MORPHOLOGY AND PATHOMORPHOLOGY

Peculiarities of Myocardial Structure in Rats with Hereditary Hypertension Reared by Normotensive Females

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 9, pp. 330-332, September, 2001
Original article submitted July 31, 2001

Morphometric analysis revealed no signs of myocardial hypertrophy at the tissue and ultrastructural levels in 3-week-old NISAG rat pups reared by normotensive Wistar females. The severity of myocardial hypertrophy in these animals aging 6 months was similar to that in NISAG rats reared by natural mothers. However, raising conditions during the early ontogeny form compensatory structural changes in the myocardium of rats with genetically determined hypertension.

Key Words: *myocardium; hypertrophy; hereditary hypertension; ontogeny; experiment*

Changes in blood pressure (BP) and other parameters of the cardiovascular system accompanying hereditary arterial hypertension are related to genetically determined characteristics, which are phenotypically expressed during the development and growth under specific environmental conditions. The contribution of genetic factors and environmental influences into the phenotype, as well as modulation of the phenotype within physiological limits can be studied on experimental models. In was demonstrated that nursing of SHR rat pups (spontaneously hypertensive rats) by normotensive Wistar or Sprague-Dawley females determined lower BP in adult rats [6]. In NISAG rats (hereditary stress-induced arterial hypertension) the onset of arterial hypertension under these experimental conditions was delayed, but not prevented [2]. The morphological basis of these functional changes and the effect of raising conditions during the early ontogeny on genetically determined peculiarities of involved organs remain unknown. Here we studied morphological characteristics of the myocardium in NISAG rats aging 3 weeks (no signs of hypertension) and 6 months (persistent increase in BP) nursed by normotensive Wistar females from the 1st day of life.

MATERIALS AND METHODS

Newborn NISAG rat pups were reared by Wistar females (Wistar rat pups were removed) from the 1st day of life until day 26. After that NISAG male and female pups were placed in different cages. Morphological analysis of the myocardium was performed on NISAG males aging 3 and 5 weeks, and 6 months and weighing 36.4±0.7, 44.2±4.1, and 259±7.4 g, respectively (5 animals per group). Age-matched Wistar and NISAG rats reared by natural mothers served as the control [3,4]. The animals were sacrificed under ether anesthesia. The samples were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M

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0007-4888/01/1323-0895$25.00

phosphate buffer (pH 7.4) at 4°C, postfixed with 1% OsO_4, and embedded into Epon and Araldite. Stereomorphometry [1] of semithin sections stained with toluidine blue was performed using a square test grid (289 points, ×700) and ocular gauge (scale interval 1.73 μ). Ultrathin sections were contrasted with uranyl acetate and lead citrate. Stereomorphometry of cardiomyocytes (CM) was performed on negatives (×5000) using a 72-point square test grid (increment 8 μ) and gauge (scale interval 0.2 μ). The results were analyzed using Statgraphics 4.0 software (Student's *t* test). The data are presented as means and standard errors (*M*±*m*).

RESULTS

In 3-week-old NISAG rat pups reared by Wistar females the relative weight of the heart and morphometric indexes of the myocardium were between the corresponding parameters in NISAG and Wistar rats reared by natural mothers. We revealed no signs of myocardial hypertrophy. The only structural peculiarity of the myocardium in these rats was low level of capillarization compared to the myocardium of control NISAG and Wistar rats. The volume of CM myofibrils did not differ from that in Wistar rats of the same age, but was lower than in control NISAG rats. At the same time, myofibrils were thicker by 40 and 30% than in control Wistar and NISAG rats, respectively.

The myocardium of 6-month-old NISAG rats reared by Wistar females was characterized by pronounced hypertrophy. The relative weight of the heart in these animals markedly surpassed that in other rats of the same age (Table 1). Histological characteristics of the myocardium in experimental NISAG rats differed from those in Wistar rats and approximated those in control NISAG rats. The only exception was high density of capillaries in NISAG rats reared by Wistar females (similar to that in Wistar rats). In hypertrophied myocardium the CM density per unit of section area is low and the number of microvessels supplying CM 2-fold surpassed the corresponding parameter in the control. Intensive blood supply to the myocardium was associated with high content of mitochondria in CM; the volume of thickened myofibrils in experimental NISAG rats was lower than in control animals. Ultrastructural morphometric indexes of CM in experimental NISAG rats markedly differed from those in animals of both control groups (Table 1).

Previous studies showed that the differences between SHR and Wistar rats in BP and the size of left ventricle CM can be revealed starting from day 28 of life, when the body weight, weight of the heart, and diameter of CM attain 50 g, 500 mg, and 13 μ, re-

TABLE 1. Morphometric Indexes of the Left Ventricular Myocardium in Rats of Different Strains Aging 3 Weeks and 6 Months (*M*±*m*)

Parameter	3 weeks			6 months		
	Wistar	NISAG		Wistar	NISAG	
		control	experiment		control	experiment
Relative heart weight, mg/g body weight	6.20±0.16	6.5±0.1	5.90±0.13*	3.00±0.07	3.6±0.1^{+}	4.50±0.16^{+*}
CM diameter, μ	10.10±0.53	9.40±0.16^{+}	10.20±0.19*	14.70±0.29	21.10±0.37^{+}	21.6±0.6^{+}
V_R of stroma/V_R of CM	0.540±0.012	0.450±0.015^{+}	0.47±0.01^{+}	0.18±0.01	0.23±0.01^{+}	0.21±0.06^{+}
Density of capillaries per 1 CM	0.500±0.012	0.470±0.015	0.41±0.01^{+*}	0.41±0.03	0.77±0.10^{+}	0.960±0.035^{+*}
In CM						
V_R, %						
myofibrils	48.30±1.04	52.50±0.85^{+}	46.1±0.8*	49.80±0.96	51.2±0.9	46.2±0.9^{+*}
mitochondria	35.9±1.0	37.90±0.72	42.0±0.8^{+*}	33.60±0.87	31.70±1.13	41.50±0.98^{+*}
sarcoplasmic reticulum	6.70±0.22	7.30±0.17^{+}	5.30±0.34^{+*}	4.50±0.08	6.00±0.19^{+}	6.10±0.29^{+}
Numerical density of mitochondrial profiles per 10 μ^2	4.5±0.1	4.5±0.1	5.60±0.13^{+*}	5.40±0.15	4.7±0.2^{+}	5.40±0.16*
Myofibril diameter, μ	0.60±0.01	0.70±0.02^{+}	1.00±0.02^{+*}	0.66±0.01	0.80±0.02	1.40±0.04^{+*}
V_R of mitochondria/V_R of myofibrils	0.79±0.03	0.77±0.03	0.91±0.03^{+*}	0.710±0.024	0.640±0.026	0.92±0.04^{+*}
V_R of sarcoplasmic reticulum/V_R of myofibrils	0.14±0.01	0.14±0.01	0.12±0.01^{+*}	0.100±0.003	0.120±0.005^{+}	0.140±0.008^{+}

Note. $p<0.05$: *compared to control NISAG rats; $^{+}$compared to Wistar rats. V_R: relative volume.

spectively [5]. In 3-week-old NISAG rats BP did not differ from that in Wistar rats [4]. However, systolic BP in 4-week-old NISAG rats was significantly higher than in Wistar rats (127±3 *vs.* 145±3 mm Hg). In 4-week-old NISAG rats reared by Wistar females basal BP (137±3 mm Hg) differed from that in control NISAG and Wistar rats, but did not reach the levels typical of hypertension. Signs of myocardial hypertrophy were found in 5-week-old NISAG rat pups reared by Wistar females: high relative weight of the heart (6.90±0.35 mg/g) and large diameter of CM (13.10±0.25 μ). The myocardial structure and CM volume did not differ between 6-month-old NISAG rats reared by natural and normotensive females, which indicates that changes in these characteristics accompany the development of hypertension. However, the formation of compensatory capacities in the heart within the genetically determined limits depends on raising conditions during the early ontogeny: hypertrophic CM are well vascularized, thick myofibrils are surrounded by numerous mitochondria and developed sarcoplasmic reticulum. These morphological characteristics and delayed development of hypertension (BP 147.0±1.8 *vs.* 171±3 mm Hg in control NISAG rats) are associated with a better prognosis for animals reared by normotensive females.

Our experiments on NISAG rats characterized by high sensitivity to stress and hypertension are consistent with published data. The development of experimental hypertension of various geneses can be modulated by rearing of rat pups by females of any normotensive rat strain (Wistar—Kyoto, Sprague—Dawley, and SR/Jr rats) [10]. The mechanisms of this effect are unclear. It was shown that hypertensive females more closely communicate with their offspring during suckling, lactation in these rats is less intensive than in normotensive animals, and their milk is not balanced by the content of sodium and calcium [8,10-12]. Hypertensive rat pups reared by normotensive females more rapidly gain weight and differ in open field behavior and reactions of the sympathoadrenal system to stress [7].

Our results suggest that the development of myocardial hypertrophy in NISAG rats is a genetically determined process accompanying hypertension. The raising conditions during the early ontogeny (nursing by normotensive females) affect these characteristics, which is manifested in the presence of structural compensatory mechanisms in the myocardium at the tissue and subcellular levels.

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