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Intracellular Reorganization and Ultrastructural Stereological Analysis of Cardiomyocytes of W/SSM Rats with Genetically Determined Cardiomyopathy

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We studied ultrastructure of cardiomyocytes of W/SSM rats with inherited hypertrophic cardiomyopathy bred from Wistar rats by hypersensitivity to cataractogenic action of high galactose doses. The observed dynamics of quantitative and qualitative changes in the main sarcoplasmic organelles reflects general principles of spatial and temporal reorganization of cardiac muscle cells induced by hypertrophy of different genesis.

Key Words: *W/SSM rats; hypertrophic cardiomyopathy; cardiomyocytes; electron microscopy; morphometry; stereology*

W/SSM rats with inherited hypertrophic cardiomyopathy were bred from Wistar rats selected by hypersensitivity to cataractogenic action of high galactose doses at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Science [8, 12,13]. These animals are characterized by spontaneous myocardium hypertrophy accompanied by diffuse stroma collagenization, focal sclerotic changes, and chronic cardiac insufficiency [6,7,9].

One of the mechanisms of pathologic process is based on changes in plasma membrane integrity resulting from enhanced hexose transport into the cells, generation of free hydroxyradicals, and intensification of lipid peroxidation [1,17]. In the myocardium of W/SSM rats with genetically determined disturbances of the carbohydrate metabolism and increased lysosome enzyme activity [17], cardiomyopathy is manifested as impaiment of muscle contractivity leading to an increase in the heart weight [10], first due to increase in cardiomyocyte (CMC) number, and then after exhaustion of their proliferative potential, due to their hypertrophy [10].

Thus, hypertrophic changes in the myocardium of W/SSM rats are similar to those observed during idiopathic human hypertrophic cardiomyopathy in the absence of anatomic or physiologic causes [11].

The aim of the present study was to reveal intracellular changes in CMC and to determine ultrastructural stereological parameters typical of W/SSM rats with genetically determined hypertrophic cardiomyopathy.

MATERIALS AND METHODS

Forty male W/SSM rats aged 3 and 10 months and 20 male Wistar rats of the same age kept under standard vivarium conditions were used in the experiment. The animals were weighed and decapitated under chloro-

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form narcosis. The weight of the heart and its ventricles was determined after 1-h fixation in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 8.0).

For survey examinations paraffin sections (histotopograms) of the right and left ventricles with ventricular septum were stained with hematoxylin and eosin followed by Perls reaction and with colloid iron-PAS-hematoxylin. For specific histological examination semithin sections (1 μ) were prepared. The tissue fragments were embedded into epon-araldite resin. Semithin sections were prepared on LKB-III ultratome, stained with azure II, and examined under a Docuval universal light microscope.

For electron microscopy left papillary muscles and left ventricle specimens were fixed for 30 min and cut into fragments with preserved fiber orientation. The fragments were fixed in a fresh portion of paraformaldehyde for 2.5-3 h at 4°C and postfixed in 2% osmium tetroxide for 2 h. The tissue specimens were dehydrated by standard technique and embedded into epon-araldite. Ultrathin sections were examined under Tesla BS-500 and JEM-1010 electron microscopes at 60 kV.

Intracellular reorganization of CMC was studied on ultrathin sections using morphometric and stereological methods [3,5]. Volume densities of myofibrils, mitochondria, smooth sarcoplasmic reticulum (SSR), T-system, and sarcoplasmic matrix (sarcoplasmic matrix itself, glycogen, ribosomes, lysosomes, other intracellular inclusions) were calculated. Surface densities of myofibrils, mitochondria, SSR, and T-system were determined. Secondary stereologic parameters (volume and surface-volume ratios of the main organelles) were calculated on the basis of the primary characteristics. Saturation of myofibrillar unit with sarcoplasmic organelles was used as a specific parameter of functional CMC state.

The results were analysed statistically as described previously [3].

RESULTS

In 3-month-old W/SSM rats, the heart weight increased by 21.8% in comparison with control Wistar rats of the same age. The weight of the left ventricle and the diameter of muscle fibers increased by 17.6 and 15.8%, respectively. Microscopic examination of the left ventricle from 10-month-old W/SSM rats revealed hypertrophy of muscle fibers in comparison with Wistar rats of the same age (23.1±0.4 *vs.* 15.1±0.5 μ, $p<0.001$).

Ultrastructural study of CMC of 3-month-old W/SSM rats reveal no significant differences in comparison with age-matched Wistar rats and spontaneously hypertensive rats (SHR) [2]. Myofibrillar bundles and mitochondria were compactly arranged in cells (Fig. 1, *a*). Most mitochondria contained electron dense matrix and densely packed pectinate cristae. Mitochondria lockated between myofibrillar bundles showed significant polymorphism. SSR vesicles were primarily seen in the myofibrillar zone of CMC. T-system tubules were localized at the level of Z-bands of myofibrils. Numerous round mitochondria, lamellar Golgi complex, and small primary lysosomes with homogenous content were found in the perinuclear zone. CMC sarcoplasm contained large quantity of glycogen represented mainly by β-form. In the subsarcolemmal zone myelin-like structures were found (Fig. 1, *b*). These structures represent residual bodies replacing degenerated mitochondria. In the subsarcolemmal zone of some CMC, cisterns and vesicles of lamellar Golgi complex were found.

TABLE 1. Ultrastructural Stereologic Analysis of CMC in W/SSM Rats with Inherited Cardiomyopathy (*M±m*)

Parameter	Wistar rats		W/SSM rats	
	3 months	10 months	3 months	10 months
Volume density, mm³/cm³				
myofibrils	473.2±13.9	510.4±14.0	533.3±10.1*	555.6± 10.2*
mitochondria	315.5±11.1	307.2±15.1	262.3±12.3*	268.5±9.6
SSR	23.4±1.0	21.0±1.1	15.2±1.2**	16.1±0.9*
T-system	21.8±0.8	18.8±1.0	17.3±0.6*	18.0±0.7
sarcoplasmic matrix	166.1±12.4	142.6±14.5	171.9±11.2	141.8±10.1
Surface density, m²/cm³				
myofibrils	1.535±0.097	1.470±0.08	1.460±0.096	1.415±0.058
mitochondria	1.683±0.057	1.421±0.098	1.320±0.069	1.268±0.059
SSR	0.422±0.038	0.383±0.050	0.274±0.036*	0.221±0.029*
T-system	0.331±0.040	0.275±0.038	0.235±0.028	0.241±0.030

Note: Here and in Table 2: *$p<0.05$ and **$p<0.01$ compared to age-matched control.

In 10-month-old W/SSM rats, the number of CMC with myofibrillar contractures of different degree, including subsegmetal contactures were found (Fig. 1, *c*). Mitochondria with destructive changes such as focal matrix lysis and cristae destruction (Fig. 1, *d*) were more abundant. The number of cristae was reduced in some mitochondria. This age group was characterized by irregular dilation of SSR vesicles, especially in the subsarcolemmal zone. The tubules of T-system were usually dilated.

Lamellar Golgi complex was characterized by hyperplasia and its fragments were seen in the perinuclear, myofibrillar, and subsarcolemmal zones. In the perinuclear zone, primary and secondary lysosomes were found, and partial lysis of the sarcoplasm and focal destruction of organelles were noted.

Stereological analysis showed that CMC hypertrophy in 3- and 10-month-old W/SSM rats was accompanied by an increase in the volume density of myofibrils by 13 and 9%, respectively, compared to control rats of the corresponding age (Table 1). At the same time, in 3-month-old rats, the volume and surface density of mitochondria decreased by 17 and 21%, respectively. In 10-month-old rats these parameters decreased by 13 and 11% (Table 1).

Disproportional changes in the myofibrillar and mitochondrial compartments decreased the mitochondria/myofibrils volume ratio by 27 and 20% in 3- and

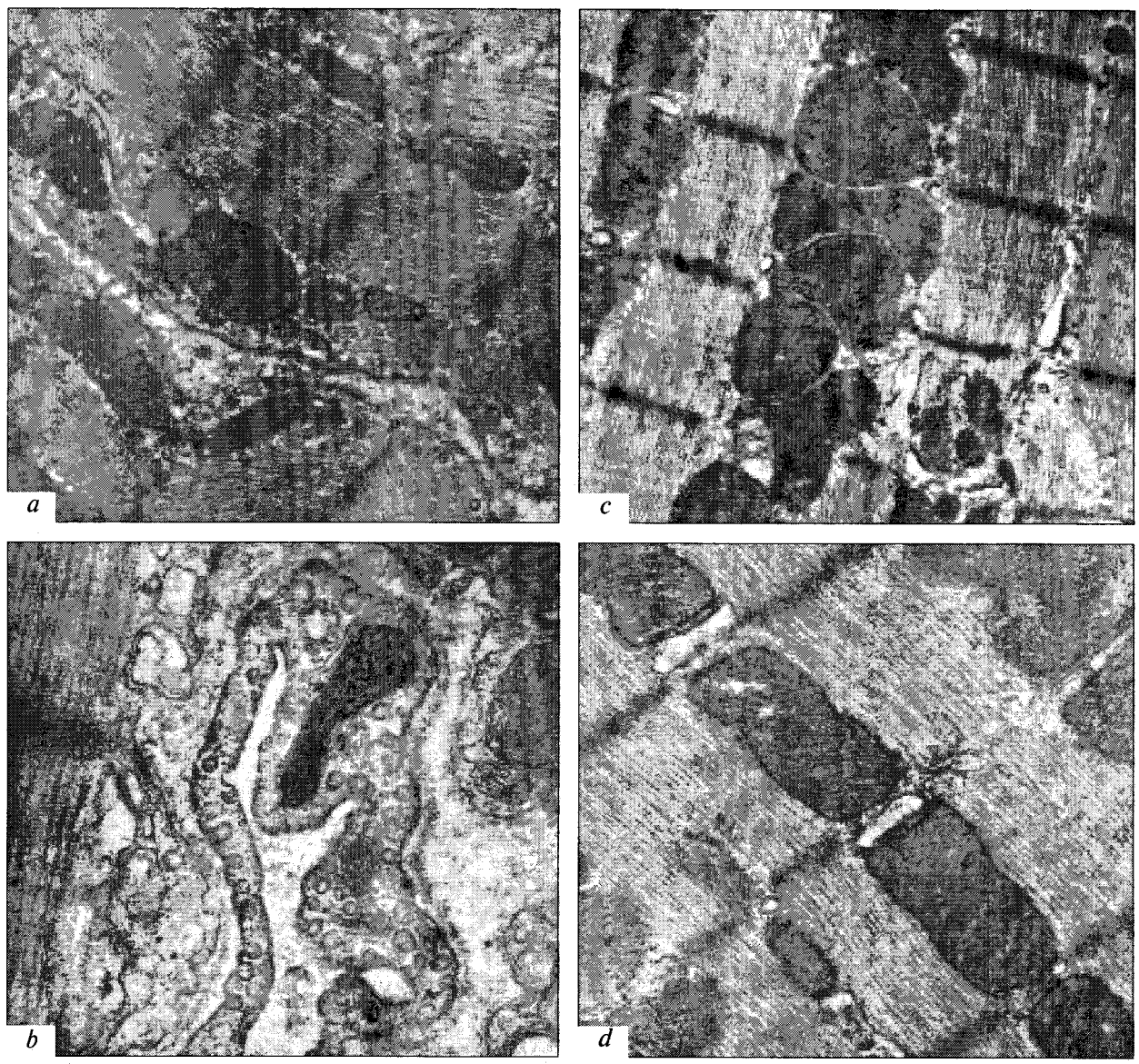

Fig. 1. Ultrastructure of cardiomyocytes (CMC) in W/SSM rats. *a*) compact distribution of myofibrils and mitochondria in CMC of 3-month-old rat, ×5000; *b*) myelin-like structures in subsarcolemmal zone of CMC of 3-month-old rat, ×8300; *c*) insignificant contracture of myofibrils in CMC of 10-month-old rat, ×5000; *d*) focal destruction of mitochondrial cristae in CMC of 10-month-old rat, ×6000.

TABLE 2. Secondary Stereological Parameters of CMC Ultrastructure of W/SSM Rats with Inherited Cardiomyopathy (*M±m*)

Parameter	Wistar rats		W/SSM rats	
	3 months	10 months	3 months	10 months
Surface-volume ratio, m^2/cm^3				
myofibrils	3.2±0.2	2.9±0.4	2.7±0.4	2.5±0.6
mitochondria	5.3±0.3	4.6±0.6	5.0±0.4	4.7±0.7
SSR	18.0±0.9	18.2±1.3	18.0±1.1	13.7±0.8
T-system	15.1±1.0	14.6±1.2	13.6±0.9	13.3±1.0
Volume ratio to myofibrils				
mitochondria	0.670±0.029	0.600±0.031	0.490±0.026**	0.480±0.022**
SSR	0.048±0.005	0.041±0.006	0.029±0.003*	0.028±0.002*
T-system	0.045±0.006	0.037±0.004	0.032±0.004	0.033±0.005
total of mitochondria, SSR and T-system	0.762±0.030	0.679±0.032	0.553±0.027*	0.545±0.023*

10-month-old W/SSM rats, respectively, compared to age-matched controls (Table 2). Similar changes in the energy-producing and contractile compartments of CMC were observed in various experimental models of myocardium hypertrophy [2,4,14-16] and can be regarded as a sequence of adaptive reactions.

CMC hypertrophy in 3- and 10-month-old W/SSM rats was accompanied by a significant decrease in the volume (by 35 and 23%, respectively) and surface (by 35 and 42%, respectively) density of SSR. In 3-month-old animals, the volume and surface density of the T-system decreased by 21 and 29%, respectively. In 10-month-old rats, volume density of the T-system did not differ from the control, while its surface density decreased by 12% (Table 1).

Changes in the volume ratio between myofibrills and mitochondria, SSR, and T-system revealed a depletion of myofibril volume of other cell organelles which was of great significance for intracellular reorganization of CMC. This parameter decreased by 27 and 20% in 3- and 10-month-old animals, respectively.

The revealed intracellular reorganization of CMC is similar to that observed in spontaneously hypertensive rats during the development of CMC hypertrophy [2,4]. It is possible that this dynamics of quantitative and qualitative changes in the main sarcoplasmic organelles of CMC reflects general principles of their spatial and temporal reorganization underlying hypertrophic changes of different genesis.

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