Structure of Zona Reticularis of Adrenal Cortex in Hypertensive NISAG Rats

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The structure of zona reticularis of the adrenal cortex in hypertensive NISAG rats was studied during the early, middle, and late periods of postnatal ontogeny. The detected morphological signs suggest that hypotrophic changes in zona reticularis of the adrenal cortex in hypertensive rats appeared before the onset of high blood pressure and accompanied the development of arterial hypertension in these animals.

Key Words: *arterial hypertension; NISAG rats; adrenocortical reticular zone; ultrastructure*

Hormones of the zona reticularis (ZR) of the adrenal cortex (AC) play an important role in intricate interactions of the regulatory systems maintaining homeostasis. For example, dihydroepiandrosterone (DEA) is a peculiar lifespan marker in humans and animals [8]. Low level of DEA in human plasma indicates predisposition to stress-induced diseases [3,4]. Cardiovascular diseases in men, specifically, arterial hypertension, is often associated with low plasma levels of DEA and DEA sulfate [5]. Experimental studies on male NISAG rats (model of human essential hypertension) showed that injection of DEA sulfate protected them from sharp elevation of blood pressure under conditions of emotional stress [2]. Preliminary studies of the structure of adrenals in adult NISAG rats showed hypertrophy of the zona glomerulosa and zona fasciculata, on the one hand, and ZR hypotrophy, on the other [1].

We carried out a morphometrical study of the structural and particularly ultrastructural organization of ZR of AC in hypertensive NISAG and normotensive Wistar rats during different periods of postnatal ontogeny.

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MATERIALS AND METHODS

Ontogenetic studies were carried out on male hypertensive NISAG and normotensive Wistar rats. Basal blood pressure was measured in these animals by the indirect tail cuff method. The study was carried out with due consideration for "Regulations for Studies on Experimental Animals".

After sacrifice at the age of 3 weeks, 6 and 12 months, both adrenals were collected for morphometric studies. One adrenal was fixed in Bouin's fluid, dehydrated in alcohols and xylene, and embedded in paraffin. In order to evaluate the volume of AC, 4-μ serial histological sections were sliced every 150 μ and stained with hematoxylin and eosin. The other adrenal was fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% OsO_4, dehydrated in alcohols and propylenoxide, and embedded in epon-araldite.

The areas of adrenocorticocytes, percentage of capillaries and periendothelial space in AC ZR were estimated on semithin sections of the adrenals stained with toluidine blue under an Axiostar plus microscope (×400) using Motic Images 2000 1.3 software. Ultrathin sections were examined under a JEM-100SX electron microscope. The percentage and numerical density of steroidogenic ultrastructures in AC ZR (mitochondria, lipid droplets, endoplasmic reticulum), and adrenocorticocyte lipofuscin were evaluated.

The results were processed using Statistica 6.0 software.

RESULTS

Blood pressure in NISAG rat pups at the age of 3 weeks was the same as in Wistar pups. The volumes of AC and ZR were virtually the same in animals of both strains. On the other hand, the volume of the capillary network and periendothelial space in ZR were lower in NISAG rats than in normotensive animals ($p<0.05$). Endocrine cells in AC ZR of NISAG rats were smaller, their mean cross-section area was decreased by 30% compared to the control (Table 1). Stereomorphometric analysis showed that endocrine cells of NISAG rats had smaller mitochondria, lower content of lipid droplets and endoplasmic reticulum, and higher content of lipofuscin (Table 1).

Blood pressure at rest and during stress was higher ($p<0.05$) in NISAG rats at the age of 6 months in comparison with Wistar rats of the same age (Fig. 1). The volume of ZR in NISAG rats was lower than in the control ($p<0.05$), while the volume of AC was appreciably higher. Decreased volume of ZR in these rats was associated with reduction of the capillary network volume and cross-section area of endocrine cells, on the one hand, and with enlargement of the periendothelial space ($p<0.05$) and connective tissue growth between the cells, on the other. The findings of stereomorphometric analysis revealed no appreciable differences in the relative volumes of steroidogenic ultrastructures in adrenocorticocytes of ZR in NISAG rats, but, similarly as in younger age, the relative volume and numerical density of lipofuscin in these cells were elevated ($p<0.05$).

By the age of 12 months, the levels of systolic blood pressure at rest and during stress were also higher ($p<0.05$) than in Wistar rats (Fig. 1). Morphological study showed simular values of ZR volumes in rats of the two strains, though structural differences in the AC ZR noted at the age of 6 months persisted (Table 1). The relative volume of the capillary network continued to decrease, while the percentage of periendothelial space was still increasing. The cross-section area of adrenocorticocytes remained lower ($p<0.05$) than in Wistar rats, but the proportion of steroidogenic ultrastructures in the cells was different. The volume and numerical densities of lipid droplets were increased in

TABLE 1. Morphometric Parameters of AC ZR in Wistar and NISAG Rats (*M*±*m*)

Parameter	Wistar, age			NISAG, age		
	3 weeks	6 months	12 months	3 weeks	6 months	12 months
ZR volume, mm^3	0.40±0.07	2.20±0.59	1.60±0.21	0.4±0.1	1.60±0.27*	1.40±0.23
Percent volume of capillaries	13.90±0.47	13.70±0.46	19.40±0.66	10.20±0.68*	11.50±0.46*	14.50±1.03*
Percent volume of pericapillary space	6.10±0.29	4.60±0.36	4.40±0.29	4.00±0.31*	6.80±0.49*	7.20±0.53*
Cell section area, μ^2	80.50±1.89	67.90±1.09	56.20±0.87	52.90±0.78*	53.70±1.07*	46.30±0.71*
Percent volume of mitochondria	45.70±1.26	36.10±2.71	39.70±1.79	47.9±1.4	34.20±1.79	30.90±1.98*
Numerical density of mitochondria, μ^{-2}	7.70±0.27	7.9±0.7	8.60±0.55	12.50±0.42*	6.10±0.29*	10.40±0.67*
Percent volume of lipid droplets	15.90±1.39	14.80±2.99	11.02±0.28	8.00±1.07*	16.10±1.89	17.00±0.34*
Numerical density of lipid droplets, μ^{-2}	3.60±0.27	1.70±0.25	1.31±0.19	2.20±0.25*	2.20±0.31	2.80±0.38*
Percent volume of lipofuscin granules	1.70±0.41	1.00±0.24	5.40±0.77	1.90±0.32	2.00±0.22*	3.40±0.69*
Numerical density of lipofuscin granules, μ^{-2}	1.10±0.17	0.90±0.25	3.20±0.35	2.00±0.18*	1.7±0.2*	1.50±0.24*
Percent volume of endoplasmic reticulum	4.70±0.38	3.10±0.18	2.10±0.11	2.40±0.14*	5.80±0.45*	2.20±0.76
Percent volume of Golgi complex	0.40±0.11	1.90±0.65	0.800±0.178	0.40±0.15	1.40±0.27	1.10±0.33

Note. *$p<0.05$ compared to Wistar rats.

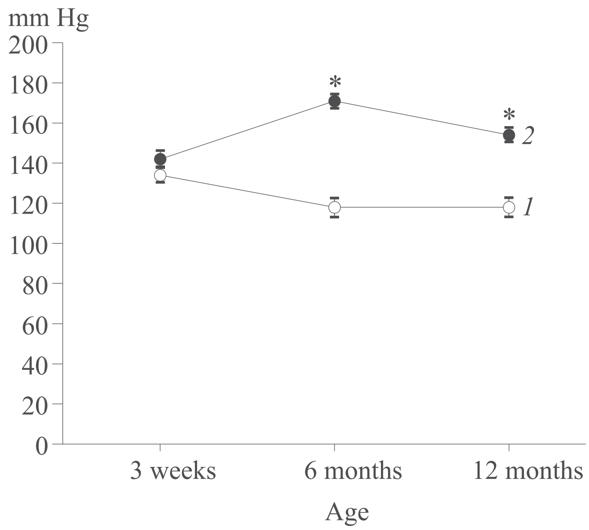

Fig. 1. Dynamics of systolic blood pressure in Wistar (*1*) and NISAG rats (*2*). *$p<0.05$ compared to Wistar rats.

NISAG rats, while the relative volumes of mitochondria and lipofuscin decreased.

The size of AC in men virtually does not change throughout the life (from 20-29 to 54-90 years) [6]. At the same time, the width of ZR undergoes significant involution transformations associated with a decrease in DEA and DEA sulfate production. Our studies showed that the first signs of hypotrophic processes in ZR of NISAG rats manifest as early as at the age of 3 weeks, when the mechanisms of increased stress sensitivity are forming. Among these signs are shrinkage of endocrine cells, accumulation of lipid droplets and lipofuscin, and decrease in relative volume of the endoplasmic reticulum containing steroidogenesis enzymes. In 6-month-old NISAG rats with elevated blood pressure, hypotrophic changes in ZR became more pronounced than in Wistar rats, against the background of rapidly increasing volumes of zona glomerulosa and zona fasciculata of AC [1]. Accumulation of lipid droplets and reduced content of mitochondria in adrenocorticocytes of 12-month-old rats also indicate lower level of steroidogenesis in this zone. Connective tissue growth in the pericapillary zone was noted in these rats. Presumably, sclerotic processes in ZR were stimulated by death of endocrine cells because of destructive changes in them. Age-associated reduction of ZR volume can be caused by predomination of apoptosis over proliferation in ZR [7] or by reduced migration of cells into this zone [9]. The fact that hypotrophic changes in ZR manifest earlier and are more pronounced in NISAG rats can be responsible for reduced production of androgens by the adrenals, which, in turn, leads to increased stress sensitivity of these animals.

Hence, comparative analysis of AC ZR in rats of two strains showed that hypotrophic changes in AC ZR of NISAG rats manifest before the onset of high blood pressure and accompany the development of arterial hypertension in these animals.

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