Effect of Chronic Stress during Early Postnatal Ontogeny on Structural Characteristics of the Myocardium and Glomerular Apparatus of the Kidneys in NISAG Rats

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We studied structural characteristics of the myocardium and glomerular apparatus of the kidneys in 3-week- and 6-month-old male NISAG rats (hereditary stress-induced arterial hypertension) subjected to handling on days 1-21 of postnatal ontogeny. The animals were daily isolated from mothers for 10 min. Handling did not modulate the development of arterial hypertension and typical morphological signs in the myocardium and kidneys.

Key Words: *hypertension; handling; myocardium; kidney; ultrastructure*

Recent studies showed that changes in the environmental conditions during the early ontogeny affect the development of experimental hereditary arterial hypertension in animals. Experiments were performed on various strains of hypertensive rats and included different treatments (*e.g.*, nourishment by normotensive females, salt-enriched diets, and stress) [7,8,14,15]. Handling, *i.e.* daily short-term isolation of rat pups from mothers, is a stress factor. This method is most convenient for studying the effects of moderate chronic stress during the early ontogeny on the development of arterial hypertension in NISAG rats (hereditary stress-induced arterial hypertension).

NISAG rats are an adequate model of stress-induced arterial hypertension in humans [12]. Adult animals are characterized by persistently elevated blood pressure and pronounced increase in this parameter during stress. Structural changes in the heart and kidneys of these rats are typical of hypertension [2,5,6]. Ontogenetic studies showed that hypertension develops in NISAG rats aging of 3-4 weeks. This period is characterized by high sensitivity of the cardiovascular and hypothalamic-pituitary-adrenal systems to stress [3]. Exposure to moderate physical and psychoemotional factors in this period can produce an antihypertensive effect [4]. It was interesting to evaluate whether moderate chronic stress (handling) in the prehypertensive juvenile period would produce these changes. We studied structural characteristics of the myocardium and glomerular apparatus of the kidneys in NISAG rats subjected to handling during the early ontogeny (suckling period). The heart and kidneys are the target organs involved in the development of arterial hypertension, and morphofunctional indexes of these organs adequately reflect the stages of this process.

MATERIALS AND METHODS

Experiments were performed on male Wistar and NISAG rats aging 3 weeks and 6 months. Experimental NISAG rats were handled from the 1st to 21st day of life. These animals were daily removed from nests and placed in plastic boxes (15×15×15 cm). Intact Wistar and NISAG rats of the same age served as the

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control. Blood pressure was measured on the caudal artery of adult animals by the sphygmographic method.

The rats aging 3 weeks and 6 months were killed under ether anesthesia. Samples of the myocardium and kidneys for morphological examination were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C, postfixed in 1% OsO_4, and embedded into Epon-araldite mixture. The diameter of cardiomyocytes and diameter and numerical density of renal glomeruli were estimated on semithin sections stained with toluidine blue using a rectangular grid (289 points), ocular ruler (increment 1.73 μ), and light microscope (×700). Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a JEM-100SX electron microscope. Ultrastructural stereomorphometry of cardiomyocytes and cellular or non-cellular components of renal glomeruli was performed on negative images (×500) using a rectangular grid (72 points, increment 8 μ) and a ruler (scale 0.2 μ).

The results were analyzed using Statgraphics 4.0 software. The significance of differences was estimated by the Student's *t* test.

RESULTS

Three-week-old NISAG rats subjected to handling were characterized by slow weight gain and proportionally decreased weights of the heart and kidneys (Table 1). Cardiomyocytes in these animals were larger than in intact rats and included loosely positioned thick myofibrils surrounded by numerous mitochondria. Capillarization of cardiomyocytes was reduced due to low density of capillaries in myocardial tissue (Table 1).

Three-week-old hypertensive rat pups living under normal conditions or subjected to handling had similar morphometric indexes of renal glomeruli (Table 1). It should be emphasized that structural characteristics of renal glomeruli typical of control NISAG rats (*i.e.*, considerable amount of the mesangium and greater thickness of the basal membranes compared to normotensive Wistar rats) were also observed in handled animals.

Taking into account that blood pressure does not increase in control and handled NISAG rats aging 3 weeks [4], high content of the mesangium can be considered as a genetically determined characteristic. J. B. Lopes de Faria *et al.* [11] showed that mesangium content in renal glomeruli increases in 4-week-old hypertensive SHR rats. They reported that this is related to the predisposition of animals to glomerular diseases, which leads to the development of arterial hypertension.

Morphological signs of myocardial hypertrophy and cardiosclerosis were observed in 6-week-old NISAG rats subjected to postnatal handling. These animals did not differ from intact hypertensive rats in the degree of muscle cell capillarization and volume ratio of mitochondria and myofibrils. In handled rats the thickness of myofibrils was 1.5 times greater than in control animals (Table 2).

Quantitative and ultrastructural characteristics of renal glomeruli in handled NISAG rats did not differ from those in intact animals (Table 2). We revealed signs of hypertrophy, disturbances in blood circulation, and functional strain in podocytes typical of hypertension. However, the thickness and volume ratio of basal membranes in renal glomeruli were relatively low in handled rats. Therefore, the severity of glomerulosclerosis in handled rats was lower than in control animals.

It should be emphasized that handling had no effect on blood pressure in adult NISAG rats (Table 2).

Our results indicate that early postnatal handling does not modulate the development of arterial hypertension and typical morphological signs in the myocardium and kidneys of NISAG rats.

These results were surprising. Published data show that stress-produced changes are less pronounced in adult animals subjected to moderate chronic stress during early ontogeny [9,10]. Moreover, blood pressure in adult male SHR rats handled daily on days 1-21 of life is much lower than in control animals [14].

L. N. Maslova *et al.* [4] reported that moderate psychoemotional load during the prepubertal period produces a long-lasting antihypertensive effect in NISAG rats. Chronic stress of daily handling or exposure to various emotiogenic or physical factors from the 21st to 32nd day of life decreases baseline blood pressure in adult animals. Previous studies showed that NISAG rats differ from Wistar rats by low content of norepinephrine in the hypothalamus and medulla oblongata [1,3]. These brain structures are involved in the regulation of the vascular tone. These differences are manifested in 4-week-old NISAG rats. It was hypothesized that chronic stress during the early ontogeny activates the noradrenergic system in the brain and serves as a training procedure for the mechanisms maintaining blood pressure at a normal level.

In our experiments daily handling produced no antihypertensive effect. Probably, the mechanisms regulating cardiovascular functions are not completely developed in the early juvenile period. The genetic defect in NISAG rats manifested after the age of 3 weeks. At this term baseline blood pressure in Wistar and NISAG rats is similar [3]. However, blood pressure in NISAG rats considerably increases during stress. Younger NISAG rats are characterized by relative irresponsiveness [13]. The regulatory mechanisms are not sufficiently sensitive to stress factors and,

TABLE 1. Morphometric Indexes of the Myocardium and Renal Glomeruli in 3-Week-Old Wistar and NISAG Rats (*M*±*m*)

Parameter	Wistar	NISAG	
		intact	handling
Body weight, g	38.77±1.30	34.87±1.61	25.9±0.5*
Blood pressure, mm Hg	—	—	—
Myocardium			
Relative weight of the heart, mg/g	6.20±0.16	6.5±0.1	6.30±0.22
Cardiomyocyte diameter, μ	10.10±0.53	9.40±0.16*	10.30±0.20+
Stroma/parenchyma ratio	0.54±0.01	0.45±0.01*	0.380±0.013*+
Numerical ratio between capillaries and cardiomyocytes	0.50±0.01	0.470±0.015	0.420±0.013*+
Mitochondrion/myofibril volume ratio	0.79±0.03	0.77±0.03	0.97±0.03*+
Myofibril diameter, μ	0.60±0.01	0.70±0.02*	1.20±0.05*+
Kidney			
Relative weight of the kidneys, mg/g	10.8±0.5	10.12±0.22	10.47±0.30
Numerical density of glomeruli, per mm^2	17.1±0.9	16.6±1.2	16.75±1.00
Relative volume of glomeruli, %	5.51±0.27	5.98±0.39	6.74±0.57
Diameter of glomeruli, μ	57.5±0.8	61.2±1.1	59.7±1.9
Relative podocyte volume, %	33.12±1.21	30.78±1.39	34.76±3.64
Relative endotheliocyte volume, %	15.73±0.88	16.46±1.00	18.91±1.12*
Relative mesangium volume, %	3.26±0.64	7.44±0.89*	5.13±0.75
Basal membrane thickness, nm	140.48±4.36	155.00±6.58	161.90±7.81*
Length of the contact between podocyte processes and basal membrane, nm	221.80±8.99	218.06±12.01	277.45±14.35*+

Note. Here and in Table 2: $p<0.05$ *compared to Wistar rats; +compared to intact animals.

TABLE 2. Morphometric Indexes of the Myocardium and Renal Glomeruli in 6-Month-Old Wistar and NISAG Rats (*M*±*m*)

Parameter	Wistar	NISAG	
		intact	handling
Body weight, g	272.2±7.6	291.6±8.5	288.50±10.65
Blood pressure, mm Hg	118±4	171±3*	177±4*
Myocardium			
Relative weight of the heart, mg/g	3.00±0.07	3.6±0.1*	3.30±0.17*
Cardiomyocyte diameter, μ	14.70±0.29	21.10±0.37*	20.70±0.45*
Stroma/parenchyma ratio	0.18±0.01	0.23±0.01*	0.29±0.09*+
Numerical ratio between capillaries and cardiomyocytes	0.41±0.03	0.77±0.10*	0.90±0.03*
Mitochondrion/myofibril volume ratio	0.710±0.024	0.640±0.026	0.61±0.02
Myofibril diameter, μ	0.66±0.01	0.80±0.02	1.20±0.02*+
Kidney			
Relative weight of the kidneys, mg/g	0.610±0.024	0.650±0.022	0.65±0.33
Numerical density of glomeruli, per mm^2	7.6±0.4	6.9±0.4	6.69±0.38
Relative volume of glomeruli, %	6.15±0.38	6.51±0.38	5.38±0.43
Diameter of glomeruli, μ	102.5±1.7	112.7±1.6*	109.93±2.01*
Relative podocyte volume, %	33.52±1.38	35.95±1.24	32.91±0.82
Relative endotheliocyte volume, %	14.46±1.00	10.14±0.70*	13.35±0.73+
Relative mesangium volume, %	6.80±0.73	9.82±1.10*	9.69±0.82*
Basal membrane thickness, nm	198.3±5.6	292.6±13.1*	246.60±5.44*+
Length of the contact between pedicles and basal membrane, nm	366.6±31.4	530.2±41.7*	507.3±26.7*

therefore, they cannot modulate the development of arterial hypertension.

Our results and published data indicate that changes in the conditions of early postnatal ontogeny can modulate the development of hereditary arterial hypertension. The modulatory effect depends not only on the type, strength, and duration of stress factors, but also on maturation of major functional systems involved in the regulation of blood pressure (*i.e.* age of stressed animals).

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