Na,K-ATPase in Isolated Nephron Segments in Rats With Experimental Heart Failure

Hanna Wald, Pnina Scherzer, and Mordecai M. Popovtzer

To characterize renal transport of Na⁺ in heart failure, urinary Na⁺ excretion (UNaV), aldosterone levels, and Na,K-ATPase activity in isolated nephron segments were determined in three groups: control rats, rats with heart failure and moderate sodium retention, and rats with heart failure and severe sodium retention. Heart failure was induced by a fistula between the aorta and vena cava. For the control group, UNaV was 0.66±0.04 (mean±SEM) μeq/min, and aldosterone was 18.4±3.5 ng%. Na,K-ATPase activity (in 10⁻¹¹ mol/mm/min) was 28.4±1.1 in the proximal convoluted tubule, 23.3±1.0 in the proximal straight tubule, 37.4±1.9 in the medullary thick ascending limb, 40.2±1.9 in the cortical thick ascending limb, 43.2±2.2 in the distal convoluted tubule, and 20.5±0.9 in the cortical collecting duct. For the group with moderate heart failure, UNaV was 0.35±0.02 (p<0.001 versus control), and aldosterone was 15.9±4.4 (p=NS versus control). Na,K-ATPase activity was unchanged in the proximal convoluted tubule, proximal straight tubule, medullary thick ascending limb, and cortical collecting duct, but it increased in the cortical thick ascending limb to 57.7±3.1 (p<0.001 versus control) and decreased in the distal convoluted tubule to 35.3±1.2 (p<0.005 versus control). For the group with severe heart failure, UNaV was 0.029±0.016 (p<0.001 versus control), and aldosterone was 186.0±14.8 (p<0.001 versus control). Na,K-ATPase activity increased in the proximal convoluted tubule and proximal straight tubule to 36.6±1.5 and 40.7±1.8, respectively (p<0.001 versus control for both). Na,K-ATPase activity decreased in the medullary and cortical thick ascending limbs and distal convoluted tubule to 21.9±3.4, 23.1±4.5, and 24.0±1.5, respectively (p<0.001 versus control for all). Angiotensin II (10⁻¹⁰ M) increased Na,K-ATPase activity in proximal convoluted and straight tubules from control values of 27.1±0.4 and 23.3±1.7 to 34.4±1.8 (p<0.001) and 36.0±1.6 (p<0.001), respectively. These results show that, in rats with heart failure, Na,K-ATPase varies with the severity of Na⁺ retention. In severe heart failure the increased Na,K-ATPase activity in the proximal nephron may be related to increased angiotensin II levels. (Circulation Research 1991;68:1051–1058)

Congestive heart failure is associated with sodium retention and edema formation. The site of altered sodium reabsorption along the nephron during congestive heart failure is not well defined. In the experimental model of high-output heart failure produced by an aorta to vena cava (A-V) fistula, Stumpe et al.¹ using a micropuncture technique, demonstrated that the site of increased sodium reabsorption is located in the thick ascending limb of Henle’s loop. In earlier studies Johnston et al.² suggested that enhanced reabsorption of sodium by the proximal tubule of dogs could be an important factor causing chronic sodium retention during congestive heart failure. These discrepant findings do not necessarily reflect contradictory results but may bear on heterogeneity in patterns of active tubular sodium reabsorption in congestive heart failure. It is quite possible that more than one tubular site participates in this process and that varying degrees of cardiac insufficiency may determine the location within the nephron in which sodium reabsorption is most augmented. The purpose of our present study was therefore to produce an experimental model of cardiac insufficiency with a varying degree of severity of heart failure. Winaver et al.³ noticed that rats with cardiac insufficiency induced by A-V fistula fall roughly into two subgroups with regard to the degree of cardiac function impairment: first, animals with mild to moderate cardiac insufficiency, and second, animals with severe heart failure. This observation, therefore, forms the basis for our experimental design.

Thus, the present study was undertaken to characterize the effect of different degrees of congestive

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heart failure on kidney function, renal handling of sodium, and Na,K-ATPase activity in six distinct nephron segments, since Na,K-ATPase activity is one of the major determinants of active sodium transport.

**Materials and Methods**

Studies were performed using male Hebrew University rats weighing 200–230 g. Both the control and experimental animals were fed a standard Purina rat chow diet containing 100 meq/kg Na⁺ and drank tap water ad libitum.

Rats were placed in metabolic cages for acclimatization. Twenty-four hours before the operation, baseline renal function and sodium excretion rates (U_{Na}V) were determined.

Under pentobarbital anesthesia (50 mg/kg) a 1.0–1.2-mm elliptical A-V fistula was placed approximately 10 mm distal to the origin of the renal arteries by using a microscope (American Optical) according to the method described in detail by Stumpe et al.[1] Control rats underwent sham operation. The fistula size was measured by an eyepiece micrometer located in the ocular of the microscope. After recovery from anesthesia the rats were returned to the metabolic cages, and kidney function and sodium handling were monitored for 3 weeks. The rats with A-V fistulas exhibited two U_{Na}V patterns. Rats with moderate heart failure with heart weights of less than 1 g and no ascites, pleural effusion, or peripheral edema excreted about 50% sodium compared with sham-operated rats. Rats with severe heart failure with heart weights equal to or above 1 g with marked ascites, pleural effusion, and peripheral edema excreted less than 5% sodium compared with sham-operated rats.

Rats with moderate heart failure survived for more than 1 month, but their kidneys were processed for Na,K-ATPase determination at 9 days after A-V fistula formation. Rats with severe heart failure did not survive more than 10 days, and their kidneys were processed for Na,K-ATPase determination at 8–9 days after A-V fistula formation.

Kidneys of sham-operated rats were processed for Na,K-ATPase determination 9 days after sham operation.

After the above-stated postoperation periods, the rats were killed by bleeding through the descending aorta under light ether anesthesia, and kidneys were removed, microdissected, and prepared for Na,K-ATPase determination.

In a separate experiment five control rats and five rats that were starved for 6 days with free access to water were studied for their ATPase activity.

**Microdissection**

Sagittal slices (0.5–1.0 mm thick) were cut and immediately immersed in dissection fluid ([mM] NaCl 136, KCl 3, K₂HPO₄ 1, MgSO₄ 1.2, CaCl₂ 2, sodium lactate 4, sodium citrate 1, L-alanine 6, glucose 5.5) plus 0.6% collagenase (145 units/mg; Millipore Corp., Freehold, N.J.). The slices were incubated for 10–12 minutes in a shaking water bath at 37°C and aerated with 100% oxygen. The slices were then removed and rinsed twice in ice-cold pure dissection fluid. Cortical semicircles cut at the corticomedullary border and medullary triangles including the papilla were used to obtain the appropriate segments. The slices were transferred to a Petri dish with dissection fluid and inserted in a cooled Lucite chamber illuminated by a transmission dark-field source as described by Schmidt and Horster.[4] Microdissection was performed as described by Burg et al[5] and modified by Schmidt and Horster.[6]

The length of each segment was measured by an eyepiece micrometer. The segment was rinsed in clean dissection fluid and transferred to a glass ampule for analysis. The tubules were analyzed in their fresh form after freezing at −20°C for 24 or 48 hours.

The distal convoluted tubule (DC) that was dissected included portions of the connecting tubule, and its length was 0.3–0.6 mm. The DC segment was dissected according to its localization peripheral to the corresponding glomerulus toward the renal surface at the continuation of the thick ascending limb. Through the microscope, this segment appeared whiter and wider in its diameter than did the proximal convoluted tubule (PC). DC looked granular and had fewer convolutions compared with the PC segment.

The following cortical and medullary segments were dissected and assayed for their ATPase activity: PC 0.3–0.6 mm, cortical proximal straight tubule (PS) 0.5–1.0 mm, medullary thick ascending limb (MTAL) 0.3–1.0 mm, cortical thick ascending limb

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### Table 1. Metabolic Data for Control Rats and Rats With Moderate or Severe Heart Failure After 9 Days

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Moderate heart failure (group b) (n=10)</th>
<th>Severe heart failure (group a) (n=8)</th>
<th>Diuresis (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/day)</td>
<td>19.4±0.9</td>
<td>21.7±1.8</td>
<td>1.2±0.8*</td>
<td></td>
</tr>
<tr>
<td>Fluid intake (ml/day)</td>
<td>30.4±1.3</td>
<td>32.7±2.6</td>
<td>13±3.2*</td>
<td></td>
</tr>
<tr>
<td>Final body wt (g)</td>
<td>248±6.6</td>
<td>259±5.6</td>
<td>214±10†</td>
<td></td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td>0.74±0.01</td>
<td>0.82±0.06</td>
<td>1.10±0.05*</td>
<td></td>
</tr>
<tr>
<td>Fistula size (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuresis (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. U_{Na}V, urinary Na⁺ excretion rate; U_{K}V, urinary K⁺ excretion rate; P_{Na}, plasma Na⁺ concentration; P_{K}, plasma K⁺ concentration; P_{ald}, plasma aldosterone level.

*p<0.001 compared with control.

†p<0.02 compared with control.
(CTAL) 0.3–0.8 mm, DC 0.3–0.6 mm, and cortical collecting duct (CCD) 0.7–2.0 mm.

In starved rats, only the PC and MTAL were studied for their ATPase activities. In an additional experiment the effect of angiotensin II added in vitro to the incubation medium at a final concentration of $10^{-10}$ M on the activity of PC and PS Na,K-ATPase and Mg-ATPase was studied.

**Determination of ATPase**

The method and apparatus for ATPase determination was previously described in detail. Briefly, the method is based on a micromodification by Czaczkes et al. The hydrolysis of ATP by ATPase is coupled to the transformation of phosphoenolpyruvate to pyruvate by pyruvate kinase. Pyruvate is reduced in lactate in the presence of lactate dehydrogenase. NADH acts as the oxygen acceptor and is oxidized to NAD$^+$. There exists a stoichiometric relation between the hydrolysis of ATP and the disappearance of NADH (by oxidation to NAD$^+$). This disappearance can be monitored fluorometrically. In experiments for which the effect of angiotensin II was studied in vitro, angiotensin II was added to the tubular segment 15 minutes before initiation of the reaction at 37°C. The reaction was started by adding the mixture of electrolytes, substrates, and enzymes and proceeded for another 15 minutes.

The assay solution contained (mM) NaCl 80, NH$_4$Cl 53, MgCl$_2$ 2.7, imidazole 40, NAAD 0.06, ATP (vanadium free, Sigma Chemical Co., St. Louis) 0.42, and phosphoenolpyruvate 0.083, as well as pyruvate kinase 6 units/assay and lactate dehydrogenase 6 units/assay; osmolarity 294 mosm/kg and pH 7.4.

Na,K-ATPase was calculated by the difference between total ATPase and Mg-ATPase. The latter was determined by the addition of ouabain (strophanthin-G) to a final concentration of 4 mM.

Na,K-ATPase activity was compared between control rats and rats with severe (group a) and moderate (group b) heart failure.

Blood and urine samples were analyzed for creatinine, sodium, and potassium. The creatinine concentration was determined by an automated picric acid method using the computer-directed Gilford 3500 system (Oberlin, Ohio). Sodium and potassium concentrations in plasma and urine were determined by flame photometry (Instrumentation Laboratories, Boston). Plasma aldosterone levels were determined using the radioimmunoassay kit (Sorim Biomedica, Saluggia, Italy). Angiotensin II was a gift from CIBA-GEIGY, Basel, Switzerland. All reagents were purchased from Sigma. Data are presented as mean±SEM. Analysis of variance was performed for statistical evaluation between the three groups. Results between individual groups were compared by a nonpaired Student’s $t$ test with a modified level of significance according to the Bonferroni method. In this setting a value of $p<0.025$ was considered significant.

**Results**

**Metabolic Data**

Metabolic data for control rats and rats with moderate and severe heart failure after 9 days of A-V fistula formation are summarized in Table 1. Control rats and rats with moderate heart failure exhibited comparable values for most parameters measured except that rats with moderate heart failure had a decreased sodium excretion rate by 47% ($p<0.001$) of that of controls. Rats with moderate heart failure had mild edema, their hearts were slightly enlarged, and their fistulas were only somewhat smaller compared with those of rats with severe heart failure. Rats with severe heart failure exhibited extreme changes in most parameters measured. Their food and fluid intake and urine output were decreased compared with those of control rats ($p<0.01$ for the above three parameters). Creatinine clearance decreased to one third that of control rats ($p<0.001$). $U_{na}V$ decreased to less than 5% of control values ($p<0.001$). Urinary K$^+$ excretion ($U_{K}V$) also decreased compared with controls ($p<0.001$). Plasma Na$^+$ was not changed compared with controls, while plasma K$^+$ was increased ($p<0.001$) and plasma aldosterone mounted to levels 10 times that of controls ($p<0.001$). The hearts of rats with severe heart failure were markedly enlarged compared with controls ($p<0.001$) and rats with moderate heart failure ($p<0.005$). The rats with severe heart failure exhibited extreme edema and ascites with pleural effusion despite their very low food intake. In Table 2 the main metabolic data on days 0, 1, 3, 5, 7, and 9 are depicted. It is also evident from this table that rats with severe heart failure ate very little during the whole period of 9 days and lost weight. It should be noted, however, that after 24 hours of A-V fistula
formation, when rats in both groups had very low food intake, $U_\text{NaV}$ was significantly lower in rats with severe heart failure ($p<0.01$). Rats with severe heart failure also drank less compared with rats with moderate heart failure, but their urine output was not significantly less compared with rats with moderate heart failure except for day 9, probably because of decreased concentrating ability. This is also reflected by their low osmolar excretion rate, which on day 9 was $6.0\pm0.5$ μosm/l in rats with severe heart failure compared with $10.7\pm1.1$ in rats with moderate heart failure ($p<0.005$).

Table 3 shows metabolic data for rats with moderate heart failure on day 20 after A-V fistula formation. $U_\text{NaV}$ on day 20 was not significantly different from control.

Average $U_\text{NaV}$ values on days 0, 1, 3, 5, 7, and 9 in rats with moderate and severe heart failure are depicted in Figure 1. In the baseline period, $U_\text{NaV}$ was between 0.65 and 0.7 μeq/min. Twenty-four

**Figure 1.** Daily sodium excretion rates ($U_\text{NaV}$) in rats with moderate or severe heart failure.

**Table 2.** Metabolic Data for Rats With Moderate or Severe Heart Failure on Days 0, 1, 3, 5, 7, and 9 After Aortocaval Fistula Formation

<table>
<thead>
<tr>
<th>Day</th>
<th>Food intake (g/day)</th>
<th>Fluid intake (ml/day)</th>
<th>Body wt (g)</th>
<th>Diuresis (ml/min)</th>
<th>$U_\text{NaV}$ (μeq/min)</th>
<th>$U_\text{K}^+$ (μeq/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe heart failure (group a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20.8±0.9</td>
<td>30.3±1.0</td>
<td>236±5.4</td>
<td>0.0064±0.0005</td>
<td>0.7±0.05</td>
<td>1.78±0.09</td>
</tr>
<tr>
<td>1</td>
<td>1.3±1.0</td>
<td>17.2±3.2</td>
<td>240±12</td>
<td>0.0066±0.0012</td>
<td>0.0074±0.0037</td>
<td>0.82±0.11</td>
</tr>
<tr>
<td>3</td>
<td>2.1±1.8</td>
<td>21.0±3.1</td>
<td>238±14</td>
<td>0.0080±0.0010</td>
<td>0.38±0.08</td>
<td>1.48±0.15</td>
</tr>
<tr>
<td>5</td>
<td>2.0±2.0</td>
<td>10.3±2.9</td>
<td>225±9</td>
<td>0.0060±0.0006</td>
<td>0.015±0.005</td>
<td>0.73±0.08</td>
</tr>
<tr>
<td>7</td>
<td>2.5±1.1</td>
<td>12.8±4.0</td>
<td>220±7</td>
<td>0.0058±0.00084</td>
<td>0.024±0.0033</td>
<td>0.97±0.23</td>
</tr>
<tr>
<td>9</td>
<td>1.2±0.8</td>
<td>13.0±3.2</td>
<td>214±10</td>
<td>0.0038±0.0007</td>
<td>0.029±0.016</td>
<td>0.69±0.2</td>
</tr>
<tr>
<td>Moderate heart failure (group b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18.8±0.8</td>
<td>26.6±1.0</td>
<td>220±3.6</td>
<td>0.0063±0.0004</td>
<td>0.67±0.036</td>
<td>1.81±0.07</td>
</tr>
<tr>
<td>1</td>
<td>2.8±1.6</td>
<td>23.0±5.6</td>
<td>223±6.4</td>
<td>0.0100±0.0026</td>
<td>0.10±0.029*</td>
<td>1.26±0.11*</td>
</tr>
<tr>
<td>3</td>
<td>15.6±1.3†</td>
<td>31.8±3.0†</td>
<td>230±7.8</td>
<td>0.0084±0.0025</td>
<td>0.38±0.067</td>
<td>1.61±0.09</td>
</tr>
<tr>
<td>5</td>
<td>18.4±1.8†</td>
<td>27.6±1.1†</td>
<td>239±5.6</td>
<td>0.0065±0.0005</td>
<td>0.48±0.04†</td>
<td>1.64±0.08†</td>
</tr>
<tr>
<td>7</td>
<td>20.2±1.2†</td>
<td>29.2±0.8†</td>
<td>248±5.0</td>
<td>0.0058±0.00035</td>
<td>0.35±0.045†</td>
<td>1.66±0.14*</td>
</tr>
<tr>
<td>9</td>
<td>21.7±1.8†</td>
<td>32.7±2.6†</td>
<td>259±5.6</td>
<td>0.0068±0.00048*</td>
<td>0.35±0.022†</td>
<td>2.02±0.08†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. $U_\text{NaV}$, urinary Na+ excretion rate; $U_\text{K}^+$, urinary K+ excretion rate.

*a* $p<0.01$ compared with group a.

†$p<0.001$ compared with group a.

‡$p<0.025$ compared with group a.
hours after A-V fistula formation both groups of rats retained sodium, but as already mentioned, for group a it was significantly less than for group b. Within the next 48 hours both groups excreted the retained load or at least a part of it. From the fourth day on, rats with moderate heart failure excreted between 40% and 70% of control sodium values, while in rats with severe heart failure sodium excretion decreased to less than 5% of control values. We took into account that these rats ate almost nothing and compared their sodium excretion to that of starving rats. Rats that were starved for 6 days had an average sodium excretion rate of 0.22±0.027 (n=9) μeq/min (p<0.001) compared with that of rats in group a.

**Na,K-ATPase and Mg-ATPase Activities**

Na,K-ATPase activity along the nephron of control rats, rats with moderate heart failure, and rats with severe heart failure are presented in Figure 2. In rats with moderate heart failure, Na,K-ATPase activity increased by 43.5% (p<0.001) in the CTAL and decreased by 17.8% (p<0.005) in the DC. In the remaining segments, Na,K-ATPase activity did not change significantly. In rats with severe heart failure, Na,K-ATPase activity increased by 28.9% (p<0.001) and 74.7% (p<0.001) in the proximal nephron PC and PS, respectively, while it dramatically decreased by 41.4% (p<0.001), 42.8% (p<0.001), and 44.4% (p<0.001) in the MTAL, CTAL, and DC, respectively. In the CCD, Na,K-ATPase activity did not change in rats with moderate or severe heart failure.

Na,K-ATPase activities in the PC and MTAL of rats that were starved for 6 days are shown in Table 4. Na,K-ATPase activity did not change in these segments in starving rats.

Mg-ATPase activity for the three groups studied is presented in Figure 3. Mg-ATPase activity did not change significantly compared with controls for most segments studied from rats with moderate or severe heart failure. However, in the PC, Mg-ATPase increased by 52.1% (p<0.001) in rats with severe heart failure. A similar increase of 41.6% (p<0.02) was observed also for PC Mg-ATPase in starving rats (Table 4).

The effect of 10^-10 M angiotensin II added to the incubation medium of PC and PS isolated from intact rats on Na,K-ATPase and Mg-ATPase is shown in Table 5. Na,K-ATPase of the PC and PS increased by 27% and 54.5%, respectively, without any change in Mg-ATPase.

**Discussion**

The results of this study exhibit two patterns of heart failure after A-V anastomosis that show distinct patterns of renal function, renal sodium handling, and single nephron Na,K-ATPase activity. The group with severe heart failure (group a) had peripheral edema, ascites, pulmonary congestion, and an enlarged myocardium. This group was characterized by markedly abnormal renal function and renal sodium handling. Data for these rats were similar to those of dogs with A-V fistulas, in which a low glomerular filtration rate had been reported. In group a, the plasma aldosterone level was 10 times that of controls, and according to Winaver et al, for the same model the plasma renin activity was also markedly increased. Single nephron Na,K-ATPase activity, which represents the active reabsorption of sodium along the nephron, shows significant increases in the proximal tubule (PC and PS) with
marked decreases in the enzyme activity in the thick ascending limb of Henle’s loop (MTAL and CTAL) and in the DC. The increase in Na\textsubscript{1},K\textsubscript{1}-ATPase in the PC was accompanied by a marked increase of Mg\textsubscript{2+}-ATPase, but in the remaining segments Mg\textsubscript{2+}-ATPase did not change significantly. In the PS a marked increase in Na\textsubscript{1},K\textsubscript{1}-ATPase activity was not accompanied by a similar change in Mg\textsubscript{2+}-ATPase. It is not clear at this point whether the increase in Na\textsubscript{1},K\textsubscript{1}-ATPase activity in the proximal nephron is responsible for, or secondary to, changes in Na\textsuperscript{+} reabsorption.

Because of the fall in glomerular filtration rate and filtered load of sodium observed in these rats, it is hardly conceivable to relate the increase in proximal tubule Na\textsubscript{1},K\textsubscript{1}-ATPase to increased sodium delivery. However, the fractional reabsorption has increased and possibly the sodium transporting system operates against a steeper luminal-peritubular sodium concentration gradient. This tendency may be reinforced by increased levels of angiotensin II, which is an activator of proximal tubular sodium reabsorption.\cite{12-15} When the direct effect of 10\textsuperscript{-10} M angiotensin II on PC and PS Na\textsubscript{1},K\textsubscript{1}-ATPase was studied, it was shown to increase this activity to a similar extent to that observed in rats with severe congestive heart failure. The angiotensin II concentration of 10\textsuperscript{-10} M (about 10 times higher than physiological) was chosen according to the increase in plasma renin activity reported in rats with severe Na\textsuperscript{+} retention.\cite{3} These observations suggest a possible direct role of angiotensin II in the increased Na\textsubscript{1},K\textsubscript{1}-ATPase activity in the proximal nephron of rats with severe congestive heart failure.

The increase in proximal sodium reabsorption with the concurrent fall in glomerular filtration rate may be expected to markedly decrease distal delivery of sodium. This may be the explanation for the striking

### Table 3. Metabolic Data for Eight Rats With Moderate Heart Failure 20 Days After Aortocaval Fistula Formation

<table>
<thead>
<tr>
<th>Food intake (g/day)</th>
<th>Fluid intake (ml/day)</th>
<th>Body wt (g)</th>
<th>Diuresis (ml/min)</th>
<th>U\textsubscript{N}V (μeq/min)</th>
<th>U\textsubscript{K}V (μeq/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5±2.7</td>
<td>28.9±3.4</td>
<td>301±3.9</td>
<td>0.0069±0.0009</td>
<td>0.54±0.08</td>
<td>1.77±0.15</td>
</tr>
</tbody>
</table>

Values are mean±SEM. U\textsubscript{N}V, urinary Na\textsuperscript{+} excretion rate; U\textsubscript{K}V, urinary K\textsuperscript{+} excretion rate.

### Figure 3. Mg\textsubscript{2+}-ATPase activity in renal tubules isolated from control rats with moderate heart failure and severe heart failure 9 days after fistula formation between the aorta and vena cava or sham operation. Data are presented as mean±SEM. The following cortical and medullary segments were used: proximal convoluted tubule (PC), cortical proximal straight tube (PS), medullary thick ascending limb (MTAL), cortical thick ascending limb (CTAL), distal convoluted tubule (DC), and cortical collecting duct (CCD).

### Table 4. Na\textsubscript{1},K\textsubscript{1}-ATPase and Mg\textsubscript{2+}-ATPase Activity in Control Rats and Rats Starved for 6 Days

<table>
<thead>
<tr>
<th>Na\textsubscript{1},K\textsubscript{1}-ATPase</th>
<th>Mg\textsubscript{2+}-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>MTAL</td>
</tr>
<tr>
<td>Control</td>
<td>28.4±1.1</td>
</tr>
<tr>
<td>Starved rats</td>
<td>26.6±2.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM (10\textsuperscript{-11} mol/mm/min). PC, proximal convoluted tubule; MTAL, medullary thick ascending limb.

*p<0.02 compared with control.
Table 5. Effect of Angiotensin II on Na,K-ATPase and Mg-ATPase Activities of the Proximal Nephron

<table>
<thead>
<tr>
<th></th>
<th>Na,K-ATPase</th>
<th></th>
<th>Mg-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>PS</td>
<td>PC</td>
</tr>
<tr>
<td>Control</td>
<td>27.1±0.4</td>
<td>23.3±1.7</td>
<td>30.9±1.4</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=12)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^-10 M)</td>
<td>34.4±1.8*</td>
<td>36.0±1.6*</td>
<td>31.0±1.3</td>
</tr>
<tr>
<td></td>
<td>(n=15)</td>
<td>(n=14)</td>
<td>(n=15)</td>
</tr>
</tbody>
</table>

Values are mean±SEM (10^-11 mol/mm/min). PC, proximal convoluted tubule; PS, cortical proximal straight tubule.

* p<0.001 compared with control.

fall in Na,K-ATPase activity in the MTAL, CTAL, and DC. This interpretation rests with the tenet that sodium entry into cells modulates V_max Na,K-ATPase activity. An argument in favor of this view may be found in the study of Hayhurst and O’Neill,16 who demonstrated that aldosterone-induced stimulation of Na,K-ATPase could be abolished by amiloride, reflecting a Na^+-dependent modulation of V_max Na,K-ATPase activity. Furthermore, these authors propose that Na^+ entry modulates a posttranslational process, which regulates the expression of the enzyme at the basolateral membrane by an exocytic process.17 On the other hand, Barlet-Bas et al.18 have shown that aldosterone may increase the number of catalytic sites of Na,K-ATPase independent of Na^+ availability. This effect of aldosterone requires the permissive effect of triiodothyronine and depends on de novo protein synthesis. However, even though aldosterone may exert its effect without a change in Na^+ entry, it certainly does not rule out a possible role of sodium per se in augmenting V_max Na,K-ATPase activity. The maintenance of Na,K-ATPase activity in the CCD in face of the decrease in distal delivery may be explained by the high levels of aldosterone. This explanation is supported by the study of Barlet-Bas et al.,18 who demonstrated a sodium-independent aldosterone effect on V_max Na,K-ATPase, as mentioned above.

We took into account that the rats in group a ate almost nothing and compared their sodium excretion to that of starved rats. Rats that were starved for 6 days excreted seven to eight times more sodium (p<0.001) than did rats in group a. Na,K-ATPase activity in the PC and MTAL of starved rats (Table 4) was not different from that of controls, whereas Mg-ATPase in the PC of starved rats increased similarly to that in the PC of rats with severe heart failure. Thus, the increase in Mg-ATPase in the PC of rats with severe heart failure may be related to their low food intake. The marked decrease in Na,K-ATPase activity in the MTAL, CTAL, and DC of rats with severe heart failure was not accompanied by a similar change in Mg-ATPase excluding a general pathological damage in these nephron segments. Taken together, these observations exclude the nutritional factor as the cause for the extreme fall in Na^+ excretion and the changes in Na,K-ATPase activity in rats with severe heart failure. Hyperkalemia and hyperaldosteronism are known to increase rather than decrease Na,K-ATPase activity, mainly in the CCD.19,20 Therefore it is unlikely that the decreases in Na,K-ATPase observed in rats with severe heart failure were due to hyperkalemia or hyperaldosteronism.

The second group with moderate heart failure (group b) did not show extreme deviations from normal in most parameters studied, including normal glomerular filtration rate, except that their sodium excretion rate decreased by 47%, similar to what was shown by Stumpe et al1 for rats with A-V fistula. The weight of these rats were not significantly increased when compared with controls. In similar rats, Winaver et al3 found normal plasma renin activity, and here we found normal aldosterone levels. The segmental analysis of Na,K-ATPase activity along the nephron of these rats showed that the main site of increased sodium reabsorption was the CTAL. These results are in accordance with the findings of Stumpe et al,1 who demonstrated by the micropuncture technique that the site of increased Na^+ reabsorption in rats with experimental heart failure was the loop of Henle. The fall in Na,K-ATPase activity in the DC may stem from avid sodium reabsorption in the loop of Henle with reduced delivery of sodium to this segment.

It may be concluded that in rats with heart failure, Na,K-ATPase varies with the severity of sodium retention. In moderate heart failure, the site of enhanced sodium reabsorption is CTAL, but in severe heart failure, sodium is avidly reabsorbed in PC and PS, while in the MTAL, CTAL, and DC reabsorption is decreased, presumably because of reduced delivery. High angiotensin II levels may play a direct role in the increased Na,K-ATPase activity and enhanced Na^+ reabsorption in the proximal nephron in severe CHF. Furthermore, the beneficial response to angiotensin converting enzyme inhibitors in patients with severe CHF may be related to the abolition of the effect of angiotensin II to increase proximal Na^+ reabsorption. These findings may explain the efficacy of loop diuretics in mild to moderate heart failure with refractoriness to these agents in severe heart failure.

References

KEY WORDS • congestive heart failure • Na+ retention • angiotensin II
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