PHYSIOLOGY

Age-Related Structural Characteristics of the Adrenal Medulla in Hypertensive NISAG Rats

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Structural characteristics of the adrenal medulla in hypertensive NISAG rats (hereditary stress-induced arterial hypertension) were studied during various periods of postnatal ontogeny. Signs of hyperplasia of the adrenal medulla were most pronounced in adult hypertensive animals with persistent arterial hypertension, as well as during the period of late ontogeny.

Key Words: *arterial hypertension; adrenal medulla; stereomorphometry*

The development of arterial hypertension in NISAG rats with hereditary stress-induced arterial hypertension [3] is related to hyperactivity of the sympathoadrenal and pituitary-adrenocortical systems playing a role in the stress response [4,5]. Previous studies showed that these animals are characterized by adrenocortical hyperplasia [1,2].

Here we studied ontogenetic characteristics of the adrenal medulla (peripheral compartment of the sympathoadrenal system) in NISAG rats.

MATERIALS AND METHODS

Experiments were performed on male hypertensive NISAG rats and normotensive Wistar rats (control). The animals were maintained in a vivarium and had free access to water and food. The rats aging 3 weeks and 6 or 12 months were killed under ether anesthesia.

Basal blood pressure (BP) in animals aging 6 and 12 months was measured by the indirect tail-cuff method. Both adrenal glands were isolated for morphological examination of the medulla. One adrenal was fixed in Bouin fluid, dehydrated, and embedded into paraffin for histological examination. It was cut into serial sections (width 5 μ). The area of the medulla was estimated in each 15th serial section of the adrenal gland stained with hematoxylin and eosin using an ocular micrometer (×40). The volume of the adrenal gland was calculated [1,2]. Another adrenal was fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffered saline, postfixed with 1% OsO_4, dehydrated, and embedded into a mixture of epoxide resins Epon and araldite. Semithin sections of the adrenal glands stained with toluidine blue was examined under a light microscope (×1600) to estimate the mean volume of chromaffin cells (CC). Ultrathin sections were examined under a Jem-100SX electron microscope. During stereomorphometric analysis of CC we evaluated the numerical density and relative volume of various ultrastructures, including chromaffin granules, mitochondria, rough endoplasmic reticulum (EPR), and ribosomes.

The results were analyzed using Statistica 6.0 software. The significance of differences was evaluated by Student's *t* test ($p<0.05$).

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RESULTS

BP in 3-week-old NISAG rats was similar to that in Wistar rats (102±4.3 and 103±3.2 mm Hg, respectively) [9]. These animals did not differ by body weight, volume of the adrenal medulla, and size of CC (Table 1). Stereomorphometry of CC in the adrenal glands from these rats revealed significant differences in the content of ultrastructures characterizing biosynthetic activity of cells. The volume ratios of rough EPR, free ribosomes, and osmiophilic (mature) chromaffin granules in CC of NISAG rats were higher compared to control animals. The ratio of these granules in CC of the adrenal glands in NISAG and Wistar rats was 83 and 61%, respectively. NISAG rat pups had a lower number of large heterogeneous (by the content) or light granules (small volume of secretion) compared to control animals (Table 1). The presence of heterogeneous and light secretory granules in CC of the adrenal glands is related to a certain stage of secretion [6]. Dense chromaffin granules with a considerable amount of secretion prevailed in NISAG rat pups. Hence, CC of the adrenal medulla in hypertensive rat pups were characterized by higher hormone-synthesizing activity compared to control animals of the same age. Increased hormone-synthesizing activity of CC in NISAG rats suggests that a changes in the adrenal medulla of these animals play an important role in the development and progression of stress-dependent arterial hypertension.

BP in 6-month-old NISAG rats was higher than in Wistar rats of the same age (171±3 and 147±2 mm Hg, respectively). Hypertrophy of the adrenal medulla was revealed in NISAG rats at this age. The volume of the adrenal medulla in NISAG rats was 3.4-fold higher compared to that in Wistar rats. These differences were associated with hyperplastic and hypertrophic changes in CC (Table 1). Chromaffin tissue overgrew and appeared in the adrenal cortex. The physiological role of anatomical proximity between medullar CC and adrenocortical cells is the increase in paracrine relationships within the adrenal gland [7]. The numerical density of secretory granules in CC increased in NISAG rats (Table 1). It was primarily related to an increase in the ratio of heterogeneous granules (*i.e.*, granules releasing the secret). The volume ratio of rough EPR and free ribosomes in NISAG rats did not differ from the control (Table 1). These data indicate that secretory activity of CC in the adrenal glands of NISAG rats is higher compared to Wistar rats.

TABLE 1. Morphometric Parameters of the Adrenal Medulla and Ultrastructure of CC in Wistar and NISAG Rats during Various Periods of Postnatal Ontogeny ($M\pm m$)

Parameters	Age					
	3 weeks		6 months		12 months	
	Wistar	NISAG	Wistar	NISAG	Wistar	NISAG
Volume of the medulla, mm^3	0.41±0.02	0.39±0.02	0.80±0.02	2.6±0.2*	1.45±0.10	2.69±0.13*
Average volume of CC, μ^3	564±20	531±20	839±23	921±23*	900±23	1117±40*
Relative volume of mitochondria, %	6.20±0.90	8.20±0.97	7.30±0.97	6.90±0.77	8.90±1.02	5.7±0.8*
Numerical density of mitochondria, μ^{-2}	3.00±0.38	3.00±0.35	3.17±0.35	3.76±0.46	3.22±0.31	2.49±0.29
Relative volume of rough EPR, %	0.28±0.05	2.00±0.27*	1.9±0.2	1.50±0.17	1.62±0.15	1.77±0.12
Relative volume of smooth EPR, %	4.8±0.5	1.7±1.2*	1.48±0.23	1.68±0.25	1.91±0.19	1.53±0.23
Relative volume of secretory granules, %	16.50±1.04	16.80±1.23	19.70±1.71	22.60±1.46	20.70±1.56	16.8±1.2*
Total numerical density of secretory granules, μ^{-2}	73±4	68±4	68.0±5.4	83.0±4.4*	65.90±3.97	62.30±3.17
Numerical density of osmiophilic secretory granules, μ^{-2}	44±4	57±4*	57.0±5.3	66.0±3.6	50.30±3.13	44.40±2.66
Numerical density of heterogeneous secretory granules, μ^{-2}	19±2	8±1*	4.0±0.4	10.0±1.4*	12.20±1.01	9.90±0.87
Numerical density of light secretory granules, μ^{-2}	9±1	3.0±0.3*	6.0±0.8	7.0±0.8	3.53±0.28	7.50±0.59*
Numerical density of granules in free ribosomes, μ^{-2}	296±24	367±21*	262±17	294±8	139±7	130±8

Note. *p<0.05 compared to Wistar rats of the same age.

These specific features of the adrenal medulla in NISAG rats are probably associated with their higher sensitivity to stress. The increased emotional excitability of NISAG rat pups results in frequent episodes of BP elevation and, therefore, development of arterial hypertension. Morphological signs are consistent with physiological characteristics of hormonal activity of the adrenal medulla in NISAG rats [4]. Epinephrine concentration in the adrenal gland of NISAG rats 2-fold exceeds that in Wistar rats. Moreover, expression of tyrosine hydroxylase gene (key enzyme of catecholamine biosynthesis) increases in the adrenal gland of NISAG rats [4]. These animals are also characterized by high activity of phenylethanolamine N-methyltransferase [5].

BP remained high in 12-month-old NISAG rats (153±3 *vs*. 118±4 mm Hg in Wistar rats of the same age). ECG showed that a slight decrease in systolic BP in 12-month-old rats (compared to 6-month-old animals) is related to decreased of cardiac contractility [8]. The adrenal gland in these animals was as large as in 6-month-old NISAG rats. The volume of the adrenal medulla significantly exceeded the control. The average size of CC increased by more than 20% (Table 1). Stereomorphometry showed that the relative volume of mitochondria and numerical density of chromaffin granules in CC decreased in hypertensive rats. Electron microscopy revealed degenerative changes in many CC. These cells were edematous and had smaller granules and vacuolized mitochondria. These signs reflected functional overload and activation of destructive processes in the adrenal glands of old NISAG rats.

Our results show that hormone-synthesizing activity of the adrenal medulla increases in prepubertal NISAG rats before persistent elevation of BP. These changes probably determine increased sensitivity of NISAG rats to stress. Adult NISAG rats with severe arterial hypertension are characterized by hyperplastic and hypertrophic changes in CC, which reflects high activity of the sympathoadrenal system. Hence, the adrenal medulla plays an important role in the maintenance of persistent arterial hypertension. Functional overload of the adrenal medulla and decrease in its function during the stress response are observed in 12-month-old NISAG rats.

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