11. C. Prys-Roberts, J. H. Kerr, J. L. Corbett, et al., Lancet, 1, 542 (1969).
12. E. S. Reynolds, J. Cell Biol., 17, 208 (1963).
13. S. B. Rosalki, J. Lab. Clin. Med., 69, 696 (1967).
14. A. G. Rose, S. Afr. Med. J., 48, 1285 (1974).
15. G. S. Sainani, K. L. Jain, V. R. D. Deshpande, et al., Prog. Drug. Res., 19, 361 (1975).
16. J. G. Stempak and R. T. Ward, J. Cell Biol., 22, 697 (1964).
17. K. Tsueda, P. B. Oliver, and R. W. Richter, Anesthesiology, 40, 588 (1974).

ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF CARDIOMYOCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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UDC 616.12-008.331.1-055.5].7-07:616.127-018.1-076.4

KEY WORDS: spontaneous hypertension; hypertrophy of the myocardium; ultrastructure of the cardiomyocyte; morphometry; stereology.

Spontaneous hypertension in experimental animals is linked with the development of hypertrophy of the heart [12, 14], in the course of which ultrastructural reorganization of the cardiomyocyte takes place [10, 15]. Quantitative changes in the mitochondrial and myofibrillary compartments have been studied in the greatest detail, for relations between these organelles are considered to play a key role in compensatory-adaptive reactions developing at the cell level [2, 5-7, 11]. However, for a fuller understanding of the processes taking place in cardiomyocyte hypertrophy it is essential to know the structural state of the transport systems of the cell and their interaction with other organelles.

The aim of this investigation was to study the intracellular organization of cardiomyocytes in rats of different ages with spontaneous genetic hypertension in the course of its development.

EXPERIMENTAL METHOD

Male albino rats (n = 34) with genetic arterial hypertension (SHR line) were used. The arterial blood pressure (BP) was measured by a transducer in the tail under superficial ether anesthesia. A morphometric study of the heart was conducted in twelve rats aged 1 month (body weight 62.0 $\pm$ 0.36 g), 4 months (body weight 273.3 $\pm$ 23.3 g), and 11 months (body weight 233.3 + 32.8 g). Immediately after sacrifice of the animals the heart was removed from the chest and placed in a cold chamber until it stopped beating completely. The absolute and relative weight of each heart was then determined. Samples of tissue from the left papillary muscle were fixed in a 4% solution of paraformaldehyde, postfixed in 2% OsO_4 solution, dehydrated with ethanol and propylene oxide, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB-III Ultratome. Ultrathin sections were stained and examined in the JEM 100 B electron microscope.

The mean diameter of the cardiomyocytes for each groups of animals was determined in semithin sections stained with azure II by means of the MOV-1-15 ocular micrometer. Stereologic analysis was carried out on electron micrographs under a final magnification of

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TABLE 1. Results of Morphometric and Stereologic Analysis of the Heart in SHR Rats (M ± m)

Parameter	Age of animals, msec			P_{1-2}	P_{1-3}	P_{2-3}
	1 mo. (1)	4 mo. (2)	11 mo. (3)			
Morphometric characteristics of heart and muscle fibers						
Body weight, g	62,0±0,36	273,3±23,3	233,3±32,8			
Absolute weight of the heart, mg	431,0±19,5	1126,7±148,6	1353,3±140,5	0,001	0,001	
Relative weight of the heart, mg/g body weight	6,96±0,22	4,09±0,22	5,87±0,35	0,001	0,05	0,05
Diameter of cardiomyocytes, μ	13,5±0,5	25,7±1,4	26,0±1,0	0,001	0,001	
Stereologic ultrastructural characteristics of cardiomyocytes						
Relative volume ($V_{V_i}^{cyt}$), mm^3/cm^3:						
Of myofibrils	496,6±8,9	549,4±7,4	545,5±9,8	0,05	0,05	
Of mitochondria	339,7±18,2	264,0±4,4	288,6±15,3	0,05		
Of SR	12,4±1,6	9,5±1,2	16,3±1,9			0,05
Of T system	16,7±2,4	16,5±2,3	18,9±0,5			
Of remaining structures of the cytoplasm	134,5±9,0	160,6±3,4	130,7±6,5			0,05
Relative surface area ($S_{V_i}^{cyt}$), m^2/cm^3:						
Of myofibrils	1,523±0,073	1,473±0,057	1,422±0,045			
Of mitochondria	1,482±0,046	1,257±0,072	1,229±0,027		0,01	
Of SR	0,199±0,026	0,197±0,009	0,328±0,012		0,05	0,01
Of T system	0,224±0,064	0,208±0,020	0,239±0,005			
Surface/volume ratio (S_{V_i}/V_{V_i}), m^2/cm^3:						
Of myofibrils	3,1±0,7	2,7±0,1	2,6±0,1			
Of mitochondria	4,4±0,2	4,8±0,3	4,2±0,2			
Of SR	16,0±0,6	21,7±3,5	20,8±2,7			
Of T system	12,9±1,9	12,7±0,6	12,8±1,0			
Ratio between bulk densities ($V_{V_i}/V_{V_{mf}}$), cm^0/cm^0 (number):						
Of mitochondria and myofibrils	0,685±0,046	0,481±0,014	0,531±0,038	0,05		
Of SR and myofibrils	0,025±0,003	0,017±0,002	0,030±0,003			0,05
Of T system and myofibrils	0,033±0,004	0,030±0,004	0,035±0,003			
Of remaining structures of cytoplasm and myofibrils	0,271±0,018	0,292±0,009	0,239±0,008			0,05

18,000. The relative volume of the myofibrils, mitochondria, sacroplasmic reticulum (SR), T system, and the remaining structures of the cytoplasm (including ribosomes, glycogen, lipid droplets, laminar complex, and amorphous substance of the cytoplasm) and the relative surface area of the organelles and their limiting membranes were estimated by methods described previously [2]. On the basis of these primary data, secondary parameters were calculated: surface/volume ratios of the ultrastructures and volume ratios of the principal cell organelles relative to the volume of the myofibrils. The results of the quantitative measurements were subjected to statistical analysis [1, 3] by Student's test.

EXPERIMENTAL RESULTS

After the age of one month the BP of the SHR rats showed a steady upward trend (118 ± 10.1 mm Hg at one month, 158 ± 4.5 mm Hg at four months), which are largely due to an increased cardiac output. In the older animals (11 months) the increase in and absolute level of BO (179 ± 5.5 mm Hg) were attributable mainly to an increase in the peripheral vascular resistance. The cardiac output in these animals was reduced mainly on account of a decrease in myocardial contractility.

In the course of ontogeny the absolute weight of the heart of the spontaneously hypertensive rats increased threefold and the mean diameter of the cardiomyocytes was doubled (Table 1). The relative weight of the heart, on the other hand, decreased a little. However, by this parameter alone it is impossible to judge the degree of hypertrophy of the heart, for the body weight of the experimental animals used in the calculations to determine the relative weight of the heart was rather variable. Accordingly, when estimating the degree of hypertrophic growth of the heart, attention was paid chiefly to the absolute weight of the heart and the diameter of the cardiomyocytes.

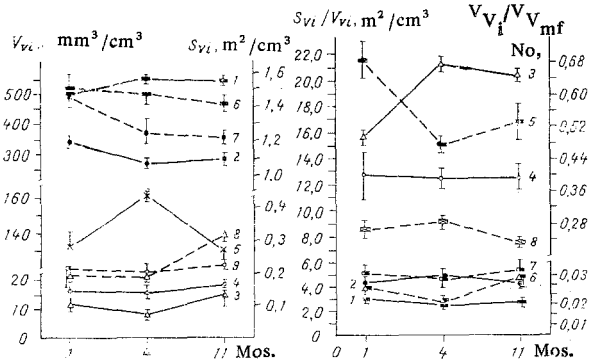

Fig. 1. Results of measurements of primary stereologic parameters of organelles of cardiomyocytes in SHR rats from the age aspect. Abscissa, age of animals (in months); ordinate: left — bulk density, right — surface density. 1, 6) myofibrils; 2, 7) mitochondria; 3, 8) SR; 4, 9) T system; 5) remaining structures of cytoplasm.

Fig. 2. Results of calculations of secondary stereologic parameters of organelles of cardiomyocytes in SHR rats from the age aspect. Abscissa, age of animals (in months); ordinate, left — surface/volume ratio of organelles, right — ratio of relative volumes of organelles to relative volume of myofibrils. 1) Myofibrils; 2) mitochondria; 3) SR; 4) T system; 5) Mitochondria/myofibrils; 6) SR/myofibrils; 7) T system/myofibrils; 8) remaining structures of cytoplasm/myofibrils.

At the ultrastructural level hypertrophy of the myocardium was manifested as a disproportionate change in the relative volumes of mitochondria and myofibrils (Fig. 1): the bulk density of the mitochondria was reduced, more especially in the rats aged 4 months (by 20%), but the bulk density of the myofibrils increased by 10% (Table 1). The relative surface area of these organelles decreased. The surface/volume ratio of the mitochondria was virtually unchanged, whereas this parameter was very slightly reduced for the myofibrils (Fig. 2). It can be concluded from the stereologic analysis that in hypertrophic growth of the cardiomyocytes in spontaneously hypertensive rats the conformational properties of these organelles are not significantly changed. Preservation of the surface/volume ratio of the intracellular structures evidently determines the adequate response of the cell to an increased load, despite the fact that the other dimensional characteristics were changed. The opposite direction of the changes in the relative volumes of the mitochondria and myofibrils led to a decrease in the volume ratio mitochondria/myofibrils, which was most marked in animals aged four months ($P < 0.05$). This process, described in experimental models of hypertrophy of the heart [4, 8, 9, 13], is evidently an essential condition for hypertrophic growth of the cardiomyocytes.

The bulk density of SR decreased a little in the animals aged 4 months, compared with those aged one month, from 12.4 $\pm$ 1.6 to 9.5 $\pm$ 1.2 mm^3/cm^3, but later it increased to 16.3 $\pm$ 1.9 mm^3/cm^2 (in 11-month-old rats). The relative surface area of SR in rats aged 11 months was almost twice that observed in rats of the other two groups. Such a marked increase in area of the membranes of SR is evidence of an increase in the power of the transport system of the cardiomyocytes and it is a compensatory-adaptive reaction. The value of the surface/volume ratio of SR, incidentally, was already increased by the age of four months and it remained about the same in rats aged 11 months (16.0 $\pm$ 0.6, 21.7 $\pm$ 3.5, and 20.8 $\pm$ 2.7 m^2/cm^3 respectively). In the period of most rapid hypertrophic growth of the cardiomyocytes, when hyperplastic growth of SR lagged behind the increase in weight of the

myofibrils, conformational changes in SR associated with an increase in the surface/volume ratios, made possible the intensification of metabolism in the cell. Later a proportionate increase in SR and of the myofibrils was observed without any change in their conformational states. The ratio of the area of the SR membranes to the volume of the myofibrils increased under these circumstances from 0.399 ± 0.045 and 0.359 ± 0.021 m^2/cm^3 in rats aged 1 and 4 months to 0.603 ± 0.032 m^2/cm^3 in animals aged 11 months ($P < 0.01$).

Hypertrophy of the cardiomyocytes in SHR rats is thus characterized not only by an ultimate increase in area of the SR membranes relative to the cell as a whole, but also by a specific increase in the area of the SR membranes relative to the volume of the myofibrils. This phenomenon can also be classed as a compensatory mechanism.

The T system does not undergo any significant quantitative changes during the development of hypertrophy of the cardiomyocytes in SHR rats. The bulk and surface density are somewhat increased in animals aged 11 months, but this causes no change in the surface/volume ratio (Table 1). Constancy of the surface/volume ratio of the T system is a compensatory mechanism of the cell in response to hypertrophic growth. With an increase in size of the cardiomyocytes the surface area of the outer sarcolemma increased per unit volume of cell: 0.1824 ± 0.0162, 0.1115 ± 0.0054, and 0.1266 ± 0.0008 m^2/cm^3 respectively for rats aged 1, 4, and 11 months. This may significantly affect the functional state of the cardiomyocytes, for the outer sarcolemma is responsible for certain vitally important functions. The T system is usually regarded as the inner sarcolemma, and for that reason maintenance of the surface/volume ratio of the inner sarcolemma must to some degree compensate for the decrease in the surface/volume ratio of the outer membrane of the heart muscle cells. It also follows from the results of the present investigation that the T system increases proportionally to the increase in size of the cardiomyocytes. This does not contradict the data obtained by other workers [13], who consider the hyperplasma of the T system is under genetic control, so as to regulate growth of the cell depending on successful performance of its basic function.

Hypertrophic growth of the heart is spontaneously hypertensive rats is thus manifested at the ultrastructural level by an initial disproportionate increase in the contractile compartment relative to the energy-producing and transport systems of the cardiomyocyte. Only after an increase in the relative volume of the myofibrils up to a certain value determined by the functional load on the heart does growth of the remaining organelles responsible for the coupled process of contraction—relaxation become possible.

LITERATURE CITED

1. G. G. Avtandilov, Introduction to Quantitative Pathological Morphology [in Russian], Moscow (1980).
2. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
3. L. M. Nepomnyashchikh, E. L. Lushnikova, and L. V. Kolesnikova, Arkh. Anat., No. 10, 94 (1981).
4. L. M. Nepomnyashchikh, E. L. Lushnikova, and M. G. Chernokalova, Byull. Éksp. Biol. Med., No. 7, 101 (1981).
5. V. S. Paukov, T. A. Kazanskaya, and V. A. Frolov, Byull. Éksp. Biol. Med., No. 4, 122 (1971).
6. D. S. Sarkisov, Outlines of the Structural Basis of Homeostasis [in Russian], Moscow (1977).
7. V. A. Frolov and G. A. Drozdova, Arkh. Patol., No. 5, 35 (1982).
8. Yu. G. Tsellarius and N. K. Eriskovskaya, Byull. Éksp. Biol. Med., No. 6, 627 (1979).
9. P. Anversa, G. Olivetti, H. Melissari, et al., Lab. Invest., 40, 341 (1979).
10. J. M. Capasso, J. E. Strobeck, and E. H. Sonnenblick, Am. J. Physiol., 241, H435 (1981).
11. M. A. Goldstein, L. A. Sordahl, and A. Schwartz, J. Molec. Cell. Cardiol., 6, 265 (1974).
12. D. D. Lund and R. J. Tomanek, Am. J. Anat., 152, 141 (1978).
13. E. Page and L. P. McCallister, Am. J. Cardiol., 31, 172 (1973).
14. R. J. Tomanek, Lab. Invest., 40, 83 (1979).
15. M. F. Wendt-Gallitelli, G. Ebrecht, and R. Jacob, J. Mol. Cell. Cardiol., 11, 275 (1979).