Renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in Okamoto and Dahl Hypertensive Rats

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SUMMARY Plasma renin activity (PRA, ng Al/ml/hr), plasma aldosterone (PA, ng%) and renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (μm PO\textsubscript{4}/mg protein/hr) were measured in two groups of eight spontaneously hypertensive rats (SHR), two groups of eight Dahl salt hypertensive rats (SS), and their controls (16 normal Wistar and 16 salt-resistant rats). Measurements were made in one group after 2 weeks on a normal (0.48% sodium) and in the other group after 2 weeks on a low (0.01% sodium) sodium diet. After a normal sodium diet, PRA and PA were lower in both groups of hypertensive rats than in control normotensive animals. Renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase was lower in SS than in controls; in SHR it was not different from control. On a sodium-free diet, SHR exhibited a rise in renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase but PRA and PA remained low. In contrast, under similar conditions PRA, PA, and renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase increased in SS rats, although to a lesser extent than in SHR. These results suggest that under basal conditions and after low salt diet, renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in SHR behaves as it does in normal rats. However, the changes are independent of PA in SHR. The reduction in PRA and PA in SS suggests volume expansion hypertension. In SHR, volume expansion is not present, and renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is not altered. Enzyme activity is lower in SS than in SHR and control. This suggests that some factor that results from volume expansion may be responsible for inhibition of renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase.

KEY WORDS • renal ATPase activity • enzyme • Okamoto rat • Dahl rat

STUDIES of the renin-angiotensin system in Dahl salt-sensitive (SS) and Okamoto-Aoki spontaneously hypertensive rats (SHR) have produced conflicting results. Most studies, however, indicate that during sustained hypertension plasma renin activity (PRA) is low in both strains. This hyporeninemic state could result from expanded extracellular fluid volume; however, plasma volume measurements in SS and SHR on a normal salt diet have not been different from normal. Despite this, small but significant changes in extracellular fluid volume may be present which are undetectable by current methodology. This assumption is supported by the results of studies of acute intragastric saline loading studies which have demonstrated an exaggerated natriuretic response in these models of experimental hypertension.

The mechanism of exaggerated natriuresis in these rats could relate in part to low plasma aldosterone as a result of a hyporeninemic state. Results supporting this hypothesis have been reported in SHR. Another possibility is that the expansion of extracellular fluid volume and the low plasma aldosterone concentration could lead to reduced activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, and this could be a factor directly involved in the exaggerated natriuresis. In support of this it has been demonstrated that renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity may be regulated by mineralocorticoids. Decreased renal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity has been described in adrenalectomized rats and in rats treated with an aldosterone antagonist. Administration of aldosterone restores Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in adrenalectomized rats. In addition, volume expansion, independent of mineralocorticoid levels, may result in decreased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in several tissues, although an influence of volume status on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in the kidney has not been clearly shown.
Materials and Methods

All experiments were conducted in adult rats (14 to 16 weeks old) weighing 200 to 250 g. Each rat was kept in a metabolic cage housed in a room at an ambient temperature of 26°C. Two strains of hypertensive rats and their controls were selected for the study: Okamoto (SHR) and normotensive Wistar rats (NR). Blood pressure averages were as follows: SHR 166 ± 4, NR 112 ± 4, SS 170 ± 6, and SR 116 ± 3 mm Hg. The Okamoto rats were bred in our laboratories from the original strain obtained from the National Institutes of Health; Dahl rats were obtained from Brookhaven National Laboratory in Upton, New York. From each strain two groups of eight rats each were studied. Group 1 was fed a normal sodium diet (Labchow, Ralston Purina, 0.48% sodium), and Group 2 was fed a sodium deficient diet (ICN Biochemicals, 0.01% sodium). All diets and tap water were fed ad libitum. On the twelfth day of the balance study, 1 ml of blood was drawn from each rat by heart puncture. Plasma renin activity (PRA) and plasma aldosterone (PA) were measured by radioimmunoassay. 12 13

The following day plasma volume and red cell volume were determined in the pentobarbital anesthetized rat by radiodisotopic dilution techniques (as described below). Upon completion of these studies the kidneys of each rat were removed for the isolation of Na+-K+-ATPase. Enzyme activity was determined in microsomal fractions (see below). The data were analyzed by Student's t test. Urinary concentrations of sodium and potassium was determined by flame photometry.

Blood Volume Determinations

Blood was drawn from normal, ether-anesthetized rats by heart puncture into a syringe containing small amounts of ACD solution. The blood was then mixed with an equal volume of ACD solution and gently stirred. The sample was centrifuged and the plasma obtained was mixed with 5 μCi of 125I-RHSA. A 0.5 ml sample of this mixture was added to a preweighed syringe, which was then reweighed. The contents of this syringe were emptied into a counting vial for use as a reference standard. Another 0.5 ml of the plasma solution was then added to the same syringe and weighed.

Ten μCi of 51Cr was added to red cells treated with ACD solution. The mixture was incubated for 30 minutes at room temperature. The red cells were washed three times with isotonic saline, after which equal volumes of red cells and isotonic saline were mixed. A 0.5 ml sample of the red cell suspension was drawn into a preweighed 1 ml syringe. The syringe was reweighed and then emptied into a counting vial. An additional 0.5 ml of the red cell suspension was added to the same syringe and weighed. Cannulas were introduced into a femoral vein and artery of rats anesthetized with sodium pentobarbital (40 mg/kg). The 51Cr-tagged red cells and 125I-albumin plasma (0.5 ml) were injected through the femoral vein catheter after which 1 ml blood samples were drawn every 10 minutes for a 30-minute period. Hematocrit was determined for all samples. Upon centrifugation, radioactive counts were measured in plasma. Radioactive counts were extrapolated to zero time to obtain plasma volume and red cell volumes, which were determined for each 10-minute interval.

Isolation of Na+-K+-ATPase

The kidneys of each rat were removed and immediately flushed with an ice cold buffer solution containing 0.25M sucrose, 30 mM histidine, and 5 mM disodium EDTA (Sigma Chemical Company) at a pH of 7.4. Kidneys were homogenized in an ice-cold solution (15 ml) of the following composition: 0.25 M sucrose, 30 mM histidine, 5 mM disodium EDTA, and 0.2% deoxycholic acid (Sigma Chemical Company). All subsequent procedures were carried out at 4°C in a cold room. Pre-chilled scissors were used to finely mince the kidneys of each rat. Homogenates of tissue suspensions were obtained by the use of a polytron (Brinkman Instruments) at a rheostat setting of 2. Each sample was homogenized for five 10-second periods, at intervals of 10 seconds. Upon dissipation of the foam layer, samples were filtered through four, then eight layers of cheesecloth. The resulting homogenates were centrifuged at 10,000 X g for 20 minutes at 4°C (Dupont Instruments, Sorvall RC-5). Afterward, the supernatant fluid was decanted, with care taken to avoid contamination by the pellets that were discarded. The supernatants were then centrifuged at 105,000 X g for 90 minutes at 4°C in a preparative ultracentrifuge (Beckman Instruments). The resulting supernatants were discarded. Pellets were resuspended 1:1 (wt/vol) in the solution containing 0.25 M sucrose, 30 mM histidine, and 5 mM disodium EDTA. Each suspension was then homogenized with a tissue homogenizer. Samples were stored immediately at −20°C. All assays were performed within 1 week.

Na+-K+-ATPase Assay

All assay tubes contained a final concentration of 100 mM NaCl, 10 mM KCl, 6 mM MgCl, 10 mM imidazole-HCl buffer (pH 7.0), and 6 mM disodium ATP, in a final assay volume of 5 ml. ATP was added to the tubes during the assay. Sodium and potassium were omitted from all tubes in which all ouabain-insensitive activity was to be measured, because rat renal Na+-K+-ATPase is markedly resistant to inhibition by cardiac glycosides. Each rack of tubes was placed in a shaker bath at 37°C for 10 minutes prior to the assay. Aliquots of enzyme isolated by above procedure were added (0.1 ml) at 15-second intervals to tubes in which total and ouabain-insensitive activity were measured. ATP was then added to all tubes. The mixtures were allowed to react for 10 minutes, after which 1 ml of cold 30% trichloroacetic acid was added to each tube to stop the reaction. The tubes were quickly agitated and placed on ice for 10 minutes and then centrifuged at 2000 rpm (4°C) for 10 minutes.
Total ATPase activity (magnesium-Na\(^+\) and K\(^+\) dependent) and ouabain-insensitive ATPase activity were determined for each sample. Inorganic phosphate concentration was determined in 1 ml of the resulting supernatants, according to the method of Fiske and Subbarow.\(^{14}\) The values for magnesium-dependent ATPase activity and ouabain-insensitive ATPase activity were then calculated as micromoles of inorganic phosphate generated per milligram of protein per hour (\(\mu\)mole PO\(_4\)/mg protein/hr). Protein concentration of each sample was measured by the method of Lowry et al.\(^{15}\) Na\(^+-K\(^+-\)ATPase activity was expressed as the difference between total activity (magnesium-dependent) and ouabain-insensitive activity.

**Results**

The data from balance studies are summarized in table 1. On both normal and low salt diets, the urinary excretion of sodium and potassium was similar in SHR and NR. Sodium intake (not shown) was also similar in both groups. The SS rats, however, excreted significantly more sodium during adaptation to low salt intake than the SR controls. In addition, sodium intake was less in SS than in SR. These data suggest a greater degree of volume contraction in SS than in SR during sodium restriction. Plasma volumes reflected the level of dietary sodium intake as well as the difference in sodium excretion observed between SS and SR during sodium restriction. On a normal salt intake, plasma volume in SHR and NR was not significantly different (SHR 3.31 ± 0.10; NR 3.43 ± 0.10, ml/100 g body weight); plasma volume decreased to the same extent in both groups during low salt intake (SHR 2.86 ± 0.11; NR 3.01 ± 0.24, ml/100 g body weight). Plasma volume was also similar in SS and SR during normal salt intake (SS 4.3 ± 0.11; SR 4.6 ± 0.34, ml/100 g body weight). However, the decrease in plasma volume following sodium restriction was greater in SS than in SR (SS 3.0 ± 0.30; SR 4.2 ± 0.12, ml/100 g body weight), demonstrating greater volume contraction in SS than in SR in response to sodium deprivation. Red cell volume did not vary with the level of sodium intake and was similar in all four groups.

**TABLE 1. Sodium and Potassium Excretions (mEq/24 hrs) of Spontaneously Hypertensive Rats (SHR) and Their Control, Normotensive Rats (NR); and Dahl Salt-Sensitive Rats (SS) and Their Respective Control, Dahl Salt-Resistant Rats (SR), on a Normal and Low Salt Intake**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Normal Na(^+) intake</th>
<th>Low Na(^+) intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(U_{Na^+})</td>
<td>(U_{K^+})</td>
</tr>
<tr>
<td>SHR</td>
<td>2.2 ± 0.21</td>
<td>3.5 ± 0.33</td>
</tr>
<tr>
<td>NR</td>
<td>2.4 ± 0.17</td>
<td>3.0 ± 0.29</td>
</tr>
<tr>
<td>SS</td>
<td>1.0 ± 0.18</td>
<td>2.2 ± 0.34</td>
</tr>
<tr>
<td>SR</td>
<td>1.1 ± 0.10</td>
<td>2.1 ± 0.12</td>
</tr>
</tbody>
</table>

Values represent the means ± SE of a 5-day balance study. Asterisk denotes significance (\(p < 0.01\)) when compared to corresponding control values.

Despite normal plasma volume in both strains of hypertensive rats during normal salt intake, PRA was significantly lower in SHR and SS than in their respective controls (figs. 1 and 2). During low salt intake, PRA increased in NR but not in SHR, despite a fall in plasma volume after sodium restriction in SHR. PRA increased in both SS and SR during low salt intake, although the rise in plasma renin was not significantly different in the two strains.
FIGURE 3. Plasma aldosterone levels were lower in spontaneously hypertensive rats (SHR) than in normotensive rats (NR) on a normal salt intake and did not increase in SHR with sodium restriction (●—● NR, ○--○ SHR). Asterisk denotes significance when compared to corresponding control value.

FIGURE 4. Both groups showed increased plasma aldosterone (PA) levels following sodium restriction. However, regardless of dietary sodium intake, PA was lower in salt-sensitive (SS) than in salt-resistant (SR) rats (●—● NR, ○--○ SR, ○--○ SS). Asterisks denote significance when compared to corresponding control value.

blunted in SS. Changes in PA concentration reflected those in PRA in all four groups of rats (figs. 3 and 4). Aldosterone levels were lower in SHR than in NR on a normal salt intake, and sodium restriction increased PA in NR, but not in SHR. Aldosterone levels were also lower in SS than in SR during normal salt intake. During sodium deprivation PA increased in SS, although not to the same extent as in SR.

Renal Na⁺-K⁺-ATPase activity in SHR was not different from that observed in NR (fig. 5), indicating no correlation in SHR with the changes in PRA and PA. Enzyme activity increased to the same extent in SHR and NR in response to sodium restriction. In contrast, enzyme activity in both SS and SR paralleled PA concentration (fig. 6). Na⁺-K⁺-ATPase was lower in SS than in SR on a normal salt intake and increased during sodium restriction, although this response was less in SS than in SR.

Discussion

These studies demonstrate that low PRA and PA are not associated with an increase in plasma volume in adult SHR and SS. In SHR, the levels of PRA and PA remained persistently suppressed after chronic sodium restriction despite a decrease in plasma volume. In contrast, we observed a significant increase in PRA and PA in SS after chronic sodium restriction, although PRA was lower in SS than in SR on any given dietary sodium intake. It is clear, then, that the PRA and PA response to volume contraction is
different in both strains. In SS, the response is a function of the volume status while this does not seem to be the case in SHR. These results suggest that SS is a model of hypertension associated with expansion of the extracellular fluid volume. The mechanism regulating PRA in SHR is not clear from these studies. However, studies in our laboratory suggest that enhanced prostaglandin synthesis may be an important factor in the suppression of renin release in SHR.

The mechanism of enhanced natriuresis observed after salt loading in SHR and SS could relate to reduced aldosterone secretion. Low aldosterone could lead to exaggerated natriuresis, by reducing the activity of renal Na+-K+-ATPase. Data from various laboratories have indicated that aldosterone plays an important part in the regulation of the activity of renal Na+-K+-ATPase. Aldosterone increases or restores the activity of the enzyme in the microsomal fraction of kidneys and isolated nephron segments obtained from adrenalecrtomized rats. 17

In the present studies in SS rats, changes in plasma aldosterone were always paralleled by changes, in the same direction, of renal Na+-K+-ATPase activity. Furthermore, in control rats there was a clear-cut relationship between PA and renal Na+-K+-ATPase. On the other hand, in SHR changes in renal Na+-K+-ATPase were independent of plasma aldosterone levels but followed the fall in extracellular fluid volume. These results indicate that the regulation of Na+-K+-ATPase activity in SHR may be volume-related but independent of aldosterone levels.

Among the physiological variables which are known to modulate Na+-K+-ATPase in the mammalian kidneys are: reduction of renal mass, high protein feeding and changes in circulating levels of certain hormones like thyroid hormone and adrenal steroids. None of these factors except aldosterone was clearly altered in our experiments. In addition, aldosterone concentration was actually low on both levels of salt intake in SHR. We are therefore inclined to believe that there is a modulator of Na+-K+-ATPase which may be related to volume changes in SHR.

Basal renal Na+-K+-ATPase activity in adult Dahl hypertensive rats was lower than that observed in controls and SHR. Moreover, the rise in Na+-K+-ATPase activity in response to sodium restriction was blunted as compared to SHR and SR. The rise in plasma aldosterone after salt restriction correlated with the increase in Na+-K+-ATPase. Aldosterone levels may play a role in the regulation of renal Na+-K+-ATPase in Dahl hypertensive rats.

Recent evidence suggests that Na+-K+-ATPase activity is suppressed in animals with various models of low renin hypertension in which extracellular fluid volume is expanded. Suppressed cardiac microsomal ATPase activity has been described in one kidney-one clip, one kidney-DOCA with salt, reduced renal mass, acute renopirval and Dahl salt sensitive models. Parallel studies of ATPase activity in renal tissue, however, have not been reported. The present studies indicate that a decrease in renal Na+-K+-ATPase accompanies reduced cardiac activity of the enzyme.

The present studies are compatible with the suggestion that a circulating inhibitor of renal Na+-K+-ATPase is present as a result of volume expansion in SS. This, in addition to aldosterone, might regulate enzyme activity in this strain. In SHR, in view of the lack of correlation between Na+-K+-ATPase and aldosterone, the circulating inhibitor may play a major role in regulation of the enzyme.

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