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Effect of Chronic Salt Loading on Renal Na-K-ATPase Activity in the Rat (41559)

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Abstract. The effect of chronic salt loading in rats fed regular chow diet on renal Na-K-ATPase was studied. The high salt intake was associated with increased filtered load of sodium (control: 126 ± 3.9 μeq/min, salt loaded: 146 ± 2.5, μeq/min, $P < 0.001$), increased net reabsorption of sodium (control: 125.3 ± 3.9 μeq/min, salt load: 134.8 ± 2.4 μeq/min, $P < 0.05$), increased urinary excretion of potassium (control: 2.4 ± 0.09 μeq/min; salt loaded: 3.0 ± 0.1 μeq/min, $P < 0.001$) and increase in single kidney weight (control: 0.798 ± 0.010 g, salt loaded: 0.937 ± 0.015 g, $P < 0.001$). The above mentioned changes were associated with significant increase in renal microsomal and whole homogenate medullary Na-K-ATPase activity in the salt loaded group (microsomes: control 74.1 ± 4.9 μmole P_i/mg prot/hr, salt loaded 112.7 ± 6.0 μmole P_i/mg prot/hr, $P < 0.001$; whole homogenate: control 22.7 ± 1.0 μmole P_i/mg prot/hr, salt load 29.4 ± 1.6 μmole P_i/mg prot/hr, $P < 0.005$), while cortical and papillary Na-K-ATPase activity remained unchanged. Taken together, these results show that increased filtered and reabsorbed load of sodium, which follows high salt intake, is associated with an increased renal Na-K-ATPase activity. The preferential rise in medullary enzymatic activity may be interpreted as suggesting that these changes may stem from increased delivery and reabsorption of sodium in the ascending limb of Henle's loop.

Sodium- and potassium-activated adenosine triphosphatase (Na-K-ATPase) plays a major role in the transport of Na^+ and K^+ across cell membranes (1, 2). There is evidence in support of the concept that Na-K-ATPase mediates at least 50% of the total amount of sodium reabsorbed by the mammalian kidney (3, 4). It has also been shown that the mammalian kidney is capable of modulating the activity of Na-K-ATPase in response to a variety of physiological conditions, e.g., reduction of renal mass (5), potassium loading (6), high protein feeding (5), and changes in the circulating levels of thyroid hormone and adrenal steroids (5, 7-9). Evidence has also been presented to support the concept that the kidney responds to changes in the filtered load of sodium with an adaptive increase in the specific activity of the transport enzyme Na-K-ATPase. This has been observed in the remaining kidney after unilateral nephrectomy (3, 5) but not under direct chronic salt loading of normal rats. In a previous paper (10) we have demonstrated a marked increase in medullary microsomal Na-K-ATPase after chronic salt loading in rats compared to rats on a low salt diet. The low-salt group was fed boiled rice and drank tap water while the high-salt group was fed boiled rice with added salt (50 g/kg dry rice) and drank 1.7% NaCl solution. Because the rice diet was also low in other nutrients including potassium, in addition to being low in sodium, one could argue that the response was not only due to altered sodium content but also due to other factors. Likewise, because the control animals received a low-salt diet, one cannot infer with certainty whether the discrepancy in Na-K-ATPase activity was due to a high salt intake in the experimental or the low salt intake in the control group.

The present study was undertaken to gain a more direct evidence regarding the effect of high salt intake on renal Na-K-ATPase activity. The experimental design was modified so as to eliminate interference by any variables other than high salt intake in the experimental group. The control group consisted of animals fed standard Purina rat chow with tap water, whereas the experimental group received the same diet with a sodium load in the form of salt supplements in the drinking water. The activity of whole homogenate and microsomal Na-K-ATPase was

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compared between the two groups in the cortex, medulla, and papilla of the rat kidney.

Materials and Methods. *In vitro studies.* Male rats of the Hebrew University strain, weighing 200–250 g, were used in all experiments. Each experimental group consisted of kidneys obtained from at least five animals. For each experimental group there was a corresponding control group with an identical number of animals. Both the control and experimental animals were fed the standard Purina rat chow diet. For 11 days the control animals drank tap water and the experimental animals drank 1.7% NaCl solution. On the 12th day the rats were sacrificed by dislocation of the neck, the kidneys were removed immediately, decapsulated, weighed, and kept on ice. Slices of cortex, medulla, and papilla were cut, and pooled separately for the enzyme preparation. The tissue pooled from at least five rats was divided in two parts, one part was processed for preparation of whole homogenate and the other for preparation of microsomes. In the papilla only microsomal Na-K-ATPase was studied.

Preparation of whole homogenate. The tissues were homogenized in 20 vol of a medium containing 0.25 *M* sucrose, 2 m*M* EDTA, and 0.1% desoxycholate buffered with 5 m*M* Tris-HCl to pH 7.4–7.5. The homogenate was kept in ice and assayed the same day.

Preparation of microsomes. Preparation of the microsomal ATPase was carried out essentially according to Jorgensen and Skou (11). The tissues were homogenized in 10 vol of a medium containing 0.25 *M* sucrose, and 2 m*M* EDTA buffered with 5 m*M* Tris-HCl to a pH of 7.4–7.5. The homogenate was centrifuged at 7000*g* for 15 min; the supernatant was decanted and centrifuged at 48,000*g* for 40 min. The pellet was resuspended in an equal volume of the above solution and again homogenized in 10 vol of desoxycholate 0.1% containing 2 m*M* EDTA and 25 m*M* Tris-HCl (pH, 7.0). After incubation at room temperature for 30 min, the suspension was centrifuged at 25,000*g* for 30 min. The pellet was suspended in the above sucrose-EDTA-Tris. This final suspension was frozen at −20°C overnight and assayed the next day.

Assay of ATPase. ATPase activity was determined by the amount of inorganic phosphate (P_i) released during incubation at 37°C in a shaking, thermostatic bath, as previously described (12). All assays were run in duplicates. The P_i released was studied with and without K^+ in the medium. The standard incubation medium consisted of: NaCl 100 m*M*, KCl 10 m*M*, $MgCl_2$ 4 m*M*, ATP 4 m*M*. Enzymatic activity was stopped by the addition of 10% trichloroacetic acid. P_i was determined according to the method of Fiske and Subbarow (13). Enzymatic protein was assayed according to Lowry *et al.* (14). Na-K-ATPase was estimated as the difference of P_i release in the medium without and with K^+.

Metabolic clearance studies. One of the groups studied for Na-K-ATPase was kept in metabolic cages for 12 days. The rats were fed the same diet as described previously. Five control rats received tap water and five experimental rats drank 1.7% NaCl. The rats were weighed daily. Fluid intake and urine output were measured at 24-hr intervals. Blood (0.5 ml) was drawn from the jugular vein three times at 3-day intervals during the experiment, and at the end of 12 days, when the animals were sacrificed, blood was withdrawn from the aorta. There were 4 blood and 11 urine samples for each rat. Sodium and potassium concentrations in serum and urine were determined by flame photometry and creatinine by an automated picric acid method. Creatinine clearance and sodium and potassium excretion rates were calculated. Kidneys were removed and weighed. Water content in kidney slices was determined by drying overnight at 105°C.

Results were pooled for the control and experimental groups over the 12-day period and compared by the student's *t* test.

Results. *Effect of chronic salt loading on whole homogenate Na-K-ATPase and Mg-ATPase.* The effect of chronic salt loading on whole homogenate Na-K-ATPase and Mg-ATPase is shown in Table I. Na-K-ATPase activity increased in the medulla (29.5%; $P < 0.005$), whereas cortical Na-K-ATPase activity was not changed significantly. Whole homogenate Mg-ATPase remained unchanged both in cortex and medulla.

Effect of chronic salt loading on microsomal Na-K-ATPase and Mg-ATPase. The effect of chronic salt loading on microsomal Na-K-ATPase and Mg-ATPase is shown in Table II. Like in the whole homogenate, Na-K-

TABLE I. WHOLE HOMOGENATE Na-K-ATPase AND Mg-ATPase ACTIVITY IN SALT-LOADED AND CONTROL RATS

	Na-K-ATPase (μmole P_i/mg prot/hr)		Mg-ATPase (μmole P_i/mg prot/hr)	
	Control	Salt load	Control	Salt load
Cortex	9.4 ± 0.76 (n = 6)[a]	10.6 ± 0.64 (n = 6)	30.8 ± 1.2 (n = 6)	32.3 ± 0.7 (n = 6)
Medulla	22.7 ± 1.0 (n = 8)	29.4 ± 1.6 (n = 8)	38.1 ± 1.35 (n = 8)	39.9 ± 1.21 (n = 8)
	$P < 0.005$			

[a] n, number of groups studied. Each group consisted of kidneys obtained from five rats.

ATPase activity specifically increased in the medulla (52%, $P < 0.001$) whereas cortical and papillary Na-K-ATPase activity were not changed significantly. Microsomal Mg-ATPase was not changed significantly in all three regions studied.

Effects of chronic salt loading in the rat on body weight, kidney weight, creatinine clearance, plasma concentration, filtered load, and excretion of sodium and potassium. Table III presents creatinine clearance, plasma Na, filtered load of Na, urinary excretion of Na, net reabsorption of Na, plasma K, urinary excretion of K, water intake, and urine volume.

The number of urine collections were 53 instead of 55, and the number of blood samples were 19 instead of 20 because of technical difficulties. Obviously each n number represents the product of the number of animals (total of five in each group), the number of urine collections (a total of 11), and the number of blood samples (four specimens for each animal).

A comparison of several values between the control and salt-loaded group is summarized in Table III. Highly significant difference between the control and experimental group is demonstrated for fluid intake, urine volumes, excretion rates of sodium and potassium, creatinine clearance, and filtered load of sodium. The difference in net sodium reabsorption was also significant. There was no significant difference in plasma sodium and potassium concentrations between the two groups. There was a significant increase in kidney weight between the control and salt-loaded group: 0.798 ± 0.01 g/kidney (n = 18) in the control group and 0.937 ± 0.015 g/kidney in the experimental group (n = 18), $P < 0.001$. Water content did not change in kidneys of the control and experimental groups; control 74.8 ± 0.25 (n = 8), salt load 74.4 ± 0.39 (n = 8), not sig-

TABLE II. MICROSOMAL Na-K-ATPase AND Mg-ATPase ACTIVITY IN SALT-LOADED AND CONTROL RATS

	Na-K-ATPase (μmole P_i/mg prot/hr)		Mg-ATPase (μmole P_i/mg prot/hr)	
	Control	Salt load	Control	Salt load
Cortex	32.4 ± 4.2 (n = 6)[a]	33.0 ± 1.3 (n = 6)	52.9 ± 4.6 (n = 6)	52.3 ± 4.09 (n = 6)
Medulla	74.1 ± 4.9 (n = 7)	112.7 ± 5.98 (n = 7)	60.4 ± 5.9 (n = 7)	68.7 ± 7.1 (n = 7)
	$P < 0.001$			
Papilla	40.2 ± 5.3 (n = 4)	35.8 ± 3.8 (n = 4)	44.0 ± 9.8 (n = 4)	44.0 ± 9.6 (n = 4)

[a] n, number of groups studied. Each group consisted of kidneys obtained from at least five rats.

TABLE III. CREATININE CLEARANCES, PLASMA CONCENTRATIONS, URINARY EXCRETION, AND REABSORPTION OF SODIUM AND POTASSIUM WATER INTAKES, AND URINE VOLUMES IN SALT-LOADED AND CONTROL RATS

	Control	Salt load	P^b
Water intake (ml/24 hr)	27.4 ± 0.95 (n = 55)[a]	70.4 ± 3.1 (n = 55)	<0.001
Urine volume (ml/24 hr)	10.4 ± 0.42 (n = 54)	53.0 ± 2.7 (n = 53)	<0.001
Creatinine clearance (ml/min)	0.87 ± 0.027 (n = 54)	1.01 ± 0.017 (n = 53)	<0.001
Plasma sodium (meq/liter)	145 ± 0.86 (n = 19)	145 ± 0.99 (n = 19)	n.s.
Filtered load of sodium (μeq/min)	126.2 ± 3.9 (n = 53)	146.5 ± 2.5 (n = 53)	<0.001
Urinary excretion of sodium (μeq/min)	0.76 ± 0.04 (n = 53)	11.9 ± 0.58 (n = 53)	<0.001
Net reabsorption of sodium (μeq/min)	125 ± 3.9 (n = 53)	134.8 ± 2.4 (n = 53)	<0.05
Plasma potassium (meq/liter)	4.1 ± 0.09 (n = 19)	4.2 ± 0.065 (n = 18)	n.s.
Urinary excretion of potassium (μeq/min)	2.43 ± 0.094 (n = 53)	3.03 ± 0.104 (n = 53)	<0.001

[a] n, total number of daily determinations in five rats.
[b] P, significance between control and experimental groups.

nificant. Animal weights did not differ significantly when compared in the beginning and end of the experiment. When the change in weight over the 12-day period was compared, the difference did not reach significance but it seemed that the salt-loaded rats gained less weight than the controls. The change in body weight over the 12-day period was 44.8 ± 6.6 g in the control group and 29.6 ± 4.3 g in the experimental group (n = 5) for both groups, P not significant.

Discussion. A variety of maneuvers, e.g., unilateral nephrectomy (5), high-protein feeding (5), administration of glucocorticoids (8) and of thyroid hormone (9, 15) are known to stimulate the activity of Na–K-ATPase. Since these maneuvers are associated with an increase in glomerular filtration rate (GFR) and tubular reabsorption of sodium, it has been suggested that the filtered load of sodium may be of critical importance in the adaptive mechanisms responsible for enzyme activation (3, 5, 15). The results reported in the present communication demonstrate an increase in crude homogenate and microsomal renal Na–K-ATPase in response to oral salt load, which was given for 12 consecutive days. The increase in enzyme activity was found in medullary but neither in cortical nor in papillary Na–K-ATPase. Mg-ATPase remained unchanged in all the kidney regions studied both in crude homogenate and microsomes. In our previous study chronic salt load was associated not only with a rise in Na–K-ATPase in the medulla but also in the papilla. Those results were at variance with our present observations which have failed to show a change in microsomal Na–K-ATPase activity in the papilla. This discrepancy, however, could be reconciled due to the difference in the methods used in each of the two experimental protocols, whereas in our previous study the animals were fed a rice diet which was not only deficient in Na but also in K^+ and other nutrients. In the present study the animals were fed regular pellet chow diet.

The relatively high Na–K-ATPase activity in microsomes of the papilla has already been

reported by us in three previous publications (20–22). Because the tissue of the papilla consists mainly of collecting tubules it seems that the activity measured by us represents mainly Na–K-ATPase activity in this segment.

The clearance studies (Table III) show significant increase in GFR, filtered load, and net tubular reabsorption of sodium in the salt-loaded group. These observations strongly support the possibility that the increase in enzyme activity was due to an augmented filtered and reabsorptive load of sodium. During acute saline load, decreased proximal reabsorption of sodium has been documented (16). If this is also the case during chronic salt loading then it follows that the bulk of filtered sodium is delivered to the distal nephron, possibly to the ascending limb of the loop of Henle, where, due to the high transport capacity, most of the reabsorptive process is taking place. The above segmental formulation is compatible with the presently observed increase in the medullary Na–K-ATPase which accordingly may reflect an increase in the enzyme activity in the ascending limb of loop of Henle. The increase in GFR in salt-loaded rats is not readily explicable by the available data. Naturally one would expect that a chronic salt load would produce a state of extracellular volume expansion with increased cardiac output and renal blood flow. The absence of direct support for this possibility, such as an increase in body weight, makes this suggestion conjectural at this time. Furthermore, surprisingly the salt-loaded animals revealed a decrease rather than an increase in body weight, probably reflecting a relative decrease in lean body weight.

The increase in absolute sodium reabsorption was associated with an increase in potassium excretion (Table III). The increase in urinary excretion of potassium was observed during chronic salt loading despite the presumable fall in plasma renin and aldosterone levels. This could reflect the operation of intratubular factors mainly, which are known to augment potassium excretion. Among them one may include: increased distal urine flow and increased distal delivery of sodium. The observed increase in the kidney weight in the salt-loaded rats can be used to draw an analogy with the hypertrophy of the residual kidney after unilateral or subtotal nephrectomy, which is also associated with an increase in renal Na–K-ATPase activity (18). This is further substantiated by the observation that there was no change in water content. Thus it can be concluded that there was an increase in the dry weight of the kidneys of salt-loaded animals.

Jacobson *et al.* (18) have recently demonstrated specific increase in medullary but not cortical Na–K-ATPase in rat kidney after reduction of renal mass, which may be considered to be a similar stimulus to salt loading. Their conclusion was that reduction in renal mass resulted in an increased number in pumping sites. Stewart *et al.* (19) have demonstrated activation of Na–K-ATPase of the salt gland of the duck in response to salt loading which also appears to represent an increase in the number of enzyme units.

Our present results cannot allow us to arrive at any definite conclusions regarding the exact nature of the change in the enzyme activity, but they conclusively show that chronic salt loading causes an increase in medullary Na–K-ATPase activity.

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