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Effect of Chronic Stress during Neonatal Ontogeny on Structural Organization of the Adrenal Gland in Hypertensive NISAG Rats

I. I. Buzueva, M. D. Shmerling, E. E. Filushina, A. L. Markel', and G. S. Yakobson

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Structural organization of the adrenal gland was studied in 3-week- and 6-month-old hypertensive NISAG rats subjected to daily handling (10-min separation from mothers) on postnatal days 1-21. Neonatal handling reduces the stress-induced blood pressure rise in adult NISAG rats and modulates the structure of the adrenal cortex and medulla.

Key Words: *hypertension; handling*; *adrenal gland*; *ultrastructure*

Previous studies showed that chronic stress exposure during the neonatal ontogeny modulates the response of the hypothalamic-pituitary-adrenal [8,9] and sympathoadrenal [7,13] systems of normotensive adult animals to stress. Little is known about the delayed effects of early stress exposure in hypertensive animals. There is no consensus on the effect of early stress on the basal and stress-induced blood pressure (BP) [4,11]. NISAG rats selected as a stress-sensitive hypertensive strain are of special interest in this respect. Systolic pressure in NISAG rats did not exceed the normal during the first few weeks after birth, but tended to decrease starting from postnatal weeks 3-4 [3]. The development of hypertension in these rats is accompanied by increased sensitivity of the hypothalamic-pituitary-adrenocortical and sympathoadrenal systems to stress [5,6]. Structural changes in the adrenal cortex of NISAG rats with signs of hypertrophy of the zona glomerulosa were observed as early as on postnatal week 3. In 6-month-old rats, when stable arterial hypertension developed [1,2], these changes became more pronounced. It is important to find out, whether neonatal stress can affect structural organization of the adrenal gland in NISAG rats at different periods of postnatal ontogeny.

MATERIALS AND METHODS

The study was carried out on 3-week- and 6-month-old hypertensive NISAG and normotensive Wistar rats. The experimental group included NISAG rats subjected to neonatal handling — daily 10-min separation from mothers in 15×15×15 cm plastic boxes on postnatal days 1-21, i.e. until weaning. Intact age-matched male NISAG and Wistar rats served as controls. BP was measured in adult rats by tail-cuff method. The rats were killed under ether anesthesia at the age of 3 weeks and 6 months, and both adrenals were fixed in a mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at 4°C. After fixation the right adrenal was embedded in paraffin and ultrathin serial sections (5 μ) were prepared. In every 15th section stained with hematoxylin-

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences; Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk. ***Address for correspondence***: i.i.buzueva@iph.ma.nsc.ru. Buzueva I.

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eosin, the volumes of the cortical and medullar zones were evaluated using an ocular micrometer (magnification 2.5×16). Adrenal volume was calculated by its weight and relative density of the adrenal tissue (1.039 g/ml^3) [10]. The fragments of the left adrenal were postfixed with 1% osmium tetroxide, dehydrated in alcohol-propylene oxide, and embedded in Epon-Araldite. Semithin and ultrathin sections were prepared using an LKB-V ultramicrotome. The semithin sections stained with toluydine blue were examined under a light microscope (100×16) to determine the mean volume of endocrine cells. The ultrathin sections were analyzed under a JEM100SX electron microscope. The relative volume densities of mitochondria and lipid droplets in adrenocorticocytes were measured using a double square grid (magnification 15,000) [12].

The data were statistically analyzed using STATGRAPHICS 4.0 software.

RESULTS

At the age of 3 weeks, NISAG rats subjected to neonatal handling were characterized by lower body weight and proportionally decreased adrenal weight (relative adrenal weight did not change, Table 1), compared to nonhandled controls of the same strain. Histological study also showed decreased volume of the zona glomerulosa, reduced population of glomerular adrenocorticocytes and volume density of mitochondria. Similarly to the zona glomerulosa, significantly smaller volumes of adrenocorticocytes and steroidogenic structures were found in the zona fasciculata of the adrenal cortex, compared to the control. By contrast, the volume of medullar chromaffinocytes in the experimental group far surpassed the corresponding parameter in controls. Thus, stress exposure in the early postnatal period induced hypertrophic changes only in the medullar chromaffinocytes.

At the age of 6 months, the basal BP in handled and nonhandled NISAG rats increased to the same extent (Table 2). However, during stress BP in NISAG rats subjected to neonatal handling was significantly lower than in nonhandled hypertensive rats. It is noteworthy that both the absolute and relative adrenal weight and the volumes of cortical zones were similar in handled and nonhandled rats. However, in experimental NISAG rats we observed increased population of small cells characterized by lower content of steroidogenic structures (mean volume decreased by 22% compared to that in intact rats). Analysis of the main morphometric indices in the zona fasciculata (volume of this zone and volumes of adrenocorticocytes and steroidogenic structures) revealed no significant differences between the experimental and intact NISAG rats, except slight decrease in the content of lipid droplets in the experimental group. In contrast to the cortex, the volume of the medulla in experimental hypertensive rats decreased by 44%, compared to that in intact rats. However it 2.4-fold surpassed the corresponding parameter in normotensive Wistar rats. The mean volume of chromaffinocytes and their density in the experimental and control groups were similar.

It was hypothesized that the development of hypertension in NISAG rats is associated with a decrease in norepinephrine level in the hypothalamus and medulla oblongata, i.e. in centers associated with regulation of

TABLE 1. Morphometric Parameters of Adrenal Glands in 3-Week-Old Rats (*M±m*)

Parameters		Wistar	NISAG	NISAG (handling)
Body weight, g		38.80±1.39	34.90±1.62	25.9±0.5*+
Absolute weight of adrenals, mg		14.30±0.76	16.60±0.73*	14.20±0.49+
Relative weight of adrenals, mg/100 g body weight		36.90±1.04	47.8±2.5*	54.90±2.16*
Volume of adrenal cortex, mm^3		6.00±0.35	7.80±0.44*	6.10±0.28*
Zona glomerulosa	volume, mm^3	1.20±0.07	1.9±0.1*	1.40±0.15+
	volume of adrenocorticocytes, μl^3	323±15	388±16*	299±10+
	relative volume of mitochondrial, %	24.60±1.56	22.62±1.38	19.16±0.38+
	relative volume of lipid droplets, %	25.90±3.15	27.80±2.74*	25.85±2.94
Zona fasciculata	volume, mm^3	4.40±0.33	5.50±0.38	4.34±0.36
	volume of adrenocorticocytes, μl^3	709±28	551±21*	487±19*+
	relative volume of mitochondrial, %	37.00±1.17	27.40±1.31*	24.30±1.08*
	relative volume of lipid droplets, %	11.40±1.25	28.70±1.85*	23.10±1.75*
Adrenal medulla	volume, mm^3	0.41±0.02	0.39±0.02	0.41±0.04
	volume of chromaffinocytes, μl^3	565±20	527±19	586±24+

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

TABLE 2. Morphometric Parameters of Adrenal Glands in 6-Month-Old Rats (*M±m*)

Parameters		Wistar	NISAG	NISAG (handling)
BP at rest, mm Hg		118±4	171±3*	177±4*
BP during stress, mm Hg		128±2	215±5*	189±4*+
Body weight, g		272.2±7.6	291.6±8.5	288.50±10.65
Absolute weight of adrenals, mg		34.70±2.05	42.40±1.99*	42.20±0.54*
Relative weight of adrenals, mg/100 g body weight		12.70±0.53	14.5±0.5*	14.70±0.78*
Volume of cortex, mm^3		13.30±0.81	17.7±0.9*	18.10±0.27*
Zona glomerulosa	volume, mm^3	1.60±0.05	3.0±0.1*	3.1±0.1*
	volume of adrenocorticocytes, μl^3	533±19	652±17*	507±15+
	relative volume of mitochondrial, %	24.20±1.78	35.90±2.54*	24.90±1.65+
	relative volume of lipid droplets, %	29.8±3.3	38.2±6.0*	20.70±2.86*+
Zona fasciculata	volume, mm^3	10.60±0.66	13.00±0.86*	13.60±0.27*
	volume of adrenocorticocytes, μl^3	770±20	763.5±18.0	730±24
	relative volume of mitochondrial, %	32.30±2.25	30.20±2.54	28.90±1.78
	relative volume of lipid droplets, %	29.90±3.11	29.40±3.26	21.5±2.3*+
Adrenal medulla	volume, mm^3	0.70±0.02	2.6±0.2*	1.80±0.17*+
	volume of chromaffinocytes, μl^3	848±22	924±25*	802±27+

the vascular tone [4]. According to published data, conventional models of chronic stress (studied usually at late periods of ontogeny, in particular, in the prepubertal period) can activate the noradrenergic system in the brain. Hence, they can serve as a specific means for training central mechanisms aimed at prevention of stress-induced BP rise. In our experiments, early stress exposure (10-min handling during postnatal days 1 to 21) did not cause expected antihypertensive effect. The possible reason is that central mechanisms of BP regulation were not formed in the early juvenile period (days 1-21). However, less pronounced hyperthrophic changes in the adrenal gland of adult NISAG rats subjected to neonatal handling suggests lower sensitivity of these rats to stress compared with nonhandled controls.

Thus, our findings indicate that neonatal handling reduces stress-induced rise of arterial blood pressure in adult hypertensive NISAG rats and produce a modulatory effect on the adrenal cortex and medulla, resulting in a less pronounced hypertrophy of these regions compared to intact NISAG rats.

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