Na^+-K^+-ADENOSINETRIPHOSPHATASE AND CERTAIN OXIDOREDUCTASES IN THE KIDNEY OF RATS WITH SPONTANEOUS HYPERTENSION

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The activity of Na^+-K^+-adenosinetriphosphatase (Na-K-ATPase), succinate dehydrogenase (SD), lactate dehydrogenase (LD), and glucose-6-phosphate dehydrogenase (G-6-PD) was studied in the cortex and the outer and inner parts of the medulla of the kidneys of rats with spontaneous hypertension (line SHR) and control Wistar rats. No changes in the activity of the above enzymes compared with the control was found in SHR rats aged 6-8 weeks and in the prehypertensive stage. SHR rats at the age of 16-22 weeks, with persistent hypertension, differed from the control rats in their low specific Na-K-ATPase, SD, and LD activity in the tissue of the outer part of the medulla. This difference may be connected with the reduced intensity of energy metabolism and cessation of active sodium transport in the ascending limb of the loop of Henle in the SHR rats and may be responsible for the phenomenon of exaggerated sodium excretion characteristic of hypertension.

KEY WORDS: *Na^+-K^+-adenosinetriphosphatase; spontaneous hypertension; exaggerated sodium excretion.*

Various functional changes suggesting the existence of a special work pattern characteristic of hypertension are found in the nephron, as in the kidney as a whole, in arterial hypertension. These changes are manifested most clearly in the phenomenon of exaggerated sodium excretion in patients with hypertension [3, 11] or in animals with experimental hypertension [10] following intravenous injection of sodium chloride. The concrete mechanism of this exaggerated sodium excretion has not been adequately studied although its explanation could be of great importance to the understanding of the pathogenesis of hypertension itself.

Because of the suggestion that these changes could be connected with changes in sodium transport in the renal tubules, it was decided to study the activity of Na^+-K^+-adenosinetriphosphatase (Na-K-ATPase) in the cortex and medulla of the kidneys of rats with spontaneous genetic hypertension (spontaneously hypertensive rats or SHR),* at present regarded as a model for human essential hypertension. At the same time, the activity of lactate, succinate, and glucose-6-phosphate dehydrogenases (LD, SD, and G-6-PD) in the kidney also was studied.

EXPERIMENTAL METHOD

Inbred male SHR rats (22 animals) were used in the experiments at two age periods:

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TABLE 1. Na-K-ATPase Activity in Kidneys of SHR and Control Inbred Wistar Rats (in μmoles P_i/mg protein/h), M ± m

	Arterial pressure	Specific activity of Na-K-ATPase					
		cortex		outer medulla		inner medulla	
		A	B	A	B	A	B
I. Rats with hypertension	143±5	15,2±0,6	20,0±2,1	35,1±0,6	22,1±1,3	3,3±0,6	2,8±0,2
n		4	10	4	10	4	10
II. Experimental normotensive rats	82±4	17,1±1,3		36,4±1,2		3,8±0,3	
n		8		8		8	
III. Control	83±4	19,1±1,7	25,7±2,2	37,1±1,9	39,9±1,8	2,8±0,5	2,0±0,06
n		4	10	4	10	4	10
P I and III		0,07	0,09	0,4	0,01	0,6	0,7
P I and II		0,25		0,2		0,2	
P II and III		0,4		0,1		0,5	

Note. A) age of rats 6-8 weeks; B) age of rats 16-22 weeks.

6-8 weeks (some of the animals were in the prehypertensive stage and others in the early hypertensive stage) and 16-22 weeks, with persistently high arterial pressure (Table 1). Inbred Wistar rats (from the Stolbovaya nursery) of the same sex and age were used as the control. After removal from their mothers, all the animals were kept under identical conditions. The systolic blood pressure was measured in the caudal artery once a week [2].

The rats were killed by decapitation, the kidneys were decapsulated and divided into segments in the cold, and the inner part of the medulla with the papilla (inner medulla), the outer part (outer medulla), and the cortex were resected. The tissue was frozen in liquid nitrogen, ground in a porcelain mortar, and homogenized in 8 ml of a solution of 0.25 M sucrose, 0.03 M histidine, and 5 mM EDTA (pH 7.3). The homogenate was centrifuged at 1500 g for 10 min and the supernatant was made up to its initial volume and centrifuged at 10,000 g for 30 min. The residue (mitochondrial fraction) was resuspended in 0.03 M Na-phosphate buffer (pH 7.6) and used for the estimation of SD [5]. The supernatant was centrifuged at 105,000 g for 60 min. The residue was resuspended in a solution containing 25 mM imidazole and 12.5 mM histidine (pH 7.3) to the required volume and used for investigation of Na-K-ATPase by Skou's method [9] in the modification described in [2], and LD [8] and G-6-PD [6] were then determined in the supernatant.

To determine total ATPase activity the composition of the incubation samples was as follows: 2.5 mM ATP (Na salt), 5 mM $MgCl_2.6H_2O$, 140 mM NaCl, 20 mM KCl, 0.03 M histidine, volume of sample 3.5 ml. To determine Mg^{++}-ATPase activity, instead of KCl 10^{-4} M ouabain was added. Activity of Na-K-ATPase was determined from the difference between the total ATPase activity and the Mg^{++}-ATPase activity. The reaction in preparations of the cortex and outer medulla was started by the addition of the enzyme preparation, so that the final protein concentration in the sample with the cortex was 25-55 ng, and in the sample with the outer medulla 18-25 ng. The incubation time was chosen so that the linear part of the curve expressing the relationship between Na-K-ATPase activity and incubation time of the samples could be used. The incubation temperature was 46°C. During determination of Na-K-ATPase activity in preparations of the inner medulla (extremely low in this case), KCl was added to all samples (i.e., to the samples for determining total ATPase and Mg^{++}-ATPase). In addition, to increase the level of Na-K-ATPase in preparations of the inner medulla (final protein concentration in the sample 40-60 ng), they were preincubated with NaCl, KCl, $MgCl_2$, and ouabain in the above-mentioned concentrations for 10 min at 37°C. The reaction was started by the addition of 2.5 mM ATP and it continued for 60 min at 46°C. The reaction was stopped by the addition of 0.35 ml cold 50% TCA and the P_i content was

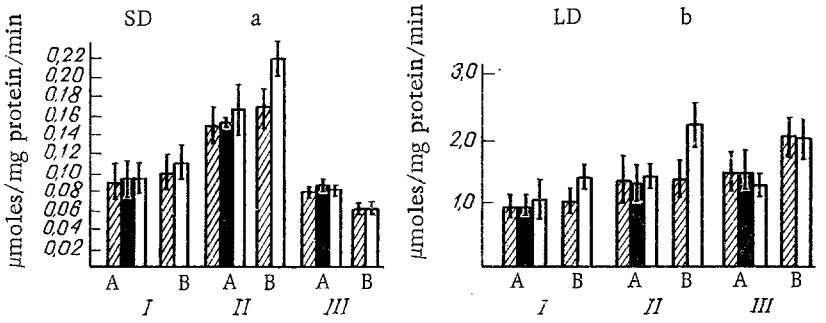

Fig. 1. SD and LD activity in kidneys of SHR rats and in kidneys of control inbred Wistar rats. Age of rats: A) 6-8 weeks; B) 16-22 weeks. I) Cortex; II) outer medulla; III) inner medulla; shaded columns represent rats with hypertension, black columns normotensive rats, unshaded columns control Wistar rats.

determined by the method of Fiske and Subbarow [4] and protein by the method of Lowry et al. [7].

For all enzyme preparations tested (from the cortex and medulla of kidneys from experimental and control rats) optimal reaction conditions were found, for which purpose curves of Na-K-ATPase activity versus Mg^{++} and ATP concentrations and incubation time and temperature were plotted, and the optimal ratio between Na^+ and K^+ ions in the incubation sample also was found. These conditions were the same for all enzyme preparations compared and they are given above.

EXPERIMENTAL RESULTS

As Table 1 shows, the specific Na-K-ATPase activity in all three layers of the kidney of the SHR rats aged 6-8 weeks (with or without hypertension) was essentially indistinguishable from that in the control group. No difference likewise was found in the specific activity of LD and SD (Fig. 1), and also of G-6-PD.

In the SHR rats with persistent hypertension and aged 16-22 weeks distinct differences compared with the control group appeared in the specific Na-K-ATPase, SD, and LD activity, chiefly in the outer part of the medulla, where it was considerably lower than in the control (40% lower for ATPase and LD and 20% lower for SD). In the cortex of the kidneys of the SHR rats, in addition, LD activity was slightly lowered, but no difference in specific G-6-PD activity could be found. The values of G-6-PD activity (in μmoles/mg protein/min) for both age groups, in both the control animals and the SHR rats, were between 0.012 and 0.016 in the cortex, 0.016-0.020 in the outer medulla, and 0.018 and 0.028 in the inner medulla.

SHR rats with persistent hypertension thus differed from the control normotensive Wistar rats in their low specific Na-K-ATPase activity and also their low SD and LD activity in the tissue of the outer part of the medulla, the mass of which consists mainly of the thick segment of the ascending limb of the loop of Henle.

This result probably reflects a decrease in the intensity of energy metabolism and a reduction of the active sodium transport in the epithelium of this segment of the nephron in these animals during the development of persistent hypertension. This view is supported by data showing a decrease in the reabsorption of water and sodium and a decrease in Na-K-ATPase activity in the outer medulla of the intact kidney of rats with hypertension produced by Goldblatt's technique [2] and in rabbits with cerebral ischemic hypertension [1] and it suggests that the changes revealed may be responsible for the exaggerated sodium excretion in response to salt loading in chronic arterial hypertension uncomplicated by renal insufficiency.

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