural–metabolic heterogeneity. The distribution of cardiomyocytes by diameter in normotensive Wistar rats and in SHR rats aged 1 month was close to normal: Most muscle cells in these animals had a diameter of 10-18 μm (Fig. 3a, b). Most cardiomyocytes in the myocardium of SHR rats aged 4 and 11 months had a diameter of 22-30 μm and the percentage of cells with a diameter of 40 μm and more was increased (Fig. 3c, d). Information analysis showed that with the development of hypertrophy of the heart the entropy and relative entropy increased, whereas the excess decreased (Table 2), evidence of an increase in disorderliness and an increase in structural–metabolic heterogeneity in the heart muscle. The changes described above were more marked in SHR rats aged 4 months, possibly as a result of marked ultrastructural changes in the myocardium at this age [6], caused by the rise of BP, and may reflect the compensatory and adaptive powers of the tissue.

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