Effect of Enalapril Injected in the Early Period of Postnatal Ontogeny on Structure of Adrenal Glands in Mature Hypertensive NISAG Rats

I. I. Buzueva

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Blood pressure in 6-month hypertensive NISAG rats daily treated with enalapril in the early postnatal period was lower than in control rats. Enalapril produced significant morphological alterations only in the zona glomerulosa of the adrenal cortex. The volumes of this area and the corresponding endocrine cells were lower than in the control. Enalapril produced a delayed modifying effect on the structure of the adrenal zona glomerulosa by moderating hyperplasic alterations, which are characteristic of intact mature NISAG rats.

Key Words: *hypertension; enalapril; adrenal gland; morphometry*

The changes in nursing (cross-suckling) during the first postnatal month modified the development of hereditary hypertension by decreasing blood pressure and decelerating structural alterations in organs of mature animals [4]. Experiments with chronic stress showed that the hypotensive effect was more pronounced, when rat pups were isolated from mothers on day 21 instead of day 1 [2]. The systems controlling blood pressure, including the renin-angiotensin-aldosterone system (RAAS), develop in prepubertal period [2,6]. At this age, the regulatory systems are most vulnerable to the action of hypotensive pharmacological agents, especially to drugs inhibiting RAAS (inhibitors of angiotensin-converting enzyme ACE, *e.g.* enalapril). Experiments on rats with hereditary arterial hypertension (SHR, GH) showed that the hypotensive effect of enalapril was retained for several months, if the drug was given to prepubertal animals [5,7].

We previously showed that the most characteristic structural alterations of adrenal glands in mature NISAG rats under conditions of sustained hypertension are developed in the zona glomerulosa of the adrenal cortex producing mineralocorticoids [1].

Our aim was to study structural changes of this adrenocortical area in mature NISAG rats receiving enalapril during prepubertal period.

MATERIALS AND METHODS

The study was carried out in the Department of Evolutionary Genetics in the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk). NISAG rat pups were isolated from their mothers on day 28 after birth. Starting from this day on, the pups daily received enalapril (25 mg/kg) for 30 days; water suspension (0.2 ml) of powered drug was administered *per os* with a syringe equipped with a blunt metal cannula. Control NISAG pups received an equivalent volume of water. Basal blood pressure was measured by the tail-cuff method at the age of 2, 3, 4, 5 and 6 months. After sacrifice both adrenal glands were isolated for morphological examination. One adrenal gland was fixed in Bowen fluid and after dehydration embedded in

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

TABLE 1. Age-Related Changes of Blood Pressure in Rats (mm Hg, *M*±*m*)

Group	2 months	3 months	4 months	5 months	6 months
NISAG (water)	183.7±2.6	171.4±3.2	182.2±5.2	173.5±2.5	184.0±5.6
NISAG (enalapril)	144.8±3.5+	160.9±3.8+	160.0±2.6+	157.5±2.7+	164.0±2.4+

Note. Here and Table 2: $p<0.05$ compared to *Wistar and +NISAG (water) rats.

paraffin. The gland was cut into 5-μ serial sections. Every 15th section was stained with hematoxylin and eosin to determine the volume of adrenal cortex and medulla using an ocular grid at 2.5×16 magnification. The volume of the adrenal gland was calculated from its weight and density coefficient of 1.039 [10]. Another adrenal gland was fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde prepared on 0.1 M phosphate buffer, then in 1% OsO_4, and after dehydration embedded in Epon-Araldite. Semithin and ultrathin sections of the adrenal glands were cut on an LKB-V ultramicrotome. Semithin sections were stained with Toluidine Blue and examined under a light microscope at 100×16 magnification to determine the mean volume of endocrine cells, the parenchymal-stromal ratio, and the relative volume of the capillary bed and periendothelial space. Ultrathin sections were examined under a JEM-100SX electron microscope. Double square grid was used as the test system (×15,000) to determine the relative volume of mitochondria, membrane structures (agranular endoplasmic reticulum and Golgi apparatus), lipid droplets, and dense granules [11]. The data were analyzed statistically using Statistica software and Student's *t* test.

RESULTS

Administration of enalapril to NISAG rat pups during the second month of life decreased blood pressure ($p<0.05$), which then remained below the control level (Table 1). In 6-month NISAG rats blood pressure was lower than in control rats of the same strain, but remained significantly higher than in Wistar rats.

In mature rats treated with enalapril during the neonatal period, the weight of the adrenal gland practically did not differ from that in control rats of the same strain, but the relative weight of this organ decreased (Table 2). The volume of adrenal cortex also slightly decreased. The most pronounced enalapril-induced alterations were observed in the zona glomerulosa. In control 6-month NISAG rats we observed pronounced hypertrophy of this area (its volume 2-fold surpassed the corresponding parameter in age-matched Wistar rats), while the volume of zona glomerulosa in NISAG rats treated with enalapril in the prepubertal period was lower by almost 40% compared to that in control NISAG rats (Table 2). Since the volumes of the adrenal medulla, zona fasciculate, and zona reticu-

TABLE 2. Morphometric Indices of Adrenal Glands (*M*±*m*)

Parameter	Wistar	NISAG (water)	NISAG (enalapril)
Weight of adrenal glands, mg	34.70±2.05	54.00±2.68*	52.80±4.31*
Relative weight of adrenal glands, mg/100 g	12.70±0.53	18.20±0.47*	16.30±0.85*+
Volume of adrenal cortex, mm^3	13.30±0.81	23.00±0.96*	21.20±2.15*
Volume of zona glomerulosa, mm^3	1.60±0.05	3.90±0.23*	2.50±0.12*+
Volume of zona fasciculata, mm^3	10.61±0.66	16.10±0.52*	15.50±1.65*
Volume of zona reticularis, mm^3	1.28±0.28	2.90±0.31*	3.20±0.39*
Volume of adrenal medulla, mm^3	0.75±0.02	2.30±0.14*	1.90±0.31*
Volume of adrenocorticocytes, mm^3	770±20	870±33*	712±30+
Parenchymal-stromal ratio	14.60±1.07	11.60±0.88*	10.10±0.63*
Relative volume of capillary bed, %	3.40±0.23	5.20±0.34*	6.30±0.31*
Relative volume of periendothelial space, %	3.50±0.32	3.30±0.38	3.40±0.28
Relative volume of mitochondria, %	24.20±1.78	18.10±1.46*	21.54±1.99
Relative volume of lipid droplets, %	29.9±3.3	34.50±3.14	32.90±3.33
Relative volume of membrane space, %	5.30±0.52	4.2±0.2*	3.80±0.34*
Relative volume of dense granules,%	0.60±0.13	1.00±0.33	2.00±0.41*+

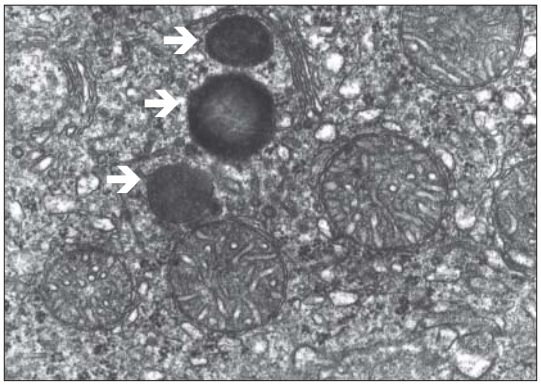

Fig. 1. Dense granules in endocrine cell from adrenocortical zona glomerulosa of experimental NISAG rats, ×35,000.

laris remained practically unchanged, zona glomerulosa was chosen for detailed morphological examination. The decrease in the volume of this area was probably caused by shrinkage of endocrine cells, whose volume was significantly below the control value, whereas parenchyma-stroma ratio in the zona glomerulosa and the relative volumes of capillary bed and periendothelial space remained unchanged.

Morphometry of endocrine cells in the zona glomerulosa revealed no significant differences in the relative volumes of mitochondria, lipid droplets, and membrane space between experimental and control NISAG rats (Table 2). However, the relative volume of dense granules 2-fold surpassed the corresponding values in hypertensive and normotensive control rats. These granules with clear-cut membranes and electron-dense homogenous matrix were found in most cells of the zona glomerulosa in these animals (Fig. 1). It was established that these dense granules are elements of autocrine regulation of steroidogenesis in endocrine cells of the zona glomerulosa, because they contain the renin-like substance [9]. It can be hypothesized that the inhibitory action of enalapril on RAAS increases the role of the autocrine mechanisms in the control of aldosterone synthesis in zona glomerulosa. The revealed structural peculiarities of the zona glomerulosa (most of all, volumetric decrease of this zone and its endocrine cells) are indicative of relative moderation of functional activity in this region in experimental rats in comparison with the controls.

Probably, this phenomenon results from the delayed action of enalapril. The hypotensive effect of this drug (ACE blocker) can be explained by its ability to dcrease the stimulatory action of angiotensin-II. This enzyme is known to stimulate the development and functional activity of the mineralocorticoid area of the adrenal cortex [3]. The experiments on regenerating adrenocortical tissue demonstrated the inhibitory action of enalapril on proliferation and steroidogenesis in the zona glomerulosa [12]. Similarly, captopril (another ACE inhibitor) decreases the level of aldosterone in the zona glomerulosa only in the animals with enhanced synthesis of this enzyme [8]. It can be hypothesized that administration of enalapril to prepubertal animals (during the development of blood pressure regulation systems) to a some extent decelerates hyperplasic processes in the zona glomerulosa of NISAG rats not only during treatment, but also at later terms.

Thus, administration of enalapril to hypertensive NISAG rats in prepubertal period produced delayed modified effects on morphological parameters of the zona glomerulosa and moderated the development of hyperplasic alterations, which are characteristic of mature rats of this strain of the rats with pronounced arterial hypertension.

REFERENCES

1. I. I. Buzueva, M. D. Shmerling, A. R. Antonov, *et al*., *Morfologiya*, **110**, No. 6, 93-96 (1996).
2. L. N. Maslova, V. V. Bulygina, and A. L. Markel', *Ross. Fiziol. Zh*., **88**, No. 6, 774-780 (2002).
3. J. Tepperman, H. Tepperman, *Physiology of Metabolism and Endocrine System* [Russian translation], Moscow (1989).
4. G. S. Yakobson, M. D. Shmerling, I. I. Buzueva, *Byull. Sib. Otd. Ross. Akad. Med. Nauk*, **112**, No. 2, 164-170 (2004).
5. S. A. Dukacz, M. A. Adams, and R. L. Kline, *Am. J. Physiol.* **276**, R10-R16 (1999).
6. G. Guron, M. A. Adams, B. Sundelin, *et al*., *Hypertension*, **29**, 91-97 (1997).
7. J. M. Ledingham and R. Laverty, *Clin. Exp. Pharmacol. Physiol*., Suppl., **22**, No. 1, S350-S352 (1995).
8. G. Mazzocchi, L. K. Malendowicz, A. Markowska, *et al*., *Endocr. Metab*., **278**, No. 6, E1027-E1030 (2000).
9. P. Rebuffat, L. K. Malendowicz, A. Kasprzak, *et al*., *Cytobios*, **68**, 7-13 (1991).
10. C. A. Swinyard, *Anat. Rec*., **74**, 71-78 (1939).
11. E. R. Weibel, *Stereological Methods*, L. (1979).
12. W. Zieleniewski, *Cytobios*, **104**, 127-132 (2001).