Morphology of the Adrenal Cortex in Normo- and Hypertensive Rats under Conditions of Salt Loading

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Adrenocorticocytes of normotensive Wistar rats and rats with spontaneous hypertension (SHR) were examined by light and electron microscopy. It is proposed that the adrenocorticocytes of rats living under conditions of permanent functional overstrain (hypertrophy of all zones) are more "fragile" during changes of water-salt homeostasis, in whose regulation they actively participate. Analysis of correlations confirmed a reduction of the adaptive potential of SHR rats to changes in water-salt homeostasis: an increase and enhancement of the bonds between adrenocorticocyte structures and a decrease of degrees of freedom.

Key Words: *adrenal cortex; adrenocorticocytes; hypertensive rats*

The adrenal cortex plays an important role in helping the organism to adapt to salt loading. Some authorities have described changes in adrenocorticocytes (ACC) under conditions of chronic salt loading specifically in the glomerular and bundle zones [5-7] and differences in ACC structures of the glomerular zone in normo- and hypertensive rats, including differences for prolonged loading with normal saline [8]. Synchronous hypertrophy of adrenocortical zones has been demonstrated in rats with spontaneous genetic hypertension (SHR strain) and in patients with arterial hypertension [3]. However, comprehensive studies with due consideration for the close relationship between water-salt metabolism, arterial pressure [2-4], and ACC of various adrenocortical zones under conditions of arterial hypertension have not been undertaken, and this prompted us to attempt such a study.

MATERIALS AND METHODS

The adrenals of 30 normotensive male Wistar rats weighing 254±5 g with arterial pressure 95±6.3 mm Hg and of 30 spontaneously hypertensive Okamoto-Aoki (SHR) rats weighing 240±12 g with stable arterial hypertension (168±5.8 mm Hg) were examined. In the first experimental series, Wistar and SHR rats, each group consisting of 10 animals, were given a single oral loading with 0.9% NaCl solution in a dose of 5% b.w.; in the second series the rats of both strains were kept on high-salt diets (peas saturated with NaCl - 1 mmol/g wet weight). The control groups each consisted of 10 animals. The animals were sacrificed 2 h after administration of liquid (series I) and after 4 days of the salt diet (series II). Aldosterone was radioimmunoassayed in the blood plasma. The size of the nuclei and nucleoli in all three adrenocortical zones was assessed by light optic examination using an ocular micrometer, the data were statistically processed, and paired correlation analysis was carried out. For electron microscopic study fragments of the adrenal cortex of 3 animals of each experimental series and control were fixed in glutaraldehyde and then in osmic acid and embedded in araldite. Slices were contrast-stained with uranyl acetate and lead citrate and examined under a 100-LM electron microscope. The area of mitochondria, relative size of mitochondrial tubules and matrix, and the size of polysomes were assessed with a point grid on electronograms.

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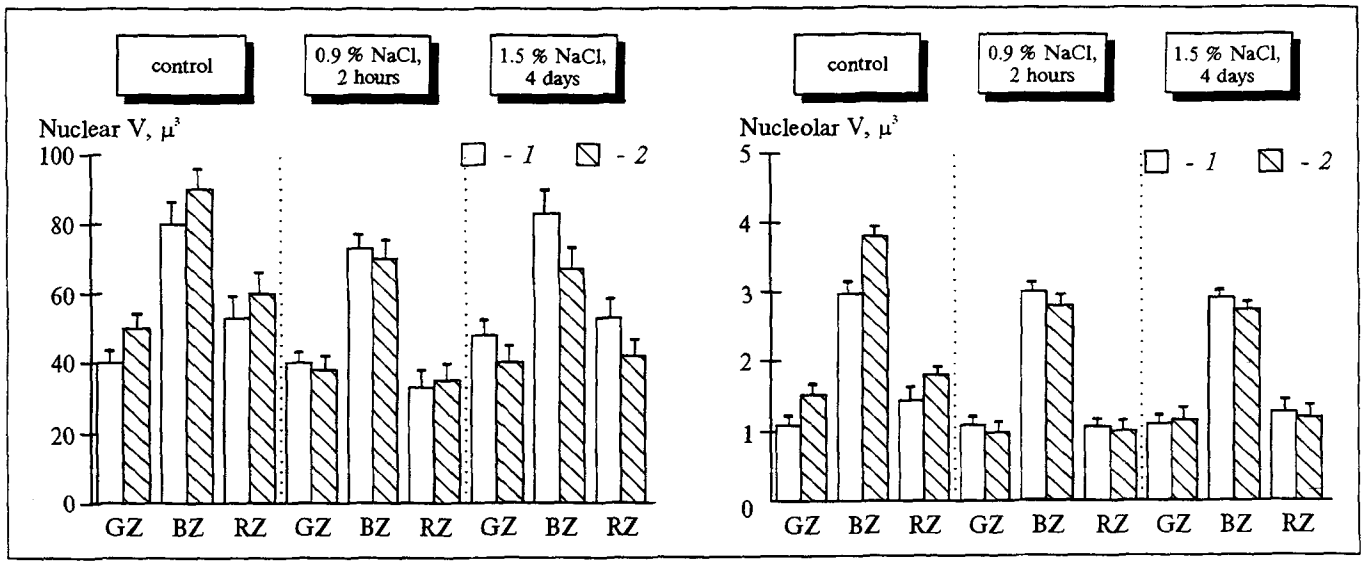

Fig. 1. Volume of ACC nuclei and nucleoli. Glomerular (*GZ*), bundle (*BZ*), and reticular (*RZ*) zones. Here and in Fig. 2: *1*) normotensive Wistar rats; *2*) hypertensive SHR rats.

RESULTS

Uniform hypertrophy of all three adrenocortical zones due to ACC hypertrophy, which was determined by an increased volume of the nuclei and nucleoli and increased mass of adrenals, was found in SHR rats (Fig. 1). The area of polysomes in the bundle zone cells was increased in SHR rats; the number of polysomes in clear cells was reduced in comparison with normotensive rats, but their total area was inceased owing to the larger mean area. The ratio of mitochondrial tubular to matrix areas was higher in SHR rats at the expense of tubules, with an overall increase in the area of the mitochondria (Fig. 2).

Salt loading with 0.9% NaCl solution resulted in a decrease of the nucleolar volume in the glomerular and reticular zones and a decrease in the volume of the nuclei in the reticular zone as soon as 2 h after loading (Fig. 1). Focal destruction of endoplasmic reticulum was observed in clear cells. The number of free ribosomes and polysomes was increased. The number of polysomes in clear cells was lower than in the control, their mean area being the same as in the control. The area of mitochondria was increased, with no change in the former relationships between tubules and matrix. In SHR rats the reaction to such loading was more pronounced. A decrease in the volume of ACC nuclei and nucleoli was synchronously observed in all adrenocortical zones. In the cytoplasm the number of polysomes was patently increased, and degradation of the reticulum was more marked than in normotensive rats. Although the increase of the mitochondrial area was unreliable, the ratios between the structures were changed at the expense of a decreased tubular area and a pronounced increase of the matrix area, its density being somewhat reduced in SHR rats (Fig. 2), possibly due to edema. In both groups the plasma aldosterone level was reduced, this being in line with the reduced volumes of the nucleoli, and in SHR rats the ACC nuclei in the glomerular zone were reduced as well.

Feeding a high-salt diet to normotensive rats for 4 days resulted in a reduction in size of the ACC nuclei only in the reticular zone, whereas in the glomerular zone the nuclei were even enlarged; the nucleoli in the glomerular zone were shrunken. The number and area of ACC polysomes in the bundle zone were unchanged, but the electron-optical density of polysomes was increased in comparison with the control and groups with 0.9% NaCl loading. The area of mitochondria was mark-

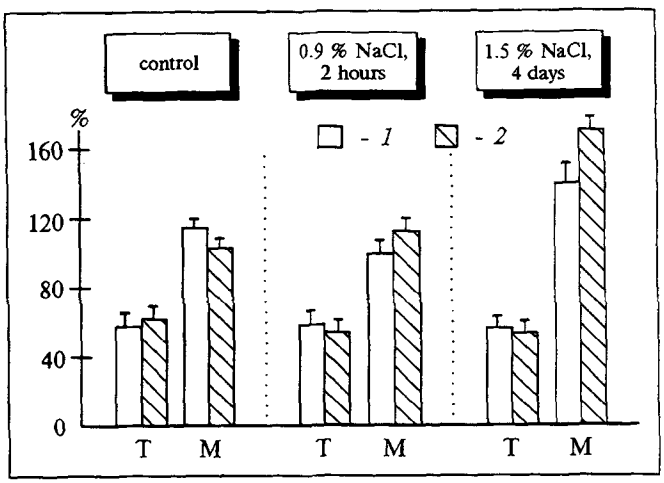

Fig. 2. Tubules (*T*) to matrix (*M*) ratio in ACC mitochondria.

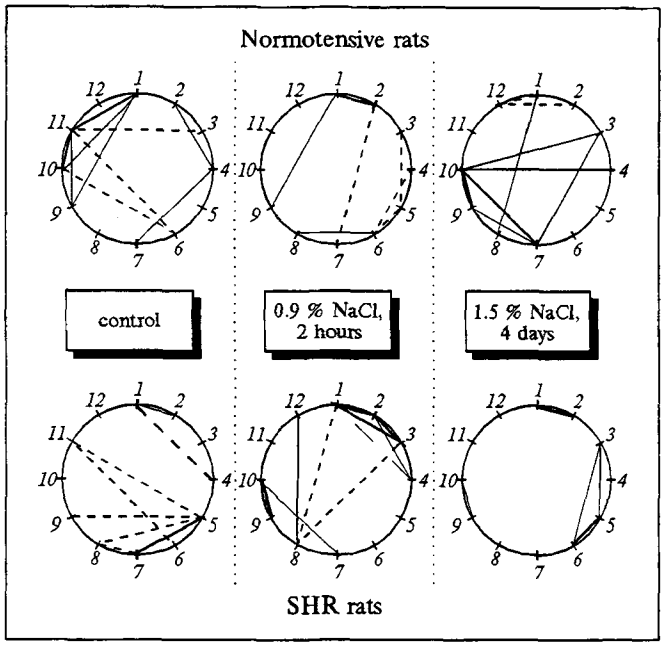

Fig. 3. Correlation matrices. ACC nuclear and nucleolar volumes in the glomerular (*1* and *2*, respectively), bundle (*3* and *4*, respectively), and reticular (*5* and *6*, respectively) zones. Adrenal weight (*7*), aldosterone level (*8*), body weight (*9*), kidney weight (*10*), heart weight (*11*), and arterial pressure (*12*).

edly increased and their density reduced, with the outer membrane lost in some places. The tubules to matrix ratio was changed (tubular area reduced and matrix area appreciably increased). Mitochondria with complete degradation were seen. On the whole, the endoplasmic reticulum looked better preserved than after the 2-h loading, possibly due to of condensation; the number of polysomes was increased. The volumes of ACC nuclei and nucleoli were decreased in all adrenocortical zones of SHR rats. Aldosterone levels were reduced in rats of both strains. No differences in polysomal changes in clear cells were detected between normo- and hypertensive rats. Mitochondrial changes were more expressed in SHR rats, as was seen in the increase of their area and matrix in clear cells and the relative reduction of the tubular area; these changes were less evident in dark cells. Hence, destructive changes of ACC in SHR rats were more pronounced, manifested in mitochondrial edema and destruction, degradation of endoplasmic reticulum fragments, and the formation of numerous free ribosomes and polysomes. The nuclei were less altered, only in some of them the perinuclear space being enlarged.

Correlation analysis revealed moderate connections between ACC nuclear volumes in the glomerular and bundle zones and between nuclear and nucleolar volumes in the reticular zone of normotensive rats in the control (Fig. 3). Heart mass is the prime indicator in them [1], whereas in SHR rats the prime indicator in the control is the ACC nucleolar volume in the reticular zone. This attests to an important role of the reticular zone ACC in the homeostasis of SHR rats. Loading with 0.9% NaCl results in an increase of the number of intraorganic connections in the adrenals of normo- and hypertensive rats as soon as 2 h after loading, but the number of these is particularly increased in SHR rats. This indicates a reaction of all adrenocortical zones to salt loading, all connections being direct and strong in SHR rats, which is characteristic of lesser degrees of freedom, whereas in 3 out of 5 normotensive animals these connections were inverse and moderate. A characteristic feature is the development of a strong connection between the glomerular zone ACC nucleoli and nuclei in normotensive rats, whereas in SHR rats such strong connections, both intra- and interzonal, are detected within the glomerular and bundle zones. A four-day high-salt diet led to the development of new connections, characterized, primarily, by separation of the glomerular zone from the bundle and reticular zones (connections between the nuclei and nucleoli of the glomerular zone, and intra- and interzonal connections in the bundle and reticular zones develop).

ACC hypertrophy in SHR rats is associated with mitochondrial hypertrophy and a change of the ratio of the specific share of mitochondrial tubules to matrix in favor of an increase in the former. Acute water-salt loading of SHR rats resulted in a synchronous decrease of the nuclear and nucleolar volumes in all zones of the cortex, whereas in normotensive rats no changes in the nuclear and nucleolar volumes of bundle zone cells were observed. Edema and destruction of the mitochondria and reticular elements were more strongly expressed in SHR rats. A longer salt loading led to a shrinking of the nuclei and nucleoli in the glomerular zone, whereas in hypertensive rats the nuclei and nucleoli became reduced in size in all zones. Destructive changes of intracellular ACC structures were more pronounced in them as well. It is possible that ACC of rats exposed to permanent functional overstrain (hypertrophy of all zones) are more "fragile" during changes of water-salt homeostasis, in whose regulation they actively participate. Correlation analysis confirmed a decline in the adaptability of SHR rats to changes of water-salt homeostasis: an increase in the number and strength of the connections between ACC structures and a reduction of the degrees of freedom.

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