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Cellular Mechanisms of Hypertrophic Cardiomyopathy in Spontaneously Hypertensive Rats (SHR)

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It is shown that the weight dynamics of the body, heart and left ventricular myocardium is similar in Wistar rats and SHR, but the number of cardiomyocytes in the 5th and 24th weeks of postnatal development is considerably higher in Wistar rats than in SHR. The earlier maturation of cardiomyocytes in SHR rats leads to their reduced proliferative activity, thus blocking the growth of the cell population.

Key Words: *cardiomyocytes; hypertrophy; spontaneously hypertensive rats (SHR); cell population size*

Modeling of hereditary cardiomyopathy is of great importance for elucidating the general pathways of heart pathology in man. Hypertrophic cardiomyopathy in spontaneously hypertensive rats (SHR) is considered to be one such model [6,10,12,13]. Myocardium hypertrophy in these animals develops at an early age and has been shown not to be directly related to the development of arterial hypertension [8,9]. Morphogenesis of myocardium hypertrophy, the pattern of ultrastructural changes in cardiomyocytes, and the spatial tissue and intracellular rearrangement of the myocardium in SHR are similar in many features to the morphological manifestation of hypertrophic cardiomyopathy in man and have been described at length [1,3-5]. However, the causes of the development of myocardium hypertrophy in SHR remain quite uncertain; in particular, little is known about the ratio between proliferative processes (or hyperplasty) and hypertrophy of cardiomyocytes in early ontogeny and their contribution to myocardium hypertrophy in SHR.

The objective of the present investigation was to compare the dynamics of cardiomyocyte population growth in postnatal ontogeny in Wistar rats and SHR.

MATERIALS AND METHODS

Thirty-two Wistar rats (controls) and 21 SHR (both 1 day old) were selected for the experiments. Ten Wistar rats and 9 SHR were killed at the start point and the others after attaining the 5- or 24-week age. All the animals were weighed before sacrifice. The hearts were fixed by perfusion with 10% paraformaldehyde in 0.1 M phosphate buffer saline (pH 7.2) until the myocardium was completely washed free of blood. After the atrii were removed, the myocardium of both ventricles (total heart weight) and of the left ventricle with the interventricular septum were weighed. Alkaline dissociation of the fixed myocardium was performed according to a standard procedure [7].

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In all animals the heart index was calculated by the formula:

$$\frac{\text{heart weight (mg)}}{\text{body weight (g)}} \times 100\%.$$

Analogously, the indexes of the left ventricle with the interventricular septum and of the right ventricle were calculated. The concentration of cardiomyocyte nuclei per milligram tissue and the number of mono-, di- and multinuclear cardiomyocytes per 1000 cells were determined.

For evaluation of the weight dynamics of the body, the heart, and the left ventricle myocardium, as well as the growth of the cardiomyocyte population, the mean specific rate of growth was computed according to the Schmalhausen formula for the weight dynamics at different stages of ontogeny [2]:

$$C=(\ln W_2-\ln W_1)/(t_2-t_1),$$

where C is the mean specific growth rate; W_1 is the initial body or heart weight or cardiomyocyte count; W_2 is the final value of the same parameters; t_1 and t_2 are the initial and final age (weeks); ln is the natural logarithm of the corresponding value.

RESULTS

The examined rats differed not only in initial values, but also in the dynamics of the body, heart, and left ventricle myocardium weight (Table 1). During the first 24 weeks of postnatal ontogeny the body weight increased 52-fold in Wistar rats and 63-fold in SHR, while the weights of the heart and the left ventricle myocardium increased 36- and 47-fold in Wistar rats, and 49- and 63-fold in SHR. Moreover, in the SHR the rate of weight gain of the left ventricle myocardium was equal to that of the body weight, while in Wistar rats it was on average 10% lower. The indexes of the heart and the left ventricle were maximal in 4-week-old rats of both strains and had dropped by the 24th week. In Wistar rats these parameters dropped more significantly, dipping below the level observed in 1-day-old rats, while in SHR the index of the left ventricle returned to the initial level. Thus the weight dynamics of the body, heart, and left ventricle myocardium over the studied period is the same in both strains, and no prevalent increase of the heart weight over the body weight was observed in SHR.

Immediately after birth, the number of cardiomyocyte nuclei and cardiomyocytes per milligram tissue in the left ventricle myocardium was equal in both strains (Table 2). Since the concentration of cells in a suspension is inversely proportional to the mean volume of the individual cardiomyocyte, it is readily seen that the volume of cardiomyocytes in SHR increases 4-fold during the first 5 weeks, and 17-fold by the 24th week vs. 2.5- and 8-fold, respectively, in Wistar rats. It follows that the volume (and, thereby, weight) of the individual cardiomyocyte is 1.6 times higher in 5-week-old SRH and twice as high in 24-week-old SHR than in Wistar rats of the corresponding age.

Hypertrophy of cardiomyocytes in SHR is directly related to their lower proliferative activity during the first 4 weeks: the cardiomyocyte count of the left ventricle increased by 2.5 and 2 times in Wistar rats and SHR, respectively. Moreover, the number of cardiomyocytes in Wistar rats slowly but reliably ($p<0.01$) increases after 5 weeks of life, attaining 290% of the initial values remains unchanged in the 24th week, whereas the number of cardiomyocytes in SHR after the first 4 weeks. It should be emphasized that the ratio between di- and multinuclear cardiomyocytes was the same in both strains.

The dynamic indexes of the specific growth rate demonstrate that the heart weight gain in 5-24-week-old SHR occurs exclusively due to the

TABLE 1. Dynamics of Morphometric Parameters in SHR and Wistar Rats (W) ($M \pm m$)

Parameter	Strain	Age		
		1-day	5 weeks	24 weeks
Body weight, g	SHR	4.5±0.2	57.2±4.2	282.7±13.5
	W	6.9±0.2	49.3±1.0	361.7±11.5
Heart weight, mg	SHR	22.1±1.3	270.2±12.3	1072.5±32.2
	W	27.2±0.8	259.5±10.4	976.1±39.8
Left ventricle weight, mg	SHR	14.3±0.7	210.8±11.0	882.8±24.4
	W	17.0±0.4	202.6±8.2	791.8±30.9
Heart index	SHR	491±20.7	476±16.9	382±12.8
	W	392±8.8	527±18.9	271±7.7
Left ventricle index	SHR	319±11.7	370±11.9	314±12.7
	W	246±5.8	411±13.4	219±6.6

TABLE 2. Dynamics of Quantitative Parameters of Cardiomyocyte (CM) Population in SHR and Wistar Rats ($M \pm m$)

Parameter	Strain	Age		
		1-day	5 weeks	24 weeks
Concentration of CM nuclei, 10^3/mg	SHR	287.0±13.8	71.7±4.1*	16.7±1.4*
	W	297.3±14.5	116.1±4.1	35.8±1.8
Concentration of CM, 10^3/mg	SHR	284.8±13.9	37.1±2.2*	8.8±0.4*
	W	294.4±13.3	50.9±2.1	18.7±0.9
Number of CM nuclei in left ventricle, 10^6	SHR	4.1±0.2	14.9±0.2	14.8±0.8
	W	5.0±0.2	23.4±0.8	27.9±1.1
Number of CM in left ventricle, 10^6	SHR	4.0±0.8	7.7±0.4	7.7±0.4
	W	5.0±0.2	12.2±0.4	14.6±0.6
Share of mononuclear cells, %	SHR	99.3±0.1	7.5±1.2	11.5±1.1
	W	99.0±0.2	7.7±0.5	10.1±0.4
Share of dinuclear cells, %	0.7±0.1	92.1±1.3	86.7±1.1	
	W	0.1±0.2	92.1±0.5	88.7±0.6
Share of multinuclear cells, %	SHR	–	0.5±0.1	1.8±0.2
	W	–	0.2±0.1	1.2±0.1

Note. An asterisk denotes significance level $p<0.001$ in a comparison of age–matched strains.

enlargement of individual cardiomyocytes (Table 3). During the first month the specific growth rate of the body, the heart, and the left ventricle myocardium was similar in Wistar rats and SHR; however, the specific growth rate of the cardiomyocyte population in the left ventricle was 30% lower in SHR, and therefore the specific growth rate of the individual cardiomyocyte had to be higher to the same extent.

During the 5th-24th weeks of postnatal ontogeny the specific growth rate of the body and the left ventricle dropped similarly: by 6 and 7 times, respectively. The weight gain of the left ventricle in Wistar rats occurred due to cardiomyocyte proliferation, while in SHR it was solely due to enlargement of individual cardiomyocytes.

Thus, the obtained results suggest that the specific growth rates of the body, heart, and left ventricle do not differ in Wistar and SHR, but that, there are considerable differences in the number of cardiomyocytes during various periods of postnatal ontogeny. It may be surmised that hypertrophy of cardiomyocytes and, consequently, heart hypertrophy in SHR are attributed in part to a reduced number of cardiomyocytes and the need to attain the species-specific organ-organism proportions. The earlier drop of proliferative activity of cardiomyocytes and their reduced number in SHR in comparison with Wistar rats may in turn reflect an earlier maturation of cardiomyocytes in SHR, induced by enhanced contractile activity due to disturbed ion transport across the sarcoplasmic membrane. Our results are somewhat contradictory to the data reported by other workers [11], who demonstrated the opposite dynamics of cardiomyocyte count in SHR and Wistar rats in the early postnatal ontogeny. These discrepancies are most likely related to genetic differences of the same strains used by Russian and Western investigators and to different methodological approaches. The common point in the interpretation of the findings is the conclusion concernins an earlier maturation of cardiomyocytes in spontaneously hypertensive rats.

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TABLE 3. Specific Growth Rate of Body, Heart, and Left Ventricle Myocardium Weight, and Cardiomyocyte Count of Left Ventricle in SHR and Wistar rats

Specific growth rate	SHR		Wistar rats	
	1 day - 5 weeks	5 - 24 weeks	1 day - 5 weeks	5 - 24 weeks
Body weight	0.5	0.08	0.5	0.08
Heart weight	0.5	0.06	0.05	0.07
Left ventricle weight	0.5	0.07	0.5	0.07
Left ventricle cardiomyocyte count	0.13	0	0.17	0.01

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Stereological Ultrastructural Analysis of Rat Cardiomyocytes after Total Hyperthermia

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Hyperthermia results in the suppression of intracellular regeneration in cardiomyocytes, manifested as intensified lysis and destruction of organelles, and leads to myocardial atrophy. Intracellular reorganization of cardiomyocytes is characterized by increases in the volume and surface density of myofibrils, sarcoplasmic reticulum, and T system.

Key Words: *cardiomyocytes; intracellular organization; total hyperthermia; stereology*

The ultrastructural changes occurring in the cells of homoiothermic animals for total hyperthermia have not been investigated in sufficient detail. Attention has been focused on the effect of hyperthermia on cultured cells, predominantly on tumor cells [1,11]. As a result of these studies, hyperthermia has found application in anticancer therapy, since tumor cells have a low resistance to heating. It has been demonstrated that hyperthermia induces irreversible structural changes in the plasma membrane leading to disturbance of membrane functions, for example, the enzyme activity and passive membrane transport [8-10]. Specific proteins with putative protective activity against heat shock are being synthesized [12,13]. Generally speaking, the direct effect of hyperthermia on cell populations has been studied. In fact, however, total hyperthermia, to which humans or animals are usually exposed, more often than not produces an indirect effect on the internal organs. Pronounced morphofunctional changes have been observed in the myocardium of animals exposed to hyperthermia [6,7], namely necrobiotic and atrophic alterations in some cardiomyocytes [3]. A clear understanding of the processes of intracellular regeneration taking place in cardiomyocytes after hyperthermia makes it possible to predict the direction in which the adaptive-compensatory reactions in the myocardium will proceed and to elucidate some patterns of intracellular reorganization in cardiomyocytes induced by unfavorable environmental factors.

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